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Reduction of total coliform numbers during vermicomposting is caused by short-term direct effects of earthworms on microorganisms and depends on the dose of application of pig slurry

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ABSTRACT

During vermicomposting of organic waste, the interactions between epigeic earthworms and the detrital microbial community lead to decreases in the abundance of some potentially pathogenic microorganisms. Despite its importance, little is known about the mechanisms involved and the factors that affect the intensity of this effect. In the present study, we carried out three experiments to test the effect of the earthworm *Eisenia fetida* on total coliform numbers in pig slurry. We firstly applied low and high doses (1.5 and 3 kg, respectively) of pig slurry to small scale vermireactors with and without earthworms. We found that *E. fetida* significantly reduced total coliform numbers after 2 weeks, but only in the low dose vermireactors. In a subsequent feeding experiment in mesocosms, we observed that the coliform population was reduced by 98% after passage through the earthworms' guts, which suggests that digestive processes in the gut of *E. fetida* are the main factors involved in the decrease in total coliforms observed in the low dose vermireactors. Decreases in total coliform numbers were not related to decreases in bacterial biomass, which indicates a specific negative effect of earthworms on the coliforms. In the third experiment, we tested the indirect effect of earthworms on total coliforms by inoculating pig slurry with either 2 or 10% vermicompost. The addition of vermicompost did not affect the number of coliforms either after 15, 30 or 60 days, which supports the idea that this bacterial group is more affected by the passage through the gut of *E. fetida* than by interactions with the earthworm-shaped microbial community.

1. Introduction

Vermicomposting is defined as an accelerated process of biooxidation and stabilization of organic wastes that involves interactions between earthworms and microorganisms (Domínguez, 2004). These interactions lead to changes in microbial biomass and activity related to changes in the structure of the microbial community (Lores et al., 2006; Aira et al., 2007a). The elimination of pathogenic microorganisms, particularly human ones, is an additional consequence of this process. Although vermicomposting has been shown to reduce the number of human pathogenic microorganisms in a variety of organic wastes (Eastman et al., 2001; Contreras-Ramos et al., 2005; Craig and Ankers, 2006), little is known about the factors involved in this.

Earthworms can interact with the soil microbial community either directly or indirectly through their feeding, burrowing and casting activities (Lavelle and Spain, 2001). Digestion of the ingested material, which occurs in a time scale of hours, is the first step in this interaction process. Specific microbial groups can respond differently to the gut environment (Schönholzer et al., 1999; Byzov et al., 2007) and selective effects on the presence and abundance of soil microorganisms have been found during the passage of material through the guts of earthworms (Pedersen and Hendriksen, 1993; Karsten and Drake, 1995). The interaction between the microorganisms delivered in the earthworm casts and the surrounding environment constitutes a further step in the earthworm-microorganism interaction (Domínguez, 2004). In the field, accumulation of casts and plant litter in small patches results in hot-spots of increased microbial activity and C assimilation (Bohlen et al., 2002). In vermicomposting systems, where the earthworm populations are raised at higher densities than in the field (Edwards and Bohlen, 1996), the gut and cast associated processes may play a key role in determining the characteristics of the microbial community (Domínguez, 2004). However, almost nothing is known about the relative contribution of these two types of processes to the observed changes in microbial populations during the decomposition of organic matter. In addition, vermicomposting, and decomposition of organic matter in general, are donor-controlled processes (Pimm, 1982), in which the rate of detrital input is expected to be a major factor influencing the interactions within the decomposer community. The availability of resources will shape the

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relationships between earthworms and microorganisms, but the outcome of these interactions may also depend on the microbial group considered (Brown and Doube, 2004).

In animal manures, potentially pathogenic microorganisms are a common fraction of the microbial community (Zhu, 2000; Sidhu and Toze, 2009). The group of bacteria known as total coliforms constitutes a good example of this. Total coliforms are all aerobes although many are facultative anaerobes, and are Gram-negative, non-spore-forming, rod-shaped bacteria that develop a red colony with a metallic sheen within 24 h at 35 °C on an Endo-type medium containing lactose (Clesceri et al., 1998). Coliforms are present in large numbers in the intestinal flora of most warm-blooded animals, and therefore their presence in the environment is associated with sources of fecal contamination. Because of this, they are used as an indicator of the potential presence of entero-pathogens, such as *Escherichia coli* O157:H7, in water and soil environments (Rompré et al., 2002; Sidhu and Toze, 2009). Although coliforms may also be found in the soil environment as part of the native microflora (Geldreich et al., 1962; Duncan and Razzell, 1972; Byappanahalli and Fujioka, 1998), their presence in agricultural amendments represents a potential threat and their screening is of special relevance in vermicomposts produced from animal manures (Smith, 2001; Contreras-Ramos et al., 2005).

In the present study, we performed three experiments to explore how the earthworm *Eisenia fetida* affects the abundance of total coliforms during the vermicomposting process. In the first experiment, we used small scale reactors to investigate the effect of the presence of earthworms, time and application rates of pig slurry on the number of total coliforms and bacterial biomass C. In the second experiment, we compared the density of total coliforms and the bacterial biomass C in the gut content of *E. fetida* and in the initial waste provided as a food source to the earthworms. Finally, in the third experiment we inoculated pig slurry with vermicompost to test for negative effects of worm-worked material on total coliforms. We discuss our results in relation to the relative contribution of direct and indirect effects of earthworms to the suppression of potentially pathogenic microorganisms.

2. Materials and methods

2.1. Earthworms, pig slurry and vermicompost

Specimens of the lumbricid earthworm *Eisenia fetida* (Savigny, 1826) were obtained from stock cultures reared under laboratory conditions (20 ± 2 ºC). Fresh pig slurry was used as food source for the earthworms and was obtained from a pig-breeding farm near the University of Vigo. The solid fraction (15% dry weight) of the slurry was selected in order to avoid any harmful effects that percolates may have on earthworms. The slurry was homogenized, and stored in sealed plastic containers at 5 ºC until use. Vermicompost, a stabilized peat-like material with low C:N ratio, was used as amendment in the inoculation experiment. The vermicompost was obtained from the *E. fetida* cultures reared in the laboratory with pig slurry as breeding medium. The main physicochemical characteristics of both the pig slurry and the vermicompost are shown in Table 1.

Table 1
Physicochemical characteristics of the pig slurry and the vermicompost used in the experiments.

	Pig slurry	Vermicompost
Moisture content (%)	85 ± 2	80 ± 1
Organic matter content (%)	86 ± 1	58 ± 2
pH	8.3 ± 1.0	6.2 ± 1.0
Electrical conductivity (mS cm^{-1})	0.25 ± 0.01	0.44 ± 0.08
Total nitrogen (mg g^{-1} dw)	24 ± 2	35 ± 2
$N\text{–}NH_4^+$ (μg g^{-1} dw)	2400 ± 100	56 ± 17
$N\text{–}NO_3^-$ (μg g^{-1} dw)	250 ± 50	271 ± 28
Total carbon (mg g^{-1} dw)	455 ± 60	363 ± 5
Dissolved organic carbon (μg g^{-1} dw)	11.1 ± 0.1	90 ± 11

dw = dry weight.

2.2. Experimental set up 1: Changes in total coliforms during vermicomposting as affected by time, presence of earthworms and the application rates of pig slurry

Temporal changes in the total coliform numbers in the pig slurry were studied by use of continuous feeding vermireactors (Aira et al., 2006). These small scale reactors were formed by PVC modules resembling sieves, with an external diameter of 30 cm. The mesh size was 5 cm, which allowed mobility of earthworms between modules. To set up the reactors, a module with fresh pig slurry was placed on top of another module containing vermicompost and earthworms. The pig slurry was applied as doses of 1.5 or 3 kg fresh weight (low and high doses, respectively). New modules with the same amount of pig slurry were added sequentially following the feeding activity of the earthworm population. This procedure allowed the addition of each module to be dated within the reactors.

The experimental set up consisted of twelve of the above-mentioned reactors. Six of them were provided with a low dose of pig slurry and the other six with a high dose. For each dose, three reactors were inoculated with 500 mature specimens of *E. fetida* and three remained without earthworms (control). By adding 500 earthworms to the vermireactors, we got an initial density ≈ 7000 ind. m^{-2}, which is within the range observed for *E. fetida* in the field (Monroy et al., 2006). After 36 weeks the reactors provided modules of increasing age, resembling a time profile. Twelve modules, added after 2, 4, 7, 8, 11, 18, 21, 25, 27, 29, 33 and 36 weeks were dismantled and isolated to avoid earthworm escape. The earthworms were then manually removed from the substrate, counted and weighed. Samples were taken from all the modules in order to quantify the bacterial biomass C. The total coliform numbers were determined in the modules added 2, 4, 7, 8, 25 and 36 weeks after the start of the experiment.

2.3. Experimental set up 2: changes in density of total coliforms after transit through the gut of E. fetida

Five mesocosms consisting of 3 L plastic containers were filled to three quarters of their capacity with sieved (>2 mm) and moistened (80% moisture content) vermiculite. Vermiculite is a hydrated silicate mineral resembling mica and does not contain any organic nutrients, which thus obliged the earthworms to ingest the pig slurry provided. Each of the mesocosms was inoculated with 50 mature specimens of *E. fetida*. A plastic mesh (1 cm pore size) was placed over the surface of the vermiculite and 200 g (fresh weight) of pig slurry were placed on top of the mesh, to avoid mixing the pig slurry with the vermiculite and to facilitate removal of the slurry. Mesocosms were checked every three days, the pig slurry was replaced and the vermiculite washed to prevent earthworms from ingesting casts. The mesocosms were maintained at a constant temperature (20 °C) in a scientific incubator and were covered with perforated aluminum foil to avoid desiccation of the pig slurry.

After 1 week, the earthworms were removed from the mesocosms, washed three times with sterile distilled water, and the gut contents released by gently pressing the bodies of intact worms with tweezers, from the last third to the posterior end (Bonkowski and Schaefer, 1997; Horn et al., 2003). The gut content corresponding to the final section of the intestine, the hindgut, was thus obtained. Several gut contents from earthworms from the same mesocosms were pooled to obtain samples that weighed approximately 50 mg (fresh weight). These samples were analyzed to estimate total coliform and total bacteria numbers.