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ACTIVITIES OF THE DIGESTIVE ENZYMES IN THE GUT AND IN TISSUE CULTURE OF A TROPICAL GEOPHAGOUS EARTHWORM, *POLYPHERETIMA ELONGATA* (MEGASCOLECIDAE)

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Summary—Endogeic geophagous earthworms from tropical areas seem to digest soil organic matter through a mutualist earthworm microflora–digestion system and the intestinal mucus produced by earthworms was supposed to play a central role in the process of digestion. A large range of glucosidic substrates characteristic of plant material was used to reveal the activities of digestive enzymes in the gut (wall and contents) of *Polypheretima elongata*. This worm consumes some plant substrates tested and is able mainly to degrade root and fungal substrates. It corroborates that tropical endogeic earthworms feed on litter debris and soils poor in organic matter. These glucosidic activities were higher than those found previously in *Pontoscolex corethrurus*. The *in vitro* tissue culture of gut wall allowed us to infer that *P. elongata* can synthesize by itself all its extra and intracellular enzymes, contrary to *P. corethrurus* which requires the microflora of the soil ingested in order to hydrolyse some substrates such as cellulose and mannan. It should be interesting to compare cellulases and mannanases of both earthworms after extraction and purification and to study the mechanisms by which *P. corethrurus* may enhance microbial activities. © 1997 Elsevier Science Ltd

INTRODUCTION

Endogeic geophagous earthworms from tropical areas are able to feed on soils poor in organic matter and have developed mutualist relationships with the ingested microflora to digest soil organic matter (Lavelle *et al.*, 1983; Lavelle, 1986; Barois, 1987; Martin *et al.*, 1987; Martin, 1989; Trigo *et al.*, 1993). They play an active part in soil organic matter dynamics and nutrient turnover and they 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 mucus produced by earthworms was supposed to play a central role in their mutualist digestion system. Part of this mucus is metabolised by micro-organisms and another part is probably reabsorbed and recycled inside the earthworm. In the gut, in presence of this mucus, the microflora which was partly inactive in the ingested soil, quickly recovers all its abilities to digest the complex substances of the soil organic matter. The earthworm will be able to absorb again through its gut walls a large part of the substances which have been degraded by the microflora. According to definitions by Lewis (1985), this anisosymbiotic mutualism among soil organisms seems to be a key feature of soil dynamics. In order to determine the role of the intestinal mucus in the interrelationship between earthworm and soil microflora, glucosidic enzymatic activities have been assayed in the gut of the tropical earthworm *P. corethrurus*. It has been established that this worm possesses a relatively complete, though weak, enzymatic system, when compared to xylophagous and fungus-growing termites (Rouland, 1986; Rouland *et al.*, 1991), to the snail *Helix aspersa* (Charrier and Rouland, 1992). In this worm the strongest enzymatic activities were located in the foregut and midgut. Organotypic culture has permitted us to demonstrate that *P. corethrurus* requires ingested microflora to degrade some substrates, like cellulose and mannan. In fact, among the main enzymes found in the gut, cellulase and mannanase were neither detected in the cultured tissues nor in the culture medium (Zhang *et al.*, 1993). Here we report on an enzymatic study with another earthworm *P. elongata*. Glucosidic enzymatic activities were measured in the gut (walls plus contents) and, in order to know the origin of the enzymes found in the gut, the wall tissues were cultured *in vitro* and enzymatic activities were measured both in the cultured tissues and in the culture medium.

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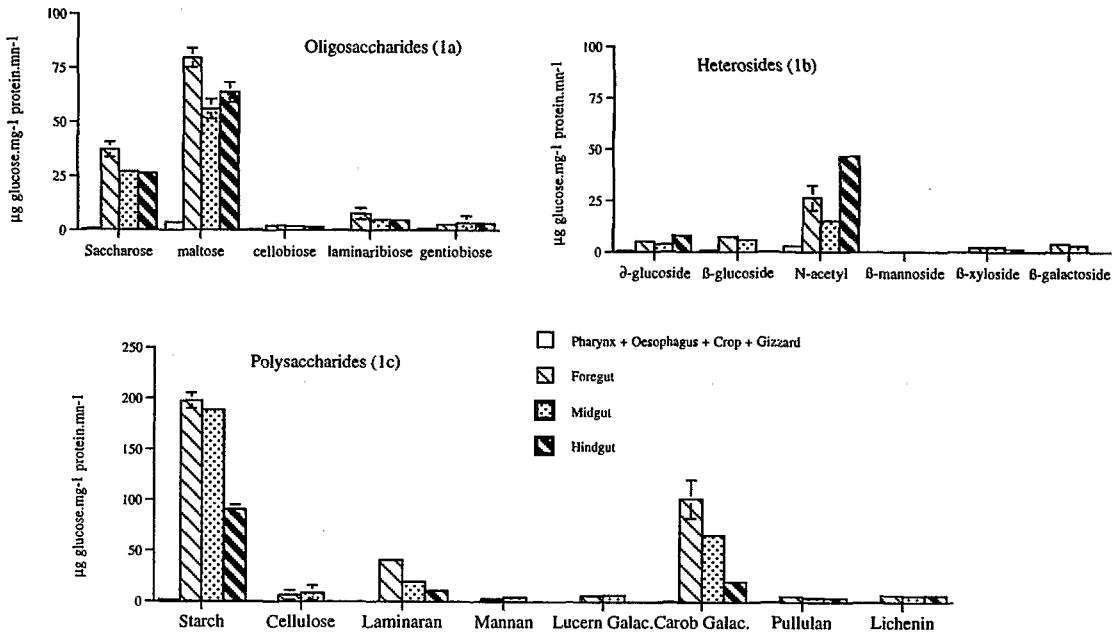

Fig. 1. Specific glucosidic activities in the gut (wall and contents) of *Polypheretima elongata*. Mean of two independent assays ± standard error for (a) oligosaccharides, (b) heterosides and (c) polysaccharides.

MATERIALS AND METHODS

The breeding stock of *P. elongata* was maintained in an environmental control chamber at 28°C, and originated from Sainte-Anne (Martinique).

Preparation of enzyme solutions

Earthworms were dissected in icy physiological Holtfreter solution (8.77‰ NaCl,w/v) (Lattaud, 1983). This solution was isotonic with the cœlomic fluid of *P. elongata*. The gut was divided into two parts: the first part comprised the pharynx, œsophagus, crop and gizzard; the second part was divided into three equivalent portions (foregut, midgut, hindgut). Fragments of each part of the gut of ten earthworms were then pooled, homogenated and dialysed as previously described (Zhang *et al.*, 1993).

Concerning *in vitro tissue culture of gut wall*, eight earthworms were placed into cellulose wool impregnated with Holfreter physiological solution for a night, then for three hours in the same wool in presence of a fungicide (Amphotericin B). The gut was then opened in the Holtfreter physiological solution and carefully washed of soil debris. Before culture, each gut fragment was placed into a rinsing physiological solution with a bactericide (sodic benzylpenicilline) for a few minutes. These explants were placed into a liquid medium including: Grace' insect medium,* fetal bovine serum† (rich in amino acids), bidistilled water, subtozan with sodic benzylpenicilline and Amphotericin B (previously mentioned concentrations, Lattaud, 1983). The tissue culture was done in tissue culture dishes. Boxes were placed in the control chamber at 28°C with agitation during 3, 4 or 6 days. Enzyme solution from tissues was prepared as indicated for the gut (wall and contents), particularly, the gut wall 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.

*Sigma Bio-Sciences
†Sigma Bio-Sciences

Enzyme activity assay

The substrates and the methods for assaying glucosidic activities were described previously (Zhang *et al.*, 1993). Specific glucosidic enzymatic activities were expressed as μg glucose released per μg of protein per minute. We also expressed the total activity of the culture medium (μg glucose mn^{-1}).

RESULTS

Specific glucosidic activities in the gut

Enzymatic glucosidic release was almost non-existent in the first part of the gut including the pharynx, oesophagus, crop and gizzard.

Maltose and saccharose were the most readily hydrolysed oligosaccharides [Fig. 1(a)] These activities were the same in all three parts of the gut. The other substrates were very weakly degraded. Among the heterosidases studied, a very high specific N-acetylglucosaminidase activity was located in the foregut and mainly in the hindgut [Fig. 1(b)]; some weak specific activities were detected on both α and β glucopyranaosides, the β xylopyranaoside and the β galactopyranaoside. The β mannosidase activity was not detected. Specific polysaccharidase activity of the gut was greatest on starch, but was also present

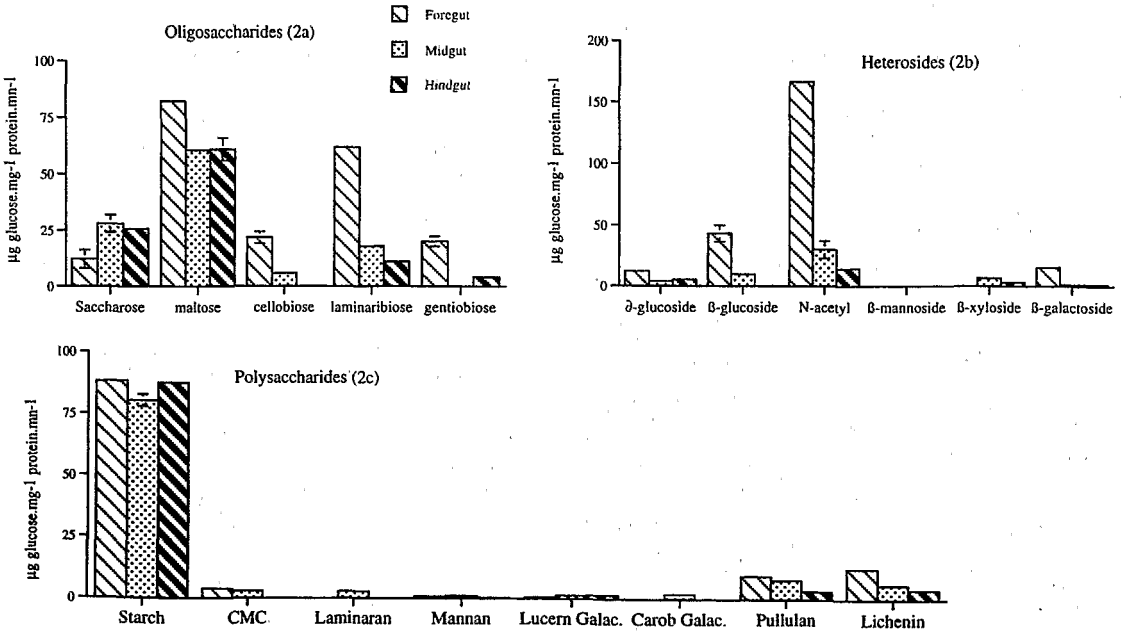

Fig. 2. Specific glucosidic activities in cultured tissues of gut wall of *Polypheretima elongata*. Mean of two independent assays ± standard error for (a) oligosaccharides, (b) heterosides and (c) polysaccharides.

on lucern galactomannan and laminaran [Fig. 1(c)]. It was essentially located in the foregut and midgut. It is important to take into account that the hydrolysis of cellulose and mannan remained weak, but was present. Other substrates were hardly ever degraded.

Specific activities in tissue culture

The maximum of glucosidic activity realeased was obtained after 3 days of culture period.

The results on oligosaccharides showed a specific activity for each of the assayed substrates, contrary to what had been found in the gut (walls and contents), but maltasic activity remained highest. [Fig. 2(a)]. For heterosides, the major specific activity was on N-acetylglucosamine, there are also a weak activity on both α and β glucopyranaosides, β xylopyranaoside and β galactopyranaoside. [Fig. 2(b)]. We should note that these activities are generaly greater than those detected in the gut and were greatest in the foregut. Only one important polysaccharidase activity was observed, the amylase one. [Fig. 2(c)]. These activities are approximately half those of the gut (walls and contents). It is interesting to take into account that, as in the gut, cellulose and mannan are degraded though weak.

Table 1. Total glucosidic activity (expressed in μg glucose mn^{-1}) in culture mediums of the gut wall of *Polypheretima elongata*. Mean of two independent assays ± standard error

	Foregut	Midgut	Hindgut
Culture mediums			
Oligosaccharides			
Saccharose	1.70 ± 0.42	5.84 ± 1.03	9.71 ± 1.51
Maltose	4.79 ± 0.97	13.51 ± 2.21	16.40 ± 2.01
Cellobiose	4.28 ± 1.1	2.58 ± 0.94	0.95 ± 0.32
Laminaribiose	5.50 ± 1.2	4.28 ± 1.12	1.70 ± 0.06
Gentiobiose	2.51 ± 0.64	2.78 ± 0.71	2.10 ± 0.23
Heterosides			
δ-Glucopyrannoside	0.26 ± 0.04	0	0
β-Glucopyrannoside	3.18 ± 0.87	1.04 ± 0.32	0
N-Acetylglucosamine	15.52 ± 1.48	1.72 ± 0.27	0.80 ± 0.04
β-Mannoside	0	0	0
β-Xylopyrannoside	0.79 ± 0.13	0	0
β-Galactopyrannoside	1.22 ± 0.48	0.42 ± 1.06	0.03 ± 0.01
Polysaccharides			
Starch	0.34 ± 0.02	0	2.52 ± 0.74
Cellulose	0.44 ± 0.08	0.42 ± 0.03	0
Laminaran	0	0	0
Mannan	0.29 ± 0.03	0.46 ± 0.04	0.071
Lucern galactomannan	0	0	0
Carob galactomannan	0	0	0
Pullulan	0	0	0
Lichenin	0	0	0

Total activity in the culture mediums

The cultured medium is full of exogenous proteins and the expression of a specific activity is impossible, then the enzymatic activities were expressed in total activity (μg of reducing sugars mn^{-1}) of 3 ml of culture medium (see Table 1).

For each part of the gut, a very good relationship was observed between the total oligosaccharidasic activities on the mediums and that of the tissue enzyme saccharidase and maltase activities increased from the foregut to the hindgut and this increase was also observed in the corresponding cultured mediums. On the contrary, cellobiase, laminaribiase and gentiobiase activities decreased at the same time on the cultured tissues and mediums.

Each cultured part of the gut showed an heterosidase activity on N-acetylglucosamine and β galactopyranaoside. This activity was greatest on N-acetylglucosamine. Activities on α and β glucopyranaosides, though weaker, were detected only in the

foregut culture medium. It is difficult to explain the β xylopyranaosidic activity detected in the foregut culture medium, when midgut and hindgut tissues were the only to show this same activity.

For polysaccharides, only the activities on starch, cellulose and mannan were present on the cultured mediums. The activities on CMC, laminaran, lucern and carob galactomannan, pullulan and lichenin were missing, although they were weakly detected in different cultured parts of the gut.

DISCUSSION AND CONCLUSION

Digestive enzymatic activities have been recognised in the Lumbricidae of the temperate areas, particularly lipase, protease, amylase, chitinase, lichenase and cellulase activities (Laverack, 1963; Tracy, 1951), but quantitative studies were limited to cellulase and chitinase (Devigne and Jeuniaux, 1961; Parle, 1963; Tracy, 1951; Loquet and Vinceslas, 1987; Urbasek, 1990).

For the first time, a study of the glucosidic activities of the geophagous endogeic tropical earthworms was conducted. It revealed a rather complete enzymatic system. The glucosidic activities were higher in *Polypheretima elongata* than in *Pontoscolex corethrurus* (Zhang *et al.*, 1993) but in both earthworms, they were lower than those detected in other invertebrates, such as the above-mentioned snail *Helix aspersa* and fungus-growing and xylophagous termites. These activities can be compared to those of humus-eating termites which are distinguished by their low content in osidases (Rouland, 1986). The geophagous endogeic tropical earthworms studied feed on litter debris and soils poor in organic matter, which is consistent with rather weak glucosidic activities detected in their gut. In the endogeic Lumbricidae, a cellulase activity lower than that of the epigeic ones had already been mentioned (Urbasek, 1990; Urbasek and Pizl, 1991). The study of the specific glucosidic activities conducted in *P. elongata* revealed major amylase and maltase activities, which shows that this earthworm is able to degrade starch, a root substrate, up to glucose. *P. elongata* as well as *P. corethrurus* showed N-acetylglucosaminase, laminarinase and laminaribiase activities which allows the degradation of β 1, 3 glucan up to glucose and chitin sub-units constituting the main substrates of the fungal cell wall. Therefore, these earthworms are likely to feed on fungi. The gut of *P. elongata*, like that of *P. corethrurus*, shows a certain specific activity on cellulose and hemicellulose: it shows that these earthworms are likely to use most of the vegetal components in the soil for their nutrition. Moreover, in *P. elongata*, the presence of cellulolytic and mannanase activities in not only the gut (wall and contents), but also in tissues and in their culture medium, allows us to infer that these enzymes are secreted by the earthworm itself without the micro-organims of the ingested soil. In nature, few organisms possess these enzymes in order to degrade cellulose and mannan which are the main plant constituents and they make use, like *P. corethrurus* either of ingested bacteria which show cellulolytic and mannanase activities, or of symbiotic bacteria in order to degrade the insoluble substrates.

It is interesting to compare the total glucosidic activity of the cultured gut walls of *P. elongata* to that of the corresponding culture mediums. The whole oligosidase activities were also distributed in each cultured part of the gut and in their culture medium. Moreover, the total activity for each oligosidase was greater in the medium than in tissues. It shows that all the oligosaccharidases were secreted in the culture medium: they are extracellular enzymes. These results are opposed to those obtained in *P. corethrurus* where maltase had proved to be an intracellular enzyme. In *P. elongata* the N-acetyloglucosaminase and β galactosidase are also extracellular enzymes, since they are observed both in cultured tissues and their medium concerning each part of the gut. The N-acetylglucosaminase is clearly the major enzyme. The α and β glucosidases seem to be extracellular enzymes which are secreted only in the foregut culture medium. Among the polysaccharidase activities, only amylase, cellulase and mannanase were detected in the culture medium. The total cellulolytic and mannanase activities were greater in the medium than in cultured tissues: they are extracellular enzymes. The case of amylase is interesting, for if it is detected on the medium, its total activity is clearly higher in cultured tissues: therefore, this enzyme would be intracellular or induced only by its substrate. Laminarinases, licheninases and pullulanases which show mean activities in the cultured walls of the gut are never detected in the culture medium: therefore, they would be intracellular enzymes. The other polysaccharidases which show a very weak activity in cultured tissues like those observed in the gut (wall and contents) do not allow us to come to a conclusion about their origin: the fact that they are not detected in the culture medium can be due to too weak an activity. Therefore, it would seem that in this species, some polysaccharidases are not secreted in the culture medium. This absence of some polysaccharidases in the culture medium can show that they are enzymes whose secretion is induced: therefore, they would be secreted only under certain food conditions and it would be interesting to know the inductions starting these secretions. These results allow us to make an approach to the mode of nutrition of *P. elongata*: this earthworm consumes most of the plant substrates tested, being able to use root and fungic substrates. It is able to synthesise by itself all its extra and intracellular enzymes, contrary to *P. corethrurus* which requires the digestive capacities of the ingested soil microflora in order to degrade substrates such as cellulose and mannan (Zhang *et al.*, 1993).

In the course of their digestion, the geophagous endogeic earthworms seem to display rather variable adaptative characters which are undoubtedly linked to the different ecological categories.

To date, our research was concentrated on glucosidic enzymes although both ligninolytic and proteolytic enzymes are to be investigated. The composition of the intestinal mucus will be analysed with particular attention to the mechanisms by which worms may enhance microbial activities.

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