Origin and activities of glycolytic enzymes in the gut of the tropical geophagous earthworm *Millsonia anomala* from Lamto (Côte d'Ivoire)

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Summary. The tropical endogeic earthworms have developed strong mutualism with the ingested microflora to digest soil organic matter. In order to determine the role of the intestinal mucus in these mutualistic relationships, glycolytic enzymatic activities have been assayed in the gut contents of adult *Millsonia anomala* from Lamto (Côte d'Ivoire). Twenty-one tested substrates selected for their plant origin were broken down, which indicates that this species may use root and fungal substrates available in soils. With the aim of comparing the origins of these glycolytic enzymes found in the gut, the intestinal wall tissues were cultured *in vitro* and enzymatic activities were measured in both cultured tissues and culture media. *M. anomala* cannot produce cellulase and mannanase and use instead the digestive enzymatic capabilities of the ingested microflora which synthesize extracellular glycolytic enzymes. These enzyme capabilities are similar to those of *Pontoscolex corethrurus*, and inferior to *Polypheretima elongata* that produces cellulase and mannanase.

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

Introduction

Endogeic geophagous earthworms seem to develop mutualistic relationships with the ingested microorganisms to digest soil organic matter (Lavelle et al. 1983; Lavelle 1986; Barois 1987; Martin et al. 1987; Martin 1989; Lavelle & Fragoso 1992; Trigo et al. 1992; Trigo & Lavelle 1993). Free soil microorganisms find favourable physico-chemical conditions for their activities in the first part of the gut: neutral pH, high water content (100 to 150 % of the dry mass of soil), high concentrations of intestinal mucus (5 to 43 % of the dry mass of ingested soil, depending on the species) and readily assimilable organic matter (Lavelle 1978; Barois & Lavelle 1986; Barois 1992). The mucus is supposed to play a central role in this mutualist digestion system; earthworms enhance microbial activities by providing in their gut an energy-rich and easily metabolizable intestinal mucus (Martin et al. 1987). In the course of in-

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testinal transit this mucus rapidly disappears. It is no longer present in the middle part of the gut. Part of it has been metabolized by microorganisms and another part has probably been reabsorbed and recycled inside the earthworm. In the posterior part of the gut, micro-organisms seem to have recovered all their metabolic capabilities, in particular by feeding on mucus, and are able to digest soil organic matter. This effect is considered as a "priming effect" *sensu* Jenkinson (1966) (Lavelle & Gilot 1994). Moreover, the efficiency of this digestion system seems to be highly dependent on temperature (Barois 1987). When temperature is decreased from 27 °C to 15 °C, the increase of microbial respiratory activity is reduced to twice the control value and earthworms fail to grow. At 28 °C the microbial activity in the gut content is 6 to 10 times higher than in the wet 2 mm – sieved soil taken as a control (Lavelle et al. 1995).

In order to determine the role of the intestinal mucus in this mutualist earthworm microflora-digestive system, glycolytic enzymatic activities have been assayed in the gut of two tropical species: *Pontoscolex corethrurus* (Müll., 1857) from Palma Sola, Veracruz (México) and *Polypheretima elongata* (Mich., 1892), from Sainte Anne (Martinique). It has been demonstrated that these earthworms possess a rather complete, though weak, enzymatic system. The *in vitro* tissue culture of gut wall showed that *P. corethrurus* requires ingested microflora in order to degrade some substrates, such as cellulose and mannan (Zhang et al. 1993), in contrast to *P. elongata* which can synthesize by itself all its extra and intracellular enzymes (Lattaud et al. 1996). In this paper, further experiments were carried out on the enzymatic system of another tropical geophagous earthworm, *Millsonia anomala*.

Materials and Methods

Adult earthworms and soil for laboratory culture were collected at Lamto (Côte d'Ivoire). In this experimental station, where the climate is tropical humid, earthworm communities are dominated by *M. anomala* (Omodeo & Vaillaud 1967), a mesohumic endogeic Megascolecidae which comprises 50 to 80 % of the overall worm biomass in the savannas and forests (Lavelle 1978). In the laboratory, these earthworms were maintained in a control chamber at 28 °C (31 g water per 100 g soil). First, glycolytic digestive enzymatic activities were scanned in the gut (wall and contents) of *M. anomala*. These activities were tested with twenty – one glycolytic substrates selected mainly for their plant origin. A large range of synthetic heteroglycoside, oligosaccharide and polysaccharide substrates were tested. They have been described previously (Zhang et al. 1993). The second step consisted in determining the origin of the main enzymes secreted by *in vitro* tissue culture of gut walls: enzymatic activities were evaluated both in the cultured tissues and in the culture medium.

Preparation of enzyme solutions

Gut walls and contents: After dissection and washing in icy physiological Holtfreter solution (8.77 ‰ NaCl, m/v) (Lattaud 1983), the gut was separated into two parts as in the study conducted previously with *P. corethrurus* and *P. elongata*. The first part included the pharynx, oesophagus, crop and gizzard; the second part was further divided into three equivalent portions referred to as foregut, midgut and hindgut. Fragments of each part of the gut (comprising wall and contents) of seven earthworms were then pooled, homogenized and dialysed as previously described (Zhang et al. 1993). The solution obtained was used as the enzyme source.

In vitro culture of intestinal tissues: Before dissection, earthworms were put overnight on cellulose wool soaked with Holtfreter physiological solution, then three or four hours in the same wool with a fungicide (fungizone i.e. amphothericin B). Seventeen earthworms were thus dissected by opening the gut in the physiological solution, washed free from intestinal content, then foregut, midgut and hindgut samples were taken. Each sample was divided into three portions according to the size of each one. The culture was limited to the foregut, midgut and hindgut. Before culture, each previous explant was placed into a rinsing physiological solution with a bactericide (sodic benzylpenicilline) for a few minutes. Concerning *P. corethrurus*, the culture had been achieved on a medium including Holtfreter solution, agar-agar, glucose, chicken embryo extracts, subtozan with a bactericide (sodic benzylpenicilline) and a fungicide (Amphotericin B) (composition previously described, Lattaud 1983). As for culture of the

gut wall tissues of *P. elongata* and *M. anomala*, we preferred to use a liquid medium whose properties are known (pH, osmotic pressure, composition into inorganic salts, amino acids, vitamins, sugars i.e. fructose, glucose, sucrose). Therefore, the explants derived from dissection were placed into 2,2 ml of a liquid medium including: Grace's insect medium*, fetal bovine serum* (rich in amino acids), bi-distilled water, subtozan with sodic benzylpenicilline and Amphotericin B. It is important to adjust the osmotic pressure of the culture medium to that of the coelomic fluid of the earthworm. The gut wall tissue culture was achieved in tissue culture dishes and the cover was sealed by a paraffin film. Boxes were agitated for 3, 5 or 6–7 days in a control chamber at 28 °C; it was shown in *P. corethrurus* that the enzymatic secretion of cultured tissues reached its maximum at 72 h. At the end of the culture, the cultured intestinal tissues and the culture medium were frozen. Enzyme solution from tissues was prepared as indicated for the gut (wall and contents), particularly, the intestinal tissues were crushed in 3 ml of ice-cold water; the culture medium was dialysed directly and the dialysate representing the enzymatic solution was used for the determination of the enzymatic activities.

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 (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 (Sedmark & Grossberg 1977).
Specific glycolytic enzymatic activities in the gut and in the cultured intestinal tissues were expressed as µg glucose released per mg of protein per minute; the explants were cultured in 2,2 ml of medium and the glycolytic activities were expressed as total activity (µg glucose · mn^{-1}).

Results

Specific glycolytic activities in the gut

Twenty-one substrates analysed were broken down by *M. anomala*. Similar to *P. elongata* and *P. corethrurus*, *M. anomala* was mainly able to degrade root and fungal substrates. Glycolytic enzymatic activities were almost non-existent in the first part of the gut comprising the pharynx, oesophagus, crop and gizzard (Fig. 1 a–c).
Maltose, laminaribiose and saccharose were the best hydrolysed oligosaccharides (Fig. 1a); a very high activity was observed on maltose and localized mainly in the foregut and midgut. The other substrates were weakly degraded. The N-acetylglucosamine was the most hydrolysed heteroglycoside, with maximum activity localized in the foregut; some weak specific activities were detected on both α and β-glucosides, the β-mannoside, the β-xyloside and on both α and β-galactosides (Fig. 1b). All the polysaccharides tested were hydrolysed (Fig. 1 c). Specific polysaccharidase activity of the gut, although greatest on laminaran, was also weakly present on starch and CMC (carboxymethylcellulose); it was essentially located in the foregut or midgut. It is worth noting that cellulose and mannan were weakly degraded. Other substrates were rarely broken down.
These results revealed that maltose, N-acetylglucosamine and laminaran were mainly hydrolysed in the gut (wall and contents) of *M. anomala*.
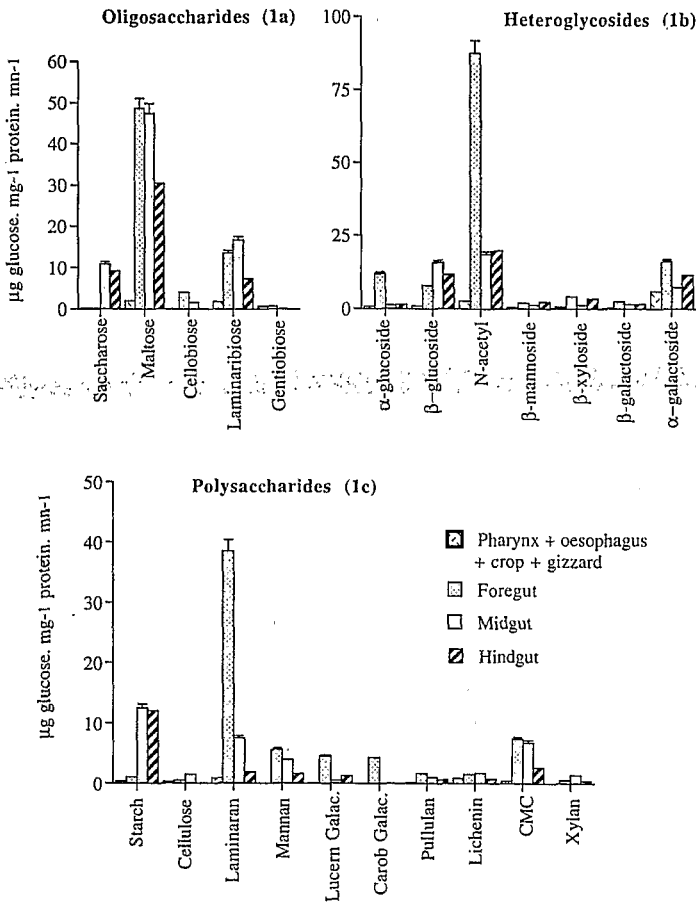
Specific activities in tissue culture

Adequate amounts of cultured tissues and culture medium allow a quantitative determination of the glycolytic enzymatic activities, as in the previous tissue cultures of *P. elongata*.
Activities of digestive enzymes in the first part of gut were nearly non-existent; therefore, only specific activities in the foregut, midgut and hindgut were evaluated. The highest glycolytic activities were detected on the assayed substrates after 3, 5 or 6–7 days of culture, according to the substrates.

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Fig. 1a–c. Specific glycolytic activities in the gut (wall and contents) of *Millsonia anomala*. Mean of two independent assays ± standard error; for (a) oligosaccharides, (b) heteroglycosides and (c) polysaccharides

In intestinal tissue culture, the results obtained on oligosaccharides (Fig. 2 a–c) confirm those concerning the enzymatic analysis of the gut: the main specific activity was detected on maltose, saccharose, laminaribiose, with highest maltase activity. However, these activities, mainly after 7 days of culture (Fig. 2 c), were on the whole higher than those observed in the gut. The main maltase activity was detected after 6–7 days in the hindgut, whereas it was also important in the foregut after 3 or 5 days. No saccharidase activity was detected after 3 or 5 days culture, this activity appearing only after 6–7 days and was highest in the hindgut. Specific activity on laminaribiose was detected after 3 days of culture with highest activity in the foregut; this activity was practically non-existent at 5 days and it was mostly in the midgut after 7 days. Some weak specific activities were observed on cellobiose and gentiobiose after 3 or 7 days. Assays on heteroglycosides (Fig. 2 d–f) confirm those carried out in the gut: main specific activity was on N acetylglucosamine, but there was also a weak activity on both α and β-glucosides, β-mannoside, β-xyloside and on both α and β-galactosides. The major activity on N-acetylglucosamine was observed after 5 days of culture and it was highest in the foregut (Fig. 2e). This activity was already present after 3 days, but it was lower than that observed in the gut (wall and contents). The weak specific activities on other heteroglycosides were detected between 3 and 6–7 days of culture, except for β-xyloside, whose activity was

not detected after 6–7 days. All the polysaccharides tested were broken down, except cellulose and mannan (Fig. 2 g–i). These results clearly show the difference between the culture and the gut where all polysaccharide substrates analysed were hydrolysed. For tissue culture, the main specific activities although rather high were present on starch, lichenin and laminaran, which is comparable to the results obtained in the gut. The amylase activity appeared after 3 days of culture (Fig. 2 g) and reached its maximum in the foregut after 5 days (Fig. 2 h). No activity on laminarin and lichenin was detected after 3 days in the cultured tissues; the activities on these substrates were detected only after 5 days of culture with a maximum in the foregut or midgut. Other substrates, i.e. lucern galactomannan, pullulan, CMC and xylan were rarely degraded regardless of the culture period.

Total activity in the culture medium

The culture medium is full of exogenous proteins and a specific activity cannot be expressed; thus the enzymatic activities were expressed in total activity (μg of reducing sugars $\cdot$ mn^{-1}) (see Table 1).

Among the oligosaccharidases studied, only high total activities on saccharose were detected in the media after 6–7 days of culture. The activities on this oligosaccharide increased from the foregut to the hindgut cultured tissues after 6–7 days reaching values similar to those detected in the media of the three cultured parts of the gut. As for, total saccharidase activities they were weak or non-existent after 3 and 5 days culture, both on the cultured tissues and media. The total activities on maltose and laminaribiose detected in the media between 3 and 7 days remained weak, with values always greater in the foregut culture medium. Inversely, these activities were always highest in the different cultured parts of the gut. Total cellobiase and gentiobiase activities were weak on both the cultured tissues and media. The same held true for heteroglycosides, although total activities on N-acetylglucosamine were important in different cultured parts of the gut. Among the polysaccharidases studied, the total activities on starch, laminaran, lucern galactomannan, pullulan, lichenin, CMC, and xylan, although weak, were generally present on the media and the tissue enzymes. The total activities on cellulose and mannan were non-existent on both media and cultured tissues.

Discussion

The tropical earthworms are able to feed on soils poor in organic matter, which allows them to enlarge their ecological niche (Lavelle 1983). Several authors assume that earthworms are able to produce enzymes to digest soil organic matter (Devigne and Jeuniaux 1961; Parle 1963; Urbasek and Pilzl 1991). But there is also strong evidence that digestion by some earthworms may be performed by microorganisms in their gut. Based on this, the hypothesis of a mutualistic digestion system between endogeic earthworms and the ingested soil microflora is possible. The worm produces assimilable carbon (intestinal mucus), which stimulates the microflora to degrade the soil organic matter and make it digestible.

The Lumbricidae of the temperate areas present various digestive enzymatic activities, particularly the amylase, cellulase, lichenase, chitinase, protease and lipase activities (Tracey 1951; Laverack 1963). However, quantitative studies have been limited to cellulase and chitinase (Tracey 1951; Devigne & Jeuniaux 1961; Parle 1963; Loquet & Vinceslas 1987; Urbasek 1990) and data about the digestion of cellulose in the gut of earthworms were in many cases contradictory. The authors cannot assume the origin of the cellulase produced by tissues of the gut walls and/or by microorganisms (Loquet & Vinceslas 1987). Urbasek (1990) concluded that all epigeic and endogeic earthworms species examined can break down the cellulose and only *Lumbricus rubellus* was dependent on symbiotic cellulolytic microflora. On the other hand, the cellulase activities detected in the gut walls were more important in the epigeic than in the endogeic earthworms, except epigeic *L. rubellus*, which had a very low enzymatic activity.
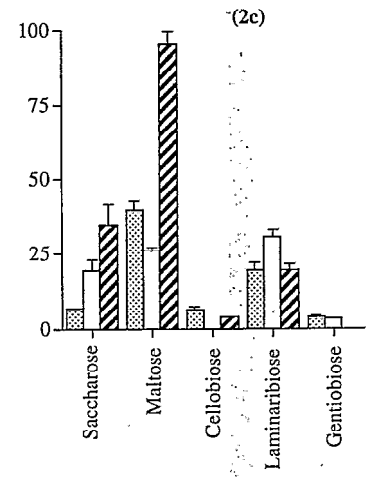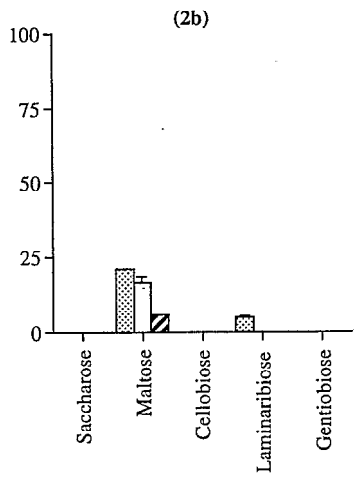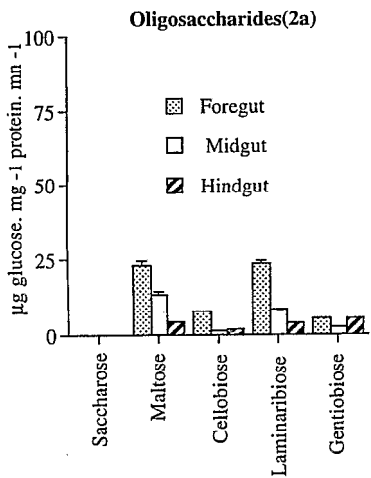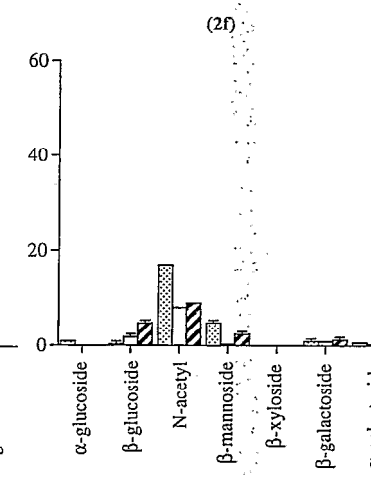
Oligosaccharides(2a) | **(2b)** | **(2c)**

Legend (2a):
▨ Foregut
☐ Midgut
▨ Hindgut

X-axis categories (2a, 2b, 2c): Saccharose, Maltose, Cellobiose, Laminaribiose, Gentiobiose

Y-axis: µg glucose. mg -1 protein. mn -1

Fig. 2a–c

Heteroglycosides (2d) | **(2e)** | **(2f)**

Legend (2d):
▨ Foregut
☐ Midgut
▨ Hindgut

X-axis categories (2d, 2e, 2f): α-glucoside, β-glucoside, N-acetyl, β-mannoside, β-xyloside, β-galactoside, α-galactoside

Y-axis: µg glucose. mg -1 protein. mn -1
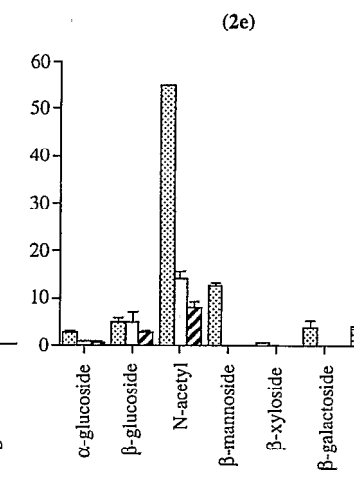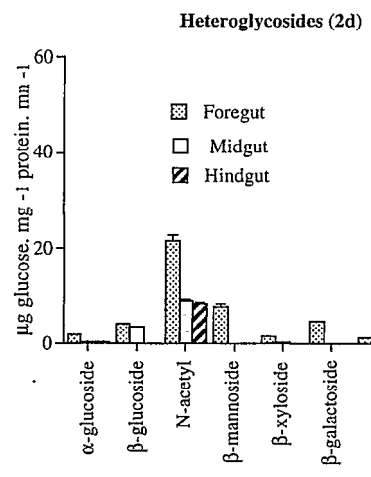
Fig. 2d–f
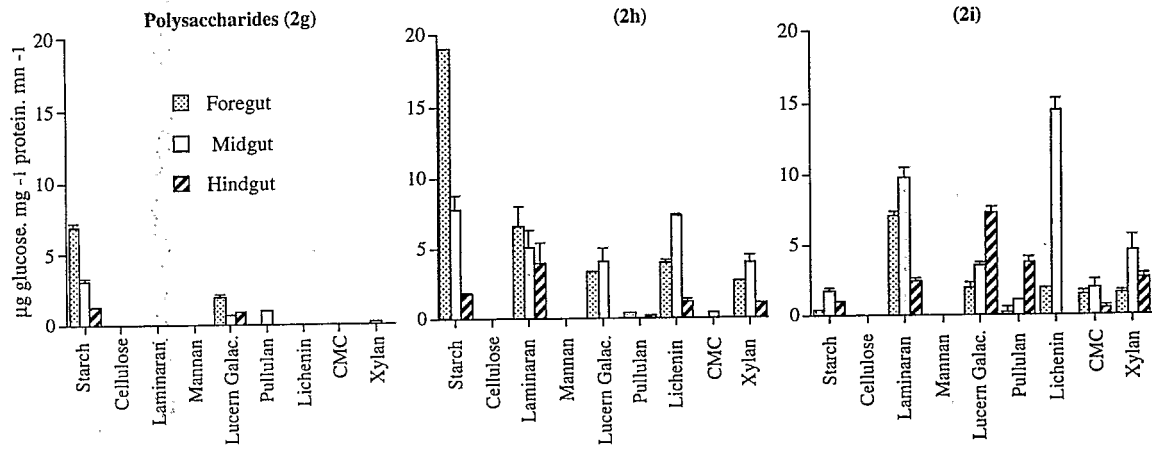
Fig. 2a–i. Specific glycolytic activities in cultured tissues of the gut wall of *Millsonia anomala* after distinct culture periods. Mean of two independent assays ± standard error. Specific activities on oligosaccharides: 3 days (a), 5 days (b) and 6–7 days (c), on heteroglycosides: 3 days (d), 5 days (e) and 6–7 days (f), on polysaccharides: 3 days (g), 5 days (h) and 6–7 days (i)

Table 1. Total glycolytic activity (expressed in µg glucose · mn^{-1}) in culture media of the gut wall of *Millsonia anomala* after culture periods (3, 5 or 6–7 days). Mean of two independent assays ± standard error

Culture mediums	3 days of culture period			5 days of culture period			6–7 days of culture period		
	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut	Foregut	Midgut	Hindgut
Oligosaccharides									
Saccharose	0	0,12 ± 0,02	1,55 ± 0,54	0	0	0	26,19 ± 0,97	27,94 ± 1,24	26,62 ± 0,77
Maltose	4,07 ± 1,20	1,12 ± 0,21	1,20 ± 0,20	1,24 ± 0,54	0	0	0,04	0,00	0
Cellobiose	1,01 ± 0,31	1,39 ± 0,62	1,05 ± 0,04	0,04	0	0	0,70 ± 0,06	0,31 ± 0,07	0,08 ± 0,02
Laminaribiose	2,75 ± 0,35	1,55 ± 0,31	1,16 ± 0,39	0,02	0,16 ± 0,01	0	0,04 ± 0,01	0,19 ± 0,03	0,04
Gentiobiose	0,58 ± 0,35	0,62 ± 0,23	0,66 ± 0,03	0	0	0	0,16 ± 0,01	0,16 ± 0,01	0,19 ± 0,01
Heteroglycosides									
δ-Glucoside	0,60 ± 0,30	0,34 ± 0,16	0,35 ± 0,17	0,17 ± 0,01	0	0,017	0,11 ± 0,01	0,03	0,06 ± 0,02
β-Glucoside	0,24 ± 0,14	0,10 ± 0,01	0,17 ± 0,06	0,66 ± 0,02	0,10 ± 0,04	0,34 ± 0,03	0,10 ± 0,01	0,00	0,15 ± 0,01
N-Acetyl	2,33 ± 0,54	1,03 ± 0,58	1,58 ± 0,62	2,69 ± 0,05	0,63 ± 0,01	2,40 ± 0,05	0,02 ± 0,01	0,05 ± 0,01	0,06 ± 0,01
β-mannoside	0,74 ± 0,07	0,21 ± 0,09	0,24 ± 0,07	0,96 ± 0,20	0	0,012	0,01	0,02	0
β-Xyloside	0,24 ± 0,02	0,10 ± 0,01	0,12 ± 0,02	0,73 ± 0,09	0	0,025	0,34 ± 0,01	0,00	0
β-Galactoside	0,56 ± 0,05	0,20 ± 0,06	0,34 ± 0,01	0,24 ± 0,14	0	0,09 ± 0,01	0,03	0,00	0
δ-Galactoside	0,52 ± 0,01	0,23 ± 0,15	0,30 ± 0,15	0,16 ± 0,03	0	0,63 ± 0,07	0,11 ± 0,01	0,10 ± 0,03	0,19 ± 0,02
Polysaccharides									
Starch	0	0,12 ± 0,02	1,55 ± 0,54	1,05 ± 0,07	2,031 ± 0,22	0	0,96 ± 0,02	0,25 ± 0,03	0,06 ± 0,02
Cellulose	0	0	0	0	0	0	0	0	0
Laminaran	1,43 ± 0,39	0	0	0,16 ± 0,01	0,67 ± 0,04	0,20 ± 0,01	0,10	0,02	0
Mannan	0	0	0	0	0	0	0	0	0
Lucern Galact.	0,01	0	0	1,04 ± 0,18	0	0,11 ± 0,01	0,11 ± 0,01	0,44 ± 0,13	0,37 ± 0,18
Pullulan	0	0,13 ± 0,01	0	0,08 ± 0,01	0	0,24 ± 0,06	0,67 ± 0,14	0,56 ± 0,07	0,49 ± 0,06
Lichenin	0,82 ± 0,11	0,64 ± 0,06	0	2,03 ± 0,04	7,04 ± 0,60	2,82 ± 0,14	0,97 ± 0,16	0,57 ± 0,16	0,57 ± 0,16
CMC	14,65 ± 0,08	10,18 ± 0,13	12,61 ± 0,31	9,28 ± 1,31	8,97 ± 0,45	9,74 ± 0,48	7,48 ± 1,19	8,08 ± 1,08	7,09 ± 0,75
Xylan	0	0	0	0	0,54 ± 0,07	0	0,76 ± 0,06	0,55 ± 0,07	0,58 ± 0,02

Our previous study of the glycolytic activities of the endogeic tropical earthworms revealed that they possessed a rather complete enzymatic system although the glycolytic enzymatic activities were rather weak as compared with other invertebrates such as the snail *Helix aspersa* (Charrier & Rouland 1992), fungus-growing and xylophagous termites. This low content in osidases was also present in the soil feeding termites (Rouland 1986). However these weak activities were consistent with the fact that endogeic earthworms feed on litter debris and soils poor in organic matter. We showed that the glycolytic activities remain higher in *P. elongata* than in *P. corethrurus* (Lattaud 1996). These results demonstrated that glycolytic activities in *M. anomala* as in *P. elongata* are more important than in *P. corethrurus*. All the substrates tested were broken down, which indicates that these three species may use root and fungal substrates available in soils. However, this study of the specific glycolytic activities in the gut of *M. anomala* revealed that among all the substrates tested the N-acetylglucosamine, laminaran and laminaribiose were the best degraded, which indicates that this earthworm more than *P. elongata* is able to hydrolyse polysaccharides which constitute the fungal cell wall. Therefore, *M. anomala* is likely to feed on fungi. In the gut of *M. anomala* the amylase and maltase activities were lower than in *P. elongata*, which demonstrates that this earthworm can degrade mainly starch, a root substrate, up to glucose.

The *in vitro* intestinal tissue culture of *M. anomala* confirms the results obtained for enzymatic analysis of the gut: main specific activity on maltose, N-acetylglucosamine and laminaran after 5 or 6–7 days of culture. Specific activities on saccharose, laminaran and lichenin were not observed after 3 days of culture and it is worth noting that these activities only appeared after 5 days for laminaran, lichenin and 6–7 days for saccharose; probably some enzymes were secreted in the culture medium by the tissues after a latent period and their secretion was perhaps induced. The specific enzymatic activities on N-acetylglucosamine, laminaran and maltose (except for hindgut after 6–7 days of culture) were higher in the gut than in the cultured tissues, but after 3 and 6–7 days the specific activities on starch and laminaribiose were more important in the tissues than in the gut. The specific activities on cellulose and mannan were observed in the gut of *M. anomala*, but they were weak, whereas these activities were non-existent in tissues and their culture medium. Thus, this earthworm, like *P. corethrurus*, relied on ingested microflora to exploit some insoluble substrates. The total activities on oligosaccharides, heteroglycosides and polysaccharides were detected even weakly in all the culture media: in *M. anomala*, oligosaccharidases, heterosidases and polysaccharidases are extracellular enzymes and only high total activities on saccharose were detected in the media after 6–7 days of culture. It has been demonstrated that *P. elongata* secreted extracellular oligosaccharidases and heterosidases. Among polysaccharidases, only amylases, cellulases and mannanases were extracellular enzymes secreted in the culture media, while laminarinases, licheninases and pullulanases were never detected in the culture media.

The *in vitro* gut wall tissue culture of the endogeic tropical earthworms showed that *M. anomala* like *P. corethrurus* made use of ingested bacteria in order to degrade cellulose and mannan of the soil, while *P. elongata* could synthesize by itself its extra and intracellular enzymes. This study allowed us to infer that endogeic earthworms seem to display variable diets and the differences between these earthworms were not linked to specific ecological niches.

References

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 Biol. Biochem. **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 Biol. Biochem. **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. Can. J. Zool. **70**, 2234–2241.

Devigne, J., Jeuniaux, C. (1961) Origine tissulaire des enzymes chitinolytiques intestinaux des lombrics. Arch. Intern. Physiol. Biochim. **69**, 223–234.

Jenkinson, D. S. (1966) The Priming Action. J. Appl. Radiat. Isotopes, Suppl. XX, 199–208.

Lattaud, C. (1983) Contrôle du sexe des cellules germinales chez l'Annélide Oligochète *Eisenia fetida f. typica* Sav., Thèse Université Paris VI.

Lattaud, C., Rouland, C., Zhang, B., Locati, S., Lavelle, P. (1996) Activities of the digestive enzymes in the gut and in tissue culture of a tropical geophagous earthworm, *Polypheretima elongata* (Megascolecidae). Soil Biol. Biochem. (in press).

Lavelle, P. (1978) Les Vers de terre de la savane de Lamto (Côte d'Ivoire): peuplements, populations et fonctions dans l'écosystème. Thèse d'Etat, Paris VI. Publication du Laboratoire de Zoologie de l'ENS 12.

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

Lavelle, P. (1986) Associations mutualistes avec la microflore du sol et richesse spécifique sous les tropiques: l'hypothèse du premier maillon. C. R. Acad. Sc. Paris **302**, 11–14.

Lavelle, P., Fragoso, C. (1992) Foodwebs in soil of the humid tropics: Importance of the mutualistic relationships. In The Soil Food-Webs (N. Ingham, Ed.).

Lavelle, P., Gilot, C. (1994) Priming effects of macroorganisms on microflora: a key process of soil function? In Beyond the Biomass. Eds. K. Ritz, J. Dighton & K. Giller. 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.

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

Laverack, M. S. (1963) The physiology of earthworms. In A. G. Kerkut (ed.), International series monograph on pure and applied biology. Oxford: Pergamon Press, 18–23.

Loquet, M., Vinceslas, M. (1987) Cellulolyse et ligninolyse liées au tube digestif d'*Eisenia fetida andrei* Bouché. Rev. Ecol. Biol. Sol **24**, 559–571.

Martin, A. (1989) Effets des vers de terre tropicaux géophages sur la dynamique de la matière organique du sol dans les savanes tropicales humides. Thèse Université Paris XI.

Martin, A., Cortez, J., Barois, I., Lavelle, P. (1987) Les mucus intestinaux de ver de terre, moteur de leurs interactions avec la microflore. Rev. Ecol. Biol. Sol **24**, 549–558.

Nelson, N. (1944) Photometric adaptation of Somogyi method for determination of glucose. J. Biol. Chem. **153**, 275–380.

Omodeo, P., Vaillaud, M. (1967) Les Oligochètes de la savane de Gpakobo en Côte d'Ivoire. Bull. IFAN, **XXIX** (A), 925–944.

Parle, J. N. (1963) Microorganisms in the intestine of earthworms. J. Gen. Microbiol. **31**, 1–11.

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 coomassie brilliant blue G 250. Anal. Biochem. **79**, 544–552.

Somogyi, M. (1945) Determination of blood sugar. J. Biol. Chem. **160**, 61–68.

Tracey, M. V. (1951) Cellulase and chitinase of earthworms. Nature **167**, 776–777.

Trigo, D., Martin, A., Lavelle, P. (1992) A mutualist system of digestion in temperate earthworms, *Allolobophora molleri* and *Octolasium lacteum* (Lumbricidae). Acta Zool. Fenn.

Trigo, D., Lavelle, P. (1993) Changes in respiration rate and some physicochemical properties of soil during gut transit through *Allolobophora molleri* (Lumbricidae, Oligochaeta). Biol. Fertil. Soil **15**, 185–188.

Urbasek, F. (1990) Cellulase activity in the gut of some earthworms. Rev. Ecol. Biol. Sol **27**, 21–28.

Urbasek, F., Pizl, V. (1991) Activity of digestive enzymes in the gut of five earthworm species (Oligochaeta; Lumbricidae). Rev. Ecol. Biol. Sol **28**, 461–468.

Werner, W., Rey, H. G., Wielinger, R. H. (1970) Proprieties of a new chromogen for determination of glucose in blood according to GOD/POD method. Frezenius Anal. Chem. **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*. Eur. J. Soil Biol. **29**, 7–11.

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