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Changes in the fatty acid profiles through the digestive tract of the earthworm *Lumbricus terrestris* L.

Luis Sampedro [a,b,*], Joann K. Whalen [b]

[a] *Department of Natural Resource Sciences, Macdonald Campus of McGill University, 21.111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, Canada H9X 3V9*

[b] *Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, E-36310 Vigo, Spain*

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Abstract

The gut of many soil arthropods contains a complex and mutualistic microbial community that usually assists the host with digestion. The same is probably true for earthworms, but the nature and function of the microbiota inhabiting their gut are virtually unknown. In this paper, we studied the microbial community in the gut content of the earthworm *Lumbricus terrestris* L. and in the bulk soil by assessing their fatty acid (FA) profiles. Our results indicated that the total FA concentration in the earthworm gut was about two orders of magnitude greater than in bulk soil, with higher concentration of bacteria (up to 500-fold), fungal and metazoan-derived FAs. Several FAs appearing in the gut were not present in bulk soil. PCA analysis revealed that the microbial community in the gut was different from that in the bulk soil, and that significant changes occurred between midgut, hindgut and proctodeum. Cluster analysis of bacterial and fungal-derived FA profiles grouped the bulk soil samples apart from the gut samples, where the hindgut profiles were more closely related to those from the proctodeum than those from the midgut. We showed important changes in the FA concentration and composition occurring at very small spatial scales inside the gut of the earthworm *L. terrestris*. These results have implications for understanding earthworm digestion, and they suggest that the microbial community in the earthworm gut is not a casual combination of microorganisms already present in the soil. Further study is needed to determine how these gut microbial communities are involved in earthworm digestion processes.

Keywords: Earthworm; Fatty acid; Gut microbiota; Digestive mutualisms

1. Introduction

The gut of many soil arthropods contains complex microbial communities that usually assist the host with digestion. These microbial–animal relationships range from commensalisms to species-specific mutualisms. Earthworms are also believed to have a mutualistic relationship with soil microorganisms passing through their digestive tract, but the nature and function of the microbiota inhabiting their gut are virtually unknown. It is hypothesized that mucus and water secretion from the earthworm gut wall into the digestive tract causes dormant, ingested microbiota to be activated, increasing microbial activity and enzyme production and hence facilitating digestion (Lavelle and Spain, 2001). This mucus-mediated, facultative mutualistic digestion was first proposed for tropical endogeic geophagous earthworms (Barois, 1992; Trigo and Lavelle, 1993). Anecic earthworms are thought to facilitate the digestion by mixing soil and plant residues to stimulate soil

* Corresponding author at: Departamento de Ecoloxía, Centro de Investigacións Ambientais de Lourizán, Apartado 127, E-36080 Pontevedra, Galicia, Spain. Tel.: +34 986 856078; fax: +34 986 856420.
E-mail address: lsampe@uvigo.es (L. Sampedro).

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microbial activity, effectively creating an ''external rumen'' before ingesting the partially decomposed materials (Lavelle and Spain, 2001). After organic substrates are consumed, earthworms also could release major enzymes necessary for decomposition into the gut, leading to ''direct digestion'' by endogenous enzymes.

Since Parle (1963) first reported the microbiology in the earthworm gut, researchers have attempted to study earthworm gut microbes using direct culture methods (e.g. Krištufek et al., 1992; Karsten and Drake, 1995), electron microscopy (Jolly et al., 1993), epifluorescence direct counts of DAPI or acridine orange stained prokaryotes (Krištufek et al., 1995; Wolter and Scheu, 1999) and more recently by fluorescent in situ hybridization with rRNA-targeted probes (FISH) specific for major bacteria phyla (Fischer et al., 1995; Schonholzer et al., 2002). Microbial numbers in the earthworm gut are higher than those in surrounding soil and denitrification, but not methane emission, occurs in the gut of *Aporrectodea caliginosa* and *Lumbricus rubellus* (Karsten and Drake, 1997). In addition, gene clone libraries of bacteria tightly associated with the gut wall were different from those in casts and in the gut content (Singleton et al., 2003, 2004). These findings suggest that the earthworm gut is favourable for the growth and activity of certain bacterial species, but whether this relationship is obligate or not, and its relevance in the energy uptake of the earthworm remains to be determined.

Fatty acid (FA) analysis is a common culture-independent tool for the study of microbial communities in sediments and soils. Depending on the procedure for extraction and the nature of the FA analyzed, it can provide information about the microbial biomass, microbial community composition, and physiological status (Haack et al., 1994; White and Ringelberg, 1998; Zelles, 1999; Schutter and Dick, 2000). The ester-linked fatty acid (EL-FA) fraction of environmental samples includes both polar lipids (membrane lipids related to biomass) and neutral lipids (storage lipids in eukaryotes) (Zelles, 1999). The EL-FA fraction has been successfully used to characterize shifts in the microbial community of soils and sediments (e.g. Schutter and Dick, 2000), but we are not aware of other published studies to examine microbial communities in the earthworm gut using FA profiles. In this study, we analyzed the FA profiles to gain insight into changes in the microbial community occurring along the digestive tract of the anecic temperate earthworm *Lumbricus terrestris* L.

2. Materials and methods

2.1. Earthworm collection and experimental design

Adult *L. terrestris* L. were collected by formalin extraction (0.25% formaldehyde) from a mixed grass–legume hayfield (loamy, mixed, typic endoaquent, pH 6.0 and 28 g C kg^{-1}), quickly washed in tap water and maintained in a culture bin at 18 °C in dark for 10 days with the original soil, thoroughly homogenized by sieving through 2 mm mesh, without added food to diminish interferences from selective feeding. Each earthworm was then cooled on ice for 5 min, which anesthetized them and prevented evacuation of the gut material, then euthanized in 50% ethanol solution, rinsed with sterile water, and immediately aseptically dissected on ice. The intestinal tract behind the gizzard was divided into midgut (closest to the mouth), hindgut and proctodeum (furthest from the mouth), and the gut content was carefully collected with dissecting needles under stereomicroscope (Stephenson, 1930; Harrison and Gardiner, 1992; Jamieson, 1992). This was quite time-consuming, so we included only 15 specimens dissected within a 48 h period to minimize the variation between individuals. Material from each gut section of five specimens was pooled to obtain at least 200 mg dry weight (dw) per replicate (there were three replicate samples from the midgut, hindgut and proctodeum). Samples of the gut content and samples of homogenised bulk soil ($n = 3$) from the culture bin (each about 2 g dw) were frozen, freeze dried and stored frozen until lipid extraction.

2.2. Lipid extraction and analysis

Total lipids were extracted with methanol and chloroform (2:1 v/v) using an accelerated solvent extraction with the Dionex ASE 200™ Extractor (Dionex Corporation, Sunnyvale, CA, USA, http://www.dionex.com) according to Macnaughton et al. (1997). Accelerated solvent extraction employs a combination of increased temperature and pressure with common solvents to increase the efficiency of the extraction process. Freeze dried samples were weighed into steel extraction cells, individually heated to 80 °C under N_2 and brought to 8.28 MPa for 5 min, then subjected to static extraction for 15 min. Three static cycles were conducted on each sample. Fatty acids methyl esters (FAMEs) were prepared by a mild alkaline methanolysis of the total lipid extract according to the standard procedure proposed by White and Ringelberg (1998). This procedure is suitable only for