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S E Biotechnol. Agron. Soc. Environ. 2012 **16**(1), 125-131

Microscale interactions between earthworms and microorganisms: a review

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Received on March 15, 2010; accepted on September 30, 2011.

Microorganisms are well adapted to their soil microhabitat where they live together in consortia, interacting with other living members, including earthworms. This literature review consists of four sections that focus on microscale interactions between earthworms and microorganisms. The first part is devoted to nephridia symbiosis. Recent discoveries show that *Verminephrobacter* spp. is present as a symbiont in earthworm nephridia. The second section deals with earthworm food preference and focuses on the major hypotheses of foraging strategies. The third section presents evidence of gut symbionts and highlights the need for additional studies in this field. The last section of this review explains why microorganism activities are enhanced in burrows and casts of earthworms.

Keywords. Earthworms, soil organisms, nephridial symbiosis, intestinal flora, feeding preference, volatile compounds, excreta, burrows.

Interactions microscopiques entre les vers de terre et les micro-organismes (synthèse bibliographique). Les micro-organismes adaptés aux microhabitats du sol interagissent avec les autres organismes du sol dont font partie les vers de terre. Cet article de synthèse s'intéresse aux interactions microscopiques entre les vers de terre et les micro-organismes, et est divisé en quatre parties. Dans la première partie, nous discutons de la symbiose néphridiale. Des découvertes récentes montrent que des symbiontes du genre *Verminephrobacter* sont présents dans les néphridies de certains vers de terre. Dans la seconde partie, la préférence alimentaire des vers de terre est considérée et des hypothèses concernant la stratégie de recherche de nourriture sont discutées. La troisième partie contient une discussion au sujet de la symbiose intestinale, quelques preuves indiquent l'existence de symbiontes intestinaux, mais de nouvelles études sont encore nécessaires. La quatrième partie montre que l'activité des micro-organismes est stimulée dans les galeries et les excréments de vers de terre.

Mots-clés. Ver de terre, organisme du sol, symbiose néphridale, flore intestinale, préférence alimentaire, composé volatil, excréments, galeries.

1. INTRODUCTION

Few soil ecology studies are focused on the prospects of linking microbes and fauna (Brown et al., 2004; Coleman et al., 2004). Without doubt, earthworms are the most important soil invertebrates in the soil ecosystem in terms of biomass and activity (Römbke et al., 2005), being often considered as ecosystem engineers (Lavelle, 1988). Moreover, soil contains a large diversity of microorganisms (Torsvik et al., 2002). Microorganisms are an unavoidable constituent of earthworms' natural diet. Three earthworm ecological groups are generally defined and earthworms feeding behavior is clearly associated to their ecological group (Bouché, 1977; Lee, 1985; Edwards et al., 1996; Brown et al., 2000):

- epigeic: earthworm species belonging to the epigeic group live on or near the soil surface, typically in the litter layers of forest soils and do not burrow. Epigeic earthworms consume plant litter and litter inhabiting organisms and ingest little or no soil. They are qualified as "litter transformers" (Lavelle et al., 1997). Several studies have shown that epigeic activities induce an increase in microbial activities due to greater surface area for decomposition, reduce immobilization by surface-litter dwelling fungi and modify the composition of microorganism communities (Scheu et al., 1994; Parkinson et al., 1998);

- anecic: earthworms from the anecic group live in permanent or semi-permanent vertical burrows in the mineral soil layers. Anecics feed on organic matter mixed with soil particles, bury surface litter, and often forming middens. Middens consist of an accumulation of surface castings mixed with organic matter in which microorganisms multiply and microbial degradation of uningested organic matter is improved (Brown, 1995; Maraun et al., 1999; Tiunov et al., 2000; Shuster et al., 2001). The amount of ingested organic matter by anecics varies with its quality (*e.g.* nitrogen, lignin or tannin quantities or C/N ratio) and its microbial composition. Anecic earthworms seem to prefer litter rich in nitrogen, without tannins or colonized by particular fungi species including *Fusarium lateritium* Nees and *Trichoderma* sp. (Cooke et al., 1980; Moody et al., 1995);
- endogeic: these earthworms typically live in mineral soil horizon, making horizontal burrows mainly at a depth of 10-15 cm. They consume more soil than other ecological categories and are often called soil organic matter feeders or geophageous.

Interactions with microorganisms are observed in earthworm burrow lining, cast, nephridia or gut, in the drilosphere (Brown et al., 2004). Drilosphere is the soil area directly or indirectly influenced by earthworms' activity and is constantly changing in space and time (Lavelle, 1988). Together, earthworms and microorganisms mineralize, humify organic matter and facilitate chelation of metal ions (Lavelle et al., 1995; Cai et al., 2002). Microorganisms help earthworms in their growth (Pizl et al., 2003). For example, Miles (1963) observed that *Eisenia fetida* Savigny hatched from microbiologically sterile cocoons could not reach sexual maturity in sterilized soil until mixed cultures of mobile protozoa were added in its food. Growth and reproduction in earthworms require carbon and nitrogen coming from litter, grit and microorganisms (Edwards et al., 1996).

In this review, we discuss four specific interactions between earthworms and microorganisms which are considered as hot spots of microbial and earthworm activities at a microscale: nephridial symbiosis, food preference, gut symbiosis and microorganisms in burrows and casts.

2. NEPHRIDIAL SYMBIOSIS

Some apparently species-specific microbial symbionts in the ampullas of the nephridia have been described on the basis of microscopic observations in earthworm species (Knop, 1926). Nephridia are paired osmoregulatory-excretory organs present in each segment of the earthworm body (Laverack, 1963). The presence of symbionts in nephridia was confirmed as being members of a monophyletic branch of the genus *Acidovorax* (Schramm et al., 2003). Their results show that different species of earthworms harbor distinct gene sequence types of the associated *Acidovorax* sp. whereas the same earthworm species from different continents present similar symbiont sequences. These bacteria might be proteolytically active during excretion, facilitating the absorption of peptides and amino acids by hosts (Pandazis, 1931). To what extent this association influences nitrogen excretion by earthworms and thus the nitrogen cycle in soil is still unknown (Schramm et al., 2003). Since the discovery of symbionts (*Acidovorax*-like bacteria) in *E. fetida* nephridia, several studies regarding bacterial colonization of earthworm nephridia have been reported (Davidson et al., 2006; Davidson et al., 2008; Pinel et al., 2008; Davidson et al., 2010; Lund et al., 2010). Firstly, Davidson et al. (2006) demonstrated that these nephridial symbionts are directly transferred from the adults to the egg capsules during mating and are not acquired from the environment in *E. fetida*. *Acidovorax* cells, present in nephridia, are dominant in mating mucus and in egg capsules. A subsequent study has shown that the model for colonization of the nephridia embryo is a sequential acquisition that begins when a nephridial canal matures in a segment and releases an attractant, inducing selective migration of the *Acidovorax*-like bacteria (Davidson et al., 2008). Gene sequence phylogenies, based on 16S rRNA comparison, revealed that all earthworm symbionts formed a cohesive and independent group (Pinel et al., 2008). After the isolation and the characterization of a relative of the genus *Acidovorax* from nephridia of *E. fetida* and based on the unique ecology of this organism, a new genus and a new species, respectively *Verminephrobacter* and *Verminephrobacter eiseniae*, have been defined (Pinel et al., 2008). The presence of symbiotic bacteria of the genus *Verminephrobacter* in several earthworm species was then investigated (Lund et al., 2010). This study shows that of over 23 earthworm species, 19 have *Verminephrobacter* symbionts in their nephridia. The studied species can be divided into three categories:

- species with nephridia only colonised by *Verminephrobacter* symbionts, including *Lumbricus terrestris* L., *Aporrectodea caliginosa* Savigny, *Aporrectodea longa* Ude;
- species with nephridia colonized by a mixed bacterial population with *Verminephrobacter* symbionts, including *Aporrectodea rosea* Savigny, *Dendrobaena veneta* Bouché;
- species with nephridia free of *Verminephrobacter* symbionts, including *Dendrobaena octaedra* Savigny and *Dendrobaena attemsi* Michaelsen.

New evidence reveals a more complex symbiosis system in *E. fetida* nephridia, involving three bacterial symbionts from different classes: *V. eiseniae*, a Microbacteriaceae and a Flexibacteriaceae. The presence of these three bacteria in the egg capsule and adults confirmed that they are associated symbionts of *E. fetida* and transmitted to the next generation (Davidson et al., 2010).

The role of these bacteria in capsule eggs remains uncertain but due to the diversity of microbial pathogen and potential predators in the soil community, a role of chemical protection is plausible (Davidson et al., 2010).

3. FOOD PREFERENCE

Microorganisms are an unavoidable constituent of earthworms' natural diet (Edwards et al., 1996). Some microbes are preferentially ingested by earthworms while others are rejected. Previous studies have highlighted selective feeding strategies in various earthworm species for certain fungal and bacterial species (Satchell, 1967; Doube et al., 1997; Doube et al., 1998; Neilson et al., 2003). In multiple choice tests, *L. terrestris* preferred apple leaves and paper discs inoculated with microorganisms and showed distinct preference for two soil fungi, *Mucor hiemalis* Wehmer and *Penicillium* sp. rather than a bacterium, *Pseudomonas fluorescens* Migula, indicating that fungal growth on food substrates may enhance the availability of carbohydrates and nitrogenous compounds to earthworms (Wright, 1972; Cooke et al., 1980). Bonkowski et al. (2000) carried out selection experiences in order to study preference of earthworm species for a variety of soil fungi. Nine fungal species were proposed to five different earthworm species. They found that two fungal species, *Fusarium nivale* Müll and *Cladosporium cladosporioides* de Vries, were preferred by earthworms. They concluded that earthworms used early successional fungal species as cues to detect fresh organic food sources in soil but the nature of this preference is unknown. Food preference tests conducted with *D. octaedra*, *A. caliginosa* and *Octolasion tyrtaeum* Savigny show that these earthworms preferred organic matter inoculated with different actinomycete species in comparison with a control (organic matter without actinomycetes) (Jayasinghe et al., 2009). This is the first example of earthworm food preference for actinomycetes. How earthworms forage and select for food resources remain unsolved. The evidence of the presence of chemoreceptors in earthworms (Laverack, 1960) combined with their ability to select specific food materials, has led to the hypothesis that olfaction could be used by earthworms in their search for adequate nutriments, including microorganisms. Indeed, most living organisms have developed sensory modalities based on principles of neural organization in order to detect and react to chemicals present in their external environment (Hildebrand, 1995). Moreover, semiochemical compounds govern intra- and inter-specific communication in a great diversity of organisms (Wyatt, 2003) including plants (Runyon et al., 2006) and insects (De Moraes et al., 2001), and are frequently used in agricultural applications (*i.e.* mating disruption, pheromone traps, push-pull strategies) (Hardie et al., 1999; Verheggen et al., 2010). Further experiments seem necessary to develop this hypothesis and enhance our knowledge on earthworm food preference.

4. GUT SYMBIOSIS

Some microorganism species were submitted to growing stimulation during gut transit (Edwards et al., 1996). Indeed, the survival of microorganisms in the earthworm gut depends on their capacity to resist to digestive enzymes of microbial or earthworm origins, intestinal mucus, $CaCO_3$, or to bacteriostatic and microbial substances (Brown, 1995) and also transit time (Scheu, 1992).

Analysis of the digestive tract contents of earthworms has revealed the presence of grass fragments and other plant leaves, roots, algal cells, seeds, fungi, bacteria, protozoa, and actinomycetes (Piearce, 1978). The microbial composition of earthworm intestine contents has been considered to reflect the composition of the soil or ingested plant remains (Morgan, 1988; Brown, 1995; Brown et al., 2004), but there is evidence of the possible existence of ecological group-specific gut microbiota in some earthworm species (Lavelle et al., 2001). Indeed, some physical links were found between bacterial cells and epithelium in the hindgut of *L. terrestris* (Jolly et al., 1993). The presence of a mutualistic digestive system was demonstrated in several tropical and temperate earthworm species in which soluble organic carbon, in the form of a mixture of low-molecular weight mucus, was added to enhance the soil microflora proliferation (Lavelle et al., 1995; Trigo et al., 1999). Intestinal mucus is composed of amino acids (about 200 Da) mixed with high-molecular weight sugars and glycoproteins (40,000-60,000 Da) (Martin et al., 1987). The mucus production and the enzyme pool depend on earthworm species and food quality. For example, epigeic species, which feed on rich substrates, need a complex enzymatic system but not an intensive mucus production in their gut (Trigo et al., 1999). More recent research has focused on the composition and structure of microbial populations of the earthworm's digestive tract (Furlong et al., 2002;

Schönholzer et al., 2002; Horn et al., 2003; Ihssen et al., 2003). Furlong et al. (2002) have shown that some microorganisms of soil (*e.g.*, *Pseudomonas* sp. and *Firmicutes* sp.) increase in abundance through the gut tract of *L. rubellus*. Automated image analysis and *in situ* hybridization were used to study the gut transit impact on bacterial community structure (Schönholzer et al., 2002). Moreover, earthworm gut was identified as an ideal habitat for N_2O-producing bacteria because earthworms activate these microorganisms during gut passage (Horn et al., 2003; Ihssen et al., 2003). However, a comprehensive description of the digestive system and the origin of different gut enzymes require further research, particularly for epigeic and anecic species (Brown et al., 2000). An increasing appreciation of the synergistic interactions between earthworms and microorganisms is observed. The main interest is focused on microorganisms that are ingested from soil and transit the gut by employing culture-based and molecular methods (Egert et al., 2004; Parthasarathi et al., 2007; Byzov et al., 2009; Thakuria et al., 2009). Despite those recent studies, the real existence of symbionts in the earthworm gut is still controversial (Curry et al., 2007). Some ones show that microbial fingerprints in the earthworm gut are associated to the microbial profile in soil and in food sources (McLean et al., 2006; Drake et al., 2007; Knapp et al., 2008; Jayasinghe et al., 2009). For instance, only small differences in bacterial communities between soil, gut, and fresh casts of *L. terrestris* have been highlighted, suggesting the existence of an indigenous earthworm microbial community as unlikely (Egert et al., 2004). Similar results were found by Knapp et al. (2009) during their study on the impact of a radical diet shift on gut microbiota of *Lumbricus rubellus* Hoffmeister and by Jayasinghe et al. (2009) where all the actinomycetes isolated from the casts occured in the natural soil of their study. However, other studies show some evidence of earthworm gut symbionts (Sampedro et al., 2007; Byzov et al., 2009). They found some microorganisms in the earthworm intestine that are absent in the surrounding soil (Byzov et al., 2009) and important changes in the fatty acid concentration and composition in the gut of the earthworm *L. terrestris* (Sampedro et al., 2007). Moreover, a recent study shows that the development of distinct gut wall-associated bacterial communities is strongly associated to earthworm ecological group, despite the shift observed with food source and habitat changes. Presence of all bacteria in earthworm gut and in soil does not allow determination of whether the bacterial communities share a symbiotic or a mutualistic metabolic interaction with earthworms (Thakuria et al., 2010).

Further studies in a large diversity of earthworm species are necessary to confirm the real presence of symbionts in earthworm gut and their functional role.

5. MICROORGANISMS IN BURROW AND CASTS

For some microorganisms, earthworms' gut represent mobile micro-habitats in which dormant microorganisms can find available food, mobility and shelter to develop (Lavelle et al., 1995). The activities of these microorganisms can continue for a short time in the casts because of the suitable amount of soluble carbon and nutrient resources. For example, an increase of microbial respiration rate (approximately 90%) is observed in fresh cast of *A. caliginosa* (Scheu, 1987). As in earthworm gut and casts, a concentration effect of microorganisms and invertebrate activity has been observed in burrows (Graff, 1971; Brown, 1995; Tiunov et al., 1999; Jégou et al., 2001). *Cellulomonas* sp. and *Promicromonospora* sp. were the dominant bacteria in *L. terrestris* burrow walls, whereas *Bacillus* sp. and *Streptomyces* sp. prevailed in the surrounding soil. But no specific fungal community was observed in the burrows of *Lumbricus terrestris* (Tiunov et al., 2002). The presence of microorganisms in earthworm burrows can probably be explained by the loss of carbon through the mucus secretion of earthworms and nitrogen secretion through nephridia (Brown et al., 2000). Recent studies confirm a more intensive microbial activity in earthworm burrow and cast (Amador et al., 2007; Jayasinghe et al., 2009). For different culture media, the number of $cells \cdot g^{-1}$ is higher in structures built by earthworms including burrows and casts than bulk soil (Amador et al., 2007). Moreover, actinomycetes counts in casts of three earthworm species are significantly higher than in the natural soil, indicating that the casts may act as excellent microhabitats for the growth of these microorganisms (Jayasinghe et al., 2009).

6. CONCLUSION

This review was intended to summarize the present knowledge of microscale interactions between earthworms and microorganisms in soil. Relations between earthworms and microorganisms are diverse and complex. Earthworms have symbionts in nephridia and some investigations tend to demonstrate symbiosis in earthworm guts, but future experiments are necessary in different species to generalize this association. Interactions between earthworms and microorganisms are also observed in earthworm casts and burrows, suggesting that these media increase microorganism activities. Moreover, food preferences are revealed for some earthworm species, indicating specific associations. However, no study explains how earthworms can choose a particular microorganism. Chemical communication through microbial volatiles in order to identify potential biologically active compounds for earthworms could be an interesting way to explore this.

Acknowledgements

Lara Zirbes was financially supported by a PhD grant from the Fonds pour la Formation à la Recherche dans l'Industrie et l'Agriculture (FRIA), Belgium.

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