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Enzymatic digestive capabilities in geophagous earthworms – origin and activities of cellulolytic enzymes

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Summary. This study compares the origin and activities of glycolytic enzymes present in the gut contents of some geophagous adult earthworms and demonstrates that all the species did not develop mutualistic relationships with the ingested microorganisms to digest soil organic matter. Although the glycolytic activities were rather weak in the earthworms studied, they possess quite a complete enzymatic system that allows to degrade root and fungal substrates available in soils. *Millsonia anomala* Omo. from Lamto (Côte d'Ivoire) and *Hormogaster elisae* Álvarez from El Molar (Madrid) developed a strong mutualistic earthworm-microflora digestion system for hydrolysing cellulose: cellulase produced by ingested microflora is able to degrade cellulose to cellobiose, cellobiase released by the worm itself breaks down cellobiose into D-glucose. Both enzymes found in intestinal contents of *Pontoscolex corethrurus* Müll. from Palma Sola, Veracruz (Mexico) were produced by ingested soil microflora since they were not found in gut tissue culture. On the contrary, *Polypheretima elongata* Perr. from Sainte Anne (Martinique), *Hyperiodrilus africanus* Bedd. and *Dichogaster terrae nigrae* Omo. et Vld. from Lamto (Côte d'Ivoire) possess a complete enzymatic system for hydrolysing cellulose. They could synthesize cellulolytic enzymes, i.e. cellulase and cellobiase by themselves. In the course of their digestion, geophagous earthworms seem to display variable adaptative characters which are undoubtedly linked to the different ecological categories and niches.

Key words: Earthworm, glycolytic digestive enzymes, *in vitro* intestinal tissue culture, ingested microflora

Introduction

Strong relationships exist between invertebrates and microorganisms to make use of soil organic matter (Rouelle 1985; Lavelle 1995). Pedofauna exerts a great influence on microbial populations. In most of the humid savannas, soil macrofauna is largely composed of geophagous species (termites and earthworms) which feed on soil organic matter (Athias et al. 1975; Lavelle et al. 1981). Endogeic earthworms have developed mutualistic relationships with ingested microflora to digest this organic matter (Barois 1987; Lavelle et al. 1995). Their effi-

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Decaëns, T., Lavelle, P., Jiménez, J. J., Escobar, G., Rippstein, G. (1994) Impact of land management on soil macrofauna in the Oriental Llanos of Colombia. European Journal of Soil Biology 30, 157–168.
Derouard, L., Tondoh, J., Vilcosqui, L., Lavelle, P. (1997) Effects of earthworm introduction on soil processes and plant growth. Soil Biology and Biochemistry 29, 541–545.
Edwards, C. A., Bohlen, P. J. (1996) Biology and Ecology of Earthworms – 3rd Edition. Chapman & Hall, London, United Kingdom, 426 p.
Gijsman, A. J. (1996) Soil aggregate stability and soil organic matter fractions under agropastoral systems established in native savanna. Australian Journal of Soil Research 34, 891–907.
Gijsman, A. J., Thomas, R. J. (1996) Evaluation of some physical properties of an Oxisol after conversion of native savanna into legume-based or pure grass pasture. Tropical Grasslands 30, 237–248.
Jiménez, J. J., Moreno, A. G., Decaëns, T., Lavelle, P., Fisher, M., Thomas, R. J. (1998a) Earthworm communities in native savannas and man-made pastures of the Eastern Plains of Colombia. Biology and Fertility of Soils 28, 101–110.
Jiménez, J. J., Moreno, A. G., Lavelle, P., Decaëns, T. (1998b) Population dynamics and adaptive strategies of *Martiodrilus carimaguensis* (Oligochaeta, Glossoscolecidae), a native species from the well-drained savannas of Colombia. Applied Soil Ecology 9, 153–160.
Lal, R. (1988) Effects of macrofauna on soil properties in tropical systems. Agriculture, Ecosystems and Environments 24, 101–116.
Lavelle, P. (1996) Diversity of soil fauna and ecosystem function. Biology International 33, 3–16.
Lavelle, P. (1997) Faunal activities and soil processes: adaptive strategies that determine ecosystem function. Advances in Ecological Research 27, 93–132.
Lavelle, P., Pashansi, B. (1989) Soil macrofauna and land management in Peruvian Amazonia (Yurimaguas, Loreto). Pedobiologia 33, 283–409.
Lee, K. E. (1985) Earthworms. Their Ecology and Relationships with Soils and Land Use. Academic Press, Orlando, U.S.A., 411 p.
Legendre, P., Vaudor, A. (1991) The R package: multidimentional analysis, spatial analysis. Université de Montréal, Département des Sciences Biologiques, Montréal, Canada.
Mytton, L. R., Cresswell, A., Colbourn, P. (1993) Improvement in soil structure associated with white clover. Grass and Forage Science 48, 84–90.
Pashanasi, B., Melendez, G., Szott, L., Lavelle, P. (1992) Effect of inoculation with the endogeic earthworm *Pontoscolex corethrurus* (Glossoscolecidae) on N availability, soil microbial biomass and the growth of three tropical fruit tree seedlings in pot experiments. Soil Biology and Biochemistry 24, 1655–1659.
Reddy, M. V., Kumar, V. P. K., Reddy, V. R., Balashouri, P., Yule, D. F., Cogle, A. L., Jangawad, L. S. (1995) Earthworm biomass response to soil management in a semi-arid tropical Alfisol agroecosystem. Biology and Fertility of Soils 19, 317–321.
Sánchez, P. A., Castilla, C. E., Alegre, J. C. (1991) Grazing pressure effects on the pasture degradation process. In: TropSoil Technical Report, 1988–1989, CIAT, Cali, Colombia, pp. 182–186.
Sokal, R. R., Rohlf, F. J. (1995) Biometry: the principles and practice of statistics in biological research – 3rd Edition. W. H. Freeman and Company, New York, USA.
Spain, A. V., Lavelle, P., Mariotti, A. (1992) Stimulation of plant growth by tropical earthworms. Soil Biology and Biochemistry 24, 1629–1633.
Thioulouse, J., Chessel, D., Doledec, S., Olivier, J. M. (1997) ADE 4: a multivariate analysis and graphical display software. Statistics and Computing 7, 75–83.

ciency seems to be highly dependent on temperature (Barois 1992). It is proposed that the mucus plays an active role in this mutualistic digestion system; earthworms enhance microbial activities by providing an energy-rich and easily metabolizable intestinal mucus in their gut (Barois & Lavelle 1986; Martin et al. 1987). In fact by ingesting soil with microflora, geophagous earthworms create suitable conditions for microbial activities in their gut by adding considerable amounts of water (80 to 150 % of the dry weight of soil) and intestinal mucus (5 to 43%). This allows the ingested soil microflora to recover all its abilities to degrade the complex substances of the soil organic matter and make it digestible (Lavelle & Gilot 1994). Previous studies suggested that geophagous earthworms had poor enzymatic capabilities and only fed on simple organic compounds (Lavelle 1983).

Previous study on glycolytic enzymatic activities present in the gut contents of geophagous adult earthworms demonstrated that these worms possess a quite complete enzymatic system (Zhang et al. 1993; Lattaud et al. 1997 a; Lattaud et al. 1997 b; Lattaud et al. 1998). These enzymes allow them to degrade root and fungal substrates available in soils and it corroborates that endogeic earthworms feed on litter debris and soils poor in organic matter. Glycolytic activities were evaluated both in the cultured gut wall tissues and in the culture media in order to compare the origin and activities of these enzymes and to determine whether they were released by the worms themselves or by the ingested microflora. It has been demonstrated that *Pontoscolex corethrurus* (Müll. 1857) from Palma Sola, Veracruz (Mexico) and *Millsonia anomala* (Omodeo & Vaillaud 1967) from Lamto (Côte d'Ivoire) require ingested microflora in order to degrade some substrates, such as cellulose and mannan (Zhang et al. 1993; Lattaud et al. 1997 b), in contrast to *Polypheretima elongata* (Mich. 1892), from Sainte Anne (Martinique) which can synthesize by itself all its extra and intracellular enzymes (Lattaud et al. 1997 a). However, among the cellulolytic activities studied in these three earthworm species, only cellulases have been dealt with, without exploiting the cellobiasic activities.

The aim of our experiments was to identify glycolytic activities in the gut of other geophagous earthworm species, *Hyperiodrilus africanus*, *Dichogaster terrae nigrae* and *Hormogaster elisae*, but also to compare them to the glycolytic activities of the three earthworm species studied previously, in particular in order to assess the origin and activities of cellulolytic enzymes.

Materials and Methods

Adult earthworms *H. africanus* (Bedd., 1891) and *D. terrae nigrae* (Omo. et Vld., 1967) from Lamto (Côte d'Ivoire) and *H. elisae* (Álvarez, 1977) from El Molar (Madrid) were maintained in their original soil in a control chamber at 28 °C (31 g water per 100 g dry soil).

Preparation of enzyme solutions

Glycolytic digestive enzymatic activities were tested with many heteroglycoside, oligosaccharide and polysaccharide substrates selected mainly for their plant origin. These activities were scanned in the first part of the gut of *H. africanus*, *D. terrae nigrae* and *H. elisae* including the pharynx, oesophagus, crop and gizzard and in the second part comprising foregut, midgut and hindgut (wall plus contents). These activities were also evaluated in cultured gut wall tissues and in the culture media. The experimental design has been previously described (Lattaud 1997b). The solution obtained was used as the enzyme source.

Enzyme activity assay

The methods for assaying glycolytic activities were described previously (Zhang et al. 1993). Oligosaccharidases were determined by the glucose oxidase method (Werner et al. 1970). Heterosidase and polysaccharidase activities were assayed as outlined by Rouland (1986). Reducing sugars released by hydrolysis were determined by the Somogyi-Nelson microdosage technique (Nelson 1944; Somogyi 1945). The protein content of the enzyme solution was assayed as outlined by Sedmark & Grossberg (1977).

Table 1. Glycolytic activities in the gut (wall plus contents) of *Hyperiodrilus africanus*, *Dichogaster terrae nigrae* and *Hormogaster elisae* as compared to those previously studied in *Pontoscolex corethrurus*, *Millsonia anomala* and *Polypheretima elongata* (see to text for references). S.A. in the GUT: specific glycolytic activity expressed as µg glucose mg^{-1} protein mn^{-1}; p, pharynx; o, oesophagus; c, crop; g, gizzard; n.d. not determined glycolytic activity; +, ++, +++, ++++, 0.1 to 3, 3 to 9, 9 to 30, 30 to 90 (and more) µg glucose mg^{-1} protein mn^{-1}

	Pontoscolex corethrurus **(Glossoscolecidae)** S.A. in the GUT				***Millsonia anomala*** **(Megascolecidae)** S.A. in the GUT				***Hormogaster elisae*** **(Hormogastridae)** S.A. in the GUT			
	p+o+c+g	*foregut*	*midgut*	*hindgut*	*p+o+c+g*	*foregut*	*midgut*	*hindgut*	*p+o+c+g*	*foregut*	*midgut*	*hindgut*
Oligosaccharides												
Maltose	++	+++	+++	+	+	++++	++++	+++	0	++++	+++	0
Cellobiose	+	+	+	+	+	++	+	+	0	++	+	0
Heterosides												
N-acetyl	+	++++	++++	+++	+	++++	+++	+++	+	++++	+++	++
a-Glucoside	+	+	+	+	+	+++	+	+	+	+	+	+
Polysaccharides												
Starch	+	+++	+++	++	+	+	+++	+++	++	++++	++	++
Cellulose	+	+	+	+	+	+	+	0	0	+	0	+
Mannan	0	++	+++	+	0	++	++	+	n.d.	n.d.	n.d.	n.d.
Laminaran	+	+++	++	+	+	++++	++	+	+	+++	+++	++
	Hyperiodrilus africanus **(Eudrilidae)** S.A. in the GUT)				***Polypheretima elongata*** **(Megascolecidae)** S.A. in the GUT				***Dichogaster terrae nigrae*** **(Megascolecidae)** S.A. in the GUT			
	p+o+c+g	*foregut*	*midgut*	*hindgut*	*p+o+c+g*	*foregut*	*midgut*	*hindgut*	*p+o+c+g*	*foregut*	*midgut*	*hindgut*
Oligosaccharides												
Maltose	++	++++	++++	+++	++	++++	++++	++++	++	++++	+++	+++
Cellobiose	0	+	++	+	+	+	+	+	+	+	0	0
Heterosides												
N-acetyl	0	+++	++++	+++	+	+++	+++	++++	+	++	++	++
a-Glucoside	+	+	+	+	+	++	++	++	+	+	+	+
Polysaccharides												
Starch	+++	+++	++++	+++	+	++++	++++	++++	+	+++	+++	++
Cellulose	0	+	++	+	+	++	++	+	0	+	+	+
Mannan	n.d.	n.d.	n.d.	n.d.	+	+	++	+	n.d.	n.d.	n.d.	n.d.
Laminaran	+	+	+++	+	+	++++	+++	+++	+	+++	+	+

Specific glycolytic activities in the gut (wall and contents) and in the cultured intestinal tissues were expressed as µg glucose released per mg of protein per minute; the gut wall tissues of *H. africanus*, *D. terrae nigrae* and *H. elisae* were cultured respectively in 2,9, 2 and 2,8 ml of medium and the glycolytic activities were expressed as total activity (µg glucose mn^{-1}).

Results

Specific glycolytic activities in the gut wall and gut contents

All the substrates tested were more or less broken down in the gut of the three geophagous earthworms studied, as was previously show for *P. corethrurus*, *M. anomala* and *P. elongata* (Zhang et al. 1993; Lattaud et al. 1997 a, b). Table 1 shows specific glycolytic activities in the gut of *H. africanus*, *D. terrae nigrae* and *H. elisae* and compares them to those previously studied in *P. corethrurus*, *M. anomala* and *P. elongata*. Only the substrates which were hydrolised significantly are mentioned in this table. Glycolytic activities were low or not present in the pharynx, oesophagus, crop and gizzard, except for *H. africanus*. On the whole, these activities essentially located in the foregut, midgut and/or hindgut, were higher in the gut of *M. anomala*, *P. elongata*, *H. africanus* and *H. elisae* than in the gut of *P. corethrurus* and *D. terrae nigrae*.

Among the oligosaccharides assayed, maltose was the most readily hydrolised substrate. A very high maltase specific activity was detected in the foregut, midgut and hindgut of *P. elongata*, in the foregut and midgut of *M. anomala* and *H. africanus* but only in the foregut of *H. elisae* and *D. terrae nigrae*. Cellobiase activity was present in the gut of all the studied species although its activity was weak. N-acetylglucosamine was the best degraded heteroglycoside, with maximum activity located in the foregut and midgut of *P. corethrurus*, in the foregut of *M. anomala* and *H. elisae*, in the midgut of *H. africanus* and in the hindgut of *P. elongata*. Polysaccharidase activity in the gut was greatest on starch and essentially located in the foregut, midgut and hindgut of *P. elongata*, in the midgut of *H. africanus* and the foregut of *H. elisae*. The major activity on laminaran was observed in the foregut of *P. elongata*. The other substrates were weakly degraded and it is important to take into account that cellulase specific activity was present in the gut of all the earthworms.

Specific activities in tissue culture and total activities in the culture media

Tables 2 to 4 show specific glycolytic activities in tissue culture and total activities in the culture media of *H. africanus*, *D. terrae nigrae* and *H. elisae* and compare them to those previously studied in *P. corethrurus*, *M. anomala* and *P. elongata*.

Glycolytic enzymatic activities were low or not present in the pharynx, oesophagus, crop and gizzard; therefore, only specific activities in the foregut, midgut and hindgut culture tissues and culture media were evaluated. The major enzymes found in the gut of all the earthworms were present in the cultured foregut, midgut and/or hindgut wall tissues and culture media. Glycolytic enzymatic activities were higher in the culture tissues of *M. anomala*, *P. elongata*, *H. africanus* and *D. terrae nigrae* than in the culture tissues of *P. corethrurus* and *H. elisae*. These activities were higher in culture tissues than in the culture media. The highest glycolytic activities were detected on the assayed substrates after 3, 5 or 6–7 days of culture.

In each earthworm species studied, the main oligosaccharidase specific activities were detected on maltose. The highest maltase activity was detected in the foregut, midgut and hindgut of *P. elongata*, in the foregut and hindgut of *M. anomala* and the foregut of *H. africanus*.

Some weak specific activities were observed on cellobiose in the culture tissues and culture media of each earthworm species, except for *P. corethrurus* where cellobiase activities were non-existent. The highest activities on heteroglycosides were detected on N-acetylglucosamine, mainly in the foregut of *P. elongata*, in the hindgut of *D. terrae nigrae* and *H. africanus*. Among the polysaccharides assayed, starch was the most readily hydrolised substrate. A very high amylase specific activity was detected in the foregut, midgut and hindgut

Table 2. Glycolytic activities in cultured gut wall tissues of *Hormogaster elisae* and in culture media after 3 to 7 days of culture as compared to that in *Millsonia anomala* which has been studied previosly (see text for references). S.A. in TISSUE CULTURE: specific glycolytic activity expressed as µg glucose mg^{-1} protein mn-1; T.A. in CULTURE MEDIA: total glycolytic activity expressed as µg glucose mn^{-1}; p, pharynx; o, oesophagus; c, crop; g, gizzard; n.d., not determined glycolytic activity; +, ++, +++, ++++, O. 1 to 3, 3 to 9, 9 to 30, 30 to 90 (and more) µg glucose mg^{-1} protein mn^{-1}

	***Millsonia anomala* (Megascolecidae)**						***Hormogaster elisae* (Hormogastridae)**					
	S.A. in TISSUE CULTURE			T.A. in CULTURE MEDIA			S.A. in TISSUE CULTURE			T.A. in CULTURE MEDIA		
	foregut	*midgut*	*hindgut*	*foregut*	*midgut*	*hindgut*	*foregut*	*midgut*	*hindgut*	*foregut*	*midgut*	*hindgut*
Oligosaccharides												
Maltose	++++	+++	++++	+	0	0	+	+	0	+	+	0
Cellobiose	+	+	+	+	+	+	+	0	0	++	0	0
Heterosides												
N-acetyl	+++	++	++	+	+	+	+	+	+	+	+	+
a-Glucoside	+	+	0	+	+	0	+	+	0	+	+	0
Polysaccharides												
Starch	+	+	+	+	+	+	+	+	+	+	+	+
Cellulose	0	0	0	0	0	0	0	0	0	0	0	0
Mannan	0	0	0	0	0	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Laminaran	++	++	+	+	+	0	+	+	+	+	+	+

Table 3. Glycolytic activities in cultured gut wall tissues of *Pontoscolex corethrurus* and in culture media after 3 to 7 days of culture. For abbreviations and legend see Table 2

	***Pontoscolex corethrurus* (Glossoscolecidae)**					
	S.A. in TISSUE CULTURE			T.A. in CULTURE MEDIA		
	foregut	*midgut*	*hindgut*	*foregut*	*midgut*	*hindgut*
Oligosaccharides						
Maltose	+++	+++	++	++	++	+
Cellobiose	0	0	0	0	0	0
Heterosides						
N-acetyl	+++	+++	++	++	++	+
a-Glucoside	+	+	+	+	+	+
Polysaccharides						
Starch	+++	++	++	++	+	+
Cellulose	0	0	0	0	0	0
Mannan	0	0	0	0	0	0
Laminaran	++	+++	++	++	++	+

of *P. elongata*. Cellulase and mannanase specific activities, though weak, were present on both media and cultured tissues of *P. elongata*, *D. terrae nigrae* and *H. africanus* and not found on both cultured tissues and media of *M. anomala*, *H. elisae* and *P. corethrurus*. Mannanase specific activities were also not present on both media and cultured tissues of *M. anomala* and *P. corethrurus*; in fact, the available mannan quantity did not allow a determination of the mannanase activities in the culture tissues of *D. terrae nigrae*, *H. africanus* and *H. elisae*.

Discussion

Geophagous earthworms are able to feed on litter debris and soils poor in organic matter (Lavelle 1983). Our study demonstrated that the endogeic earthworm species possess a rather complete enzymatic system although the glycolytic activities were weak as compared to other invertebates such as the snail *Helix aspersa* (Charrier & Rouland 1992) and fungus-growing and xylophagous termites (Rouland 1991). Specific glycolytic activities detected in the gut and in the cultured gut wall tissues were higher in *M. anomala*, *P. elongata* and *H. africanus* than in *P. corethrurus*, *D. terrae nigrae* and *H. elisae*. Maltose, N-acetylglucosamine, starch and laminaran were the substrates most efficiently broken down. These results suggest that geophagous earthworms feed mainly on root and fungal substrates available in the soils and this is in concordance with the decreasing fungus number during the intestinal transit (Barois & Lavelle 1986).

The hypothesis of mutualism between endogeic earthworms and the ingested soil microflora to digest organic matter has been established (Lavelle 1986; Barois 1987; Martin et al. 1987; Trigo et al. 1992; Lavelle et al. 1995). These mutualistic relationships have been revealed for the first time with the endogeic earthworm species *P. corethrurus* (Barois & Lavelle 1986). The intestinal mucus produced by this species stimulates the microflora to degrade the soil organic matter and make it digestible.

Although several authors have concluded that the earthworms of the temperate areas secrete numerous enzymes to digest soil organic matter (Urbasek & Pilzl 1991), they have not been able to determine the origin of the cellulase produced by tissues of the gut walls and / or by micro-organisms (Loquet & Vinceslas 1987). Nevertheless it has been demonstrated that all epigeic and endogeic earthworm species could break down cellulose and only *Lumbricus*

Table 4. Glycolytic activities in cultured gut wall tissues of *Dichogaster terrae nigrae* and *Hyperiodrilus africanus* and in culture media after 3 to 7 days of culture as compared to that in *Polypheretima elongata* which has been studied previously (see text for references). For abbreviations and legend see Table 2

	Polypheretima elongata **(Megascolecidae)**						***Dichogaster terrae nigrae*** **(Megascolecidae)**						***Hyperiodrilus africanus*** **(Eudrilidae)**					
	S.A. in TISSUE CULTURE			T.A. in CULTURE MEDIA			S.A. in TISSUE CULTURE			T.A. in CULTURE MEDIA			S.A. in TISSUE CULTURE			T.A. in CULTURE MEDIA		
	fore-gut	*mid-gut*	*hind-gut*	*fore-gut*	*mid-gut*	*hind-gut*	*fore-gut*	*mid-gut*	*hind-gut*	*fore-gut*	*mid-gut*	*hind-gut*	*fore-gut*	*mid-gut*	*hind-gut*	*fore-gut*	*mid-gut*	*hind-gut*
Oligosaccharides																		
Maltose	++++	++++	++++	++	+++	+++	+	+	+	+	++	+	++++	0	+	+	0	++
Cellobiose	+++	++	+	++	+	+	+	+	0	+	+	0	0	+	+	0	+	0
Heterosides																		
N-acetyl	++++	++	+++	+++	+	+	+++	+++	++++	0	+	0	+++	+++	++++	+	+	+
a-Glucoside	+++	++	++	+	0	0	+	+	++	+	+	+	+	+	++	+	+	+
Polysaccharides																		
Starch	++++	++++	++++	+	0	+	+	+	+	++	++	+	+	++	++	++	++	++
Cellulose	+	+	+	+	+	0	+	+	0	+	+	+	0	+	0	0	+	0
Mannan	+	+	+	+	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Laminaran	+	+	+	0	0	0	+	+	0	++	+	+	+	+	+++	+	+	+

rubellus was dependent on symbiotic cellulolytic microflora (Urbasek 1990). Recent experiments on cellulolytic activities in the gut of *Eisenia fetida andrei* clearly demonstrated that this earthworm species possesses a complete enzymatic system for hydrolysing cellulose (Vinceslas 1998). It is able to release by itself cellulase whereas a cellullase specific activity was detected in the cultured foregut, midgut and hindgut wall tissues and culture media. On the contrary, cellobiase was secreted by ingested soil microflora. Our experiments on geophagous earthworms reveal that the enzymatic system for degrading cellulose was species dependent, with in some cases synergy between microflora and earthworm. For example, *M. anomala* and *H. elisae* developed a strong mutualistic relationship with the ingested soil microorganisms for hydrolysing cellulose: cellulase produced by ingested microflora is able to degrade cellulose to cellobiose and cellobiase released by the earthworm itself breaks down cellobiose into D-glucose (Lattaud et al. 1997 b). Absence of cellulase and cellobiase activities in gut wall tissues and culture media of *P. corethrurus* indicates that these cellulolytic enzymatic activities found in the gut are of microbial origin. *P. elongata*, *D. terrae nigrae* and *H. africanus* produced by themselves the glycolytic enzymes and possessed a complete enzymatic system to hydrolyse cellulose, particularly cellulase and cellobiase. These glycolytic enzymes were secreted by cultured gut wall tissues in culture media, which indicates that they are extra-cellular enzymes. Some enzymes were released in the medium only after a latent period of culture and their secretion was perhaps induced. It is worth noting that *P. elongata* can synthesize by itself mannanase, contrary to *P. corethrurus* and *M. anomala* which require the microflora of the soil ingested to hydrolyse mannan.

This study allowed us to infer that endogeic earthworms seem to display rather variable diets and adaptative characters which are undoubtedly linked to specific ecological niches.

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References

Athias, F., Josens, G., Lavelle, P. (1975) Traits généraux du peuplement endogé de la savane de Lamto (Côte d'Ivoire). In: Vanek, S. J. (ed) Progress in Soil Zoology, pp. 389–397. Praha.

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

Barois, I. (1992) Mucus production and microbial activity in the gut of two species of *Amynthas* (Megascolecidae) from cold and warm tropical climates. Soil Biology and Biochemistry 24, 1507–1510.

Barois, I., Lavelle, P. (1986) Changes in respiration rate and some physicochemical properties of a tropical soil during transit through *Pontoscolex corethrurus* (Glossoscolecidae, Oligochaeta). Soil Biology and Biochemistry 18, 539–541.

Charrier, M., Rouland, C. (1992) Les osidases digestives de l'escargot *Helix aspersa*: localisations et variations en fonction de l'état nutritionnel. Canadian Journal of Zoology 70, 2234–2241.

Lattaud, C., Zhang, B., Locati, S., Rouland, C., Lavelle, P. (1997a) 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.

Lattaud, C., Locati, S., Mora, P., Rouland, C. (1997b) 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., Locati, S., Mora, P., Rouland, C. (1998) The diversity of digestive systems in tropical geophagous earthworms. Applied Soil Ecology 9, 189–195.

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

Lavelle, P. (1986) Associations mutualistes avec la microflore du sol et richesse spécifique sous les tropiques: l'hypothèse du premier maillon. Comptes Rendus de l'Académie des Sciences de Paris 302, 11–14.

Lavelle, P., Maury, M. E., Serrano, V. (1981) Estudio cuantitativo de la fauna del suelo en la región de Laguna Verde, Veracruz. Epocas de lluvias. Instituto de Ecología México 6, 75–105.
Lavelle, P., Gilot, C. (1994) Priming effects of macroorganisms on microflora: a key process of soil function? In: Ritz, K., Dighton, J., Giller, K. (eds) Beyond the Biomass, p. 6. Wiley-Sayce, UK.
Lavelle, P., Lattaud, C., Trigo, D., Barois, I. (1995) Mutualism and biodiversity in soils. Plant and Soil 30, 23–33.
Loquet, M., Vinceslas, M. (1987) Cellulolyse et ligninolyse liées au tube digestif d'*Eisenia fetida andrei* Bouché. Revue d'Ecologie et de Biologie du Sol 24, 559–571.
Martin, A., Cortez, J., Barois, I., Lavelle, P. (1987) Les mucus intestinaux de ver de terre, moteur de leurs interactions avec la microflore. Revue d'Ecologie et de Biologie du Sol 24, 549–558.
Nelson, N. (1944) Photometric adaptation of Somogyi method for determination of glucose. Journal of Biological Chemistry 153, 275–380.
Rouelle, J., Pussard, M., Randriamamonjizaka, J. L., Loquet, M., Vinceslas, M. (1995) Interactions microbiennes (bactéries et protozoaires), alimentation des vers de terre et minéralisation de la matière organique. Bulletin of Ecology 16 (1), 83–88.
Rouland, C. (1986) Contribution à l'étude des osidases digestives de plusieurs espèces de Termites africains. Thèse Université Paris VI.
Rouland, C., Lepage, F. (1991) The role of the symbiotic fungus in the digestive metabolism of several species of fungus-growing termites. Comparative Biochemistry and Physiology 99A, 657–663.
Sedmark, J. J., Grossberg, S. E. (1977) A rapid, sensitive and versatile assay for protein using coomassie brilliant blue G 250. Analytical Biochemistry 79, 544–552.
Somogyi, M. (1945) Determination of blood sugar. Journal of Biological Chemistry 160, 61–68.
Trigo, D., Martin, A., Lavelle, P. (1992) A mutualist system of digestion in temperate earthworms, *Allolobophora molleri* and *Octolasium lacteum* (Lumbricidae). Acta Zoologica Fennica 196, 53–59.
Urbášek, F. (1990) Cellulase activity in the gut of some earthworms. Revue d'Ecologie et de Biologie du Sol 27 (1), 21–28.
Urbášek, F., Pižl, V. (1991) Activity of digestive enzymes in the gut of five earthworm species (Oligochaeta; Lumbricidae). Revue d'Ecologie et de Biologie du Sol 28 (4), 461–468.
Vinceslas, M. (1998) Activité cellulolytique du tube digestif d'*Eisenia fetida andrei*. Impact sur la dynamique de la matière organique de déchets lignocellulosiques. Thèse 3e cycle, Université de Rouen.
Werner, W., Rey, H. G., Wielinger, R. H. (1970) Properties of a new chromogen for determination of glucose in blood according to GOD/POD method. Frezenius Analytical 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 (1), 7–11.