IDENTIFICATION OF THE INTESTINAL MICROBIAL COMMUNITY OF *EISENIA ANDREI* (ANNELIDA: LUMBRICIDAE) RAISED IN DIFFERENT SUBSTRATES

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ABSTRACT: Earthworms actions are favored by microbial activity of communities living in the gut of these organisms. Identification of the microbial flora of the digestive tract of *Eisenia andrei*, was performed in order to find differences that can be attributed to the different food substrates and similarities by the presence of endogenous microorganisms. The food substrates: goat manure (95%) + corn stubble (5%) and chicken manure (85%) + corn stubble (15%), composted for 1 month. Strains were isolated and identified by the use of biochemical tests in the key of Bergey's Manual. In animals bred in goat manure substrate, 11 strains were isolated: 1 strain of *Micrococcus* sp,. *Pseudomonas* sp., *Neisseria* sp. and *Sporolactobacillus* sp.; 4 strains of *Oscillospira* spp.; and 3 dubious strains. In animals bred in substrate with chicken manure and corn residue 12 strains were isolated: 1 strain of *Oscillospira* sp., *Bacillus* sp. and *Syntrophospora* sp.; 3 strains of *Beijerinckia* spp.; 4 strains of *Pseudomonas* spp.; and 2 dubious strains. The differences in species of microorganisms found are attributed to the different diets. *Pseudomonas* and *Oscillospira* strains matched on both treatments, so that could be part of the endogen communities of the intestinal track of this species of earthworm.

KEY WORDS: Intestinal microorganisms, diversity, Lumbricidae, *Eisenia andrei*, different substrates, vermiculture.

Earthworms (Annelida, Oligochaeta) play an important role in terrestrial ecosystems, they contribute to soil fertility modifying the physical, chemical and biological properties such as texture, organic matter decomposition and the regulation of biogeochemical cycles (Edwards & Lofty, 1972; Sprinngett Syers, 1984; Krishnamoorthy, 1985; Hoogertkamp et al., 1983;. Lavelle, 1983;. Lavelle & Martin, 1992; Egert et al., 2004). These modifications are evident when finding large amounts of organic Carbon, Nitrogen, inorganic Phosphorus, Magnesium, Potassium among others, in excreta or "casts" of earthworms (Burk et al., 1999; Schrader & Zhang, 1997; Brussaard et al., 1996; Kolmans & Vasquez, 1996). With these findings, it has been attempted to use earthworms for practical purposes, improving fertility in cultivated soils (Stockdill, 1982), stimulating pedogenesis in soil recovery (Hoogertkamp et al., 1983), evaluating the changes and identifying the different types of soil (Römbke & Jänsch, 2004).

It is also known, that in order to conduct the actions of earthworms, the relationship with other components of the soil ecosystem, such as the microflora (bacteria, fungi, etc.) are necessary. This relationship is not only limited to detritivorous food, but there are microorganisms which are not affected by the earthworms digestive enzymes and probably some of the digestive enzymes are produced by the microorganisms themselves in the intestine, giving it greater ability to degrade complex substances found in soil organic matter (Lee, 1985;

Edwards & Fletcher, 1988; Vinotha et al., 2000; Fukatsu & Nikot, 1998; Santiago, 1995; Alonzo et al., 1999).

Studies have demonstrated the presence of bacteria in the digestive tract of earthworms and some of the strains were not found in the surrounding soil, thus it has been suggested the existence of a mutual relationship between a particular species of earthworm and soil microorganisms, proposing that this would allow the annelid to adapt to different habitats (Hubers, 1993).From these researches, several authors have analyzed each fraction of the digestive tube for greater understanding of this relationship and therefore their ecological function (Parle, 1963; Márialigeti, 1979; Toyota & Kimura, 1994, 2000; James, 1995).

More recent researches have been conducted to determine the communities of micro organisms that are specific or endogenous of each species of earthworm (Toyota & Kimura, 2000; Idowu et al., 2006) to distinguish them from those that could be ingested or inoculated with the soil they inhabit. It has been recently identified the bacterial flora of two species of earthworms found in the same plot of sugar cane cultivation; found that each species has a characteristic and exclusive microflora, despite sharing some species of bacteria among themselves and with the surrounding soil (Picón & Teisaire, 2008, 2009, 2012). Other observations showed that earthworms can act as vectors of bacteria and promote the re colonization of sterilized soil (Teisaire & Picón, 2010).

These results reinforced the idea that both in the intestine and the "casts" there is a great microbiological density and diversity. These organisms also have high activity in the soil, which would be very useful in the recovery or transformation of the waste substrate of agricultural practices.

The purpose of this work is to isolate and identify the microbial flora of the digestive tract of *Eisenia andrei*. Since this species is used in earthworm hatcheries or vermiculture in which different food substrates are used, some of these objectives is to isolate bacterial strains in different feeding conditions to establish differences in microbial populations attributable to different substrates and to identify the presence of endogenous strains of this species of earthworm.

MATERIALS AND METHODS

Specimens of *Eisenia andrei* of the family Lumbricidae, were raised in the laboratory and were fed with different substrates for 4 months. Food substrates corresponded to: (1) goat manure (95%) + corn stubble (5%) and (2) chicken manure (85%) + corn stubble (15%), composted for 1 month. Each substrate was placed in boxes of 40 x 30 x 15 cm. The substrates were watered daily for the first two weeks in order to maintain the wet litter to stabilize the pH, remove the soluble material and plant worms in each one of them. The pH was measured every week and the substrate was removed, so that the washing was homogeneous. At 30 days into the trial, 100 worms were seeded to each substrate. When selecting earthworms it was taken into account the size and the presence of clitellar ring developed and visible, which indicates reproductive maturity. From that moment the level of watering to the substrate bed was reduced and it was covered with a mesh of 80% greenhouse shade. *Eisenia andrei* was fed for four months with the named substrates. The worms were started to be provided food for 15 days after the start of the trial.

We proceeded to separate adult specimens from the different substrates; dissection was performed to 5 exemplars of each substrate to remove the portions of intestine. The procedure was made under anesthesia by cold in refrigerator at 7 °C, once immobilized a cut was made in the ventral area of about 15 segments long from the clitellum towards the back part of the body, allowing to expose the gut. Then, a section of the digestive tract of approximately 10 segments long was dissected. Once removed the sections of the digestive tract, with its content were macerated into physiological solution and proceeded to carry out successive dilutions.

Colonies were isolated in petri dishes, with general medium solid nutrient agar according to the technique of striatum and finally incubations were carried in oven at 30 °C. These strains were signaled and then were exposed to different tests for identification according to the keys of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

RESULTS

In worms raised in substrate (1), with goat manure and corn stubble, 11 stumps of bacteria in total were isolated, from which the following have been identified: 1 strain *Micrococcus* sp.; *Pseudomonas* sp.; *Neisseria* sp. And *Sporolactobacillus* sp..; 4 strains of *Oscillospira* spp.; and 3 strains which identification is uncertain.

In worms raised in substrate (2), with chicken manure and corn stubble, 12 strains in total were isolated and were identified: 1. *Oscillospira* sp. strain; *Bacillus* sp. and *Syntrophospora* sp.; 2 strains *Beijerinckia* spp.; 4 strains of *Pseudomonas* spp.; and 2 strains of doubtful identification.

When comparing the results obtained in the intestinal contents of *Eisenia andrei,* raised on both substrates, it was possible to differentiate the strains that are provided by the substrate from those which are endogenous to this species of earthworms.

Pseudomonas and *Oscillospira* strains are present in samples from both tests or substrates, so that they could be part of the endogenous gut communities of this earthworm species. On the other hand, the other strain found, would be coming or provided by the substrate. For the test substrate prepared with goat manure, the strains *Micrococcus* sp., *Neisseria* sp. and *Sporolactobacillus* sp. would come from the substrate. In the case of worms raised in substrate prepared with chicken manure, the strains: *Bacillus* sp., *Beijerinckia* spp. and *Syntrophospora* sp. would come from this substrate.

DISCUSSION

The results of this work show that there is a wide variety of bacterial genus in *E. andrei,* when compared with the results of other authors´ researches, such as those made by Santiago (1995) on the intestinal bacterial flora of *Onychochaeta borincana,* the Glossoscolecidae family, who has only identified the genus *Bacillus* with 7 different species, being this genus a common inhabitant of the soil where it is *O. borincana.* Valle Molinares (2006) identified in the intestinal microflora of *O. Borincana* to *Bacillus cereus* species. This same genus *Bacillus* was found by Picón and Teisaire (2012) in the intestinal contents of *Enantiodrilus borelli* together with other strains, in this case the genus *Bacillus* is considered endogenous, since it is not found in the surrounding soil.

This study found the strain *Bacillus* sp. in the digestive tract of *E. andrei,* raised in the substrate (2) prepared with chicken manure and corn stubble, and it is considered as given by the substrate.

The results set forth above and to our findings in these studies highlight the differences there are between different species of worms, some species may have endogenous strains and others take it from the substrate they inhabit. This comparison would strengthen the idea of endogenous strains of each species of earthworm, being opposed to the views of Brito-Vega and Espinosa-Victoria (2009) who analyzed the results of several authors and concluded that the bacterial communities of the digestive tract of earthworms are variable, depending on climate, type of soil and organic matter. In this paper we evidence the presence of endogenous strains of the *Eisenia andrei* and strains that are introduced by the substrate where they are raised, expanding in this way the concepts of endogenous strains.

In other researches among which we mention Márialigeti (1979) who found with some frequency exemplars of *Vibrio* sp. and *Aeromonas hydrophila* in the guts of *Eisenia lucens*. Like Toyota and Kimura (1994), also found *Aeromonas hydrophila* while working with the genus *Pheretima*. These same authors (Toyota & Kimura, 2000) had similar results in the digestive tract of *Eisenia fetida*, identifying the same bacterial species *Aeromonas hydrophila*. These results led to the interpretation that endogenous bacterial flora is not necessarily specific since the species *A. hydrophila* has been found in different species of earthworms and in the digestive tract of other animals such as leeches and juvenile salmonids, which function and ecological significance still remain to be elucidated.

Our results are similar to those of Mendez et al. (2003) who supports the concept that bacteria are present in the gut considered as endogenous bacteria that are not in the ground in which they live, these authors also show that with a pre-wash of the gut these bacteria continue to be closely associated to the inner wall of the intestine.

All this leads to the interpretation that there is not a proper bacterial flora of each species and therefore the current research could not conclude that the endogenous micro flora is specific to each species of earthworms. Yet today there are few scattered studies and so it is possible that with more knowledge and more advanced methodologies this concept is reviewed.

Bacteria that were identified in this work have different functions, for example: *Pseudomonas* sp., *Micrococcus* sp., and *Bacillus* sp. among others, promote germination, vegetative growth and development, nitrogen fixation, and increase the absorption of nutrients such as nitrogen, phosphorus and potassium, are also able to produce phytohormone in order to serve as biocontrol of phytopathogenic fungi. These strains are considered of great importance for its potential as biofertilizers for plants of agricultural importance (Diaz Vargas et al., 2001; Beringe, 1984; Ferrera-Cerrato, 1995; Rodríguez, 1995; Chanway et al., 1989; Knudson, 1922; Peter et al., 1987;. Alexander, 1981; Asea et al., 1988; Salih et al., 1989; Wilkinson et al., 1989, 1994; Frankenberger & Arshad, 1995; Fuentes et al., 2003; Tsawkelova et al., 2004a,b; Jimenez Salgado et al., 2004). Specifically, we found that the amylolytic function in soil and water bodies is in charge of the genus *Bacillus* sp., as well as the proteolytic and lipolytic function is in charge of the genus *Pseudomonas* sp., which is often found in environments which are rich in organic matter (Cárdenas, 1995).

Everything that was expressed by the different authors merely highlights the important role of earthworms in the decomposition and degradation of waste which derives from intensive agricultural activities.

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