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Efficiencies of Earthworm through It's Burrowing; Ingesting and Digesting Actions

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Abstract

The earthworm gut is an ideal vicinity for microorganisms such as bacteria, fungi etc. Earthworms ingest plant growth-promoting rhizospheric bacteria such as *Pseudomonas, Rhizobium, Bacillus, Azosprillium, Azotobacter*, etc. along with rhizospheric soil, and they might get activated or increased due to the ideal micro-environment of the gut. Therefore earthworm activity increases the population of plant growth-promoting rhizobacteria (PGPR). This specific group of bacteria stimulates plant growth directly by solubilization of nutrients, production of growth hormone, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, nitrogen fixation, and indirectly by suppressing fungal pathogens. Antibiotics, fluorescent pigments, siderophores and fungal cell-wall degrading enzymes namely chitinases and glucanases produced by bacteria mediate the fungal growth-suppression. Earthworms are reported to have association with such free living soil bacteria and constitute the drilosphere. Earthworm microbes mineralize the organic matter and also facilitate the chelation of metal ions. Gut of earthworms *L. terrestris, Allolobophora caliginosa* and *Allolobophora terrestris* were reported to contain higher number of aerobes compared to soil. Earthworms increased the number of microorganisms in soi1 as much as five times and the number of bacteria and 'actinomycetes' contained in the ingested material increased upto 1,000 fold while passing through their gut. Similar increase was observed in plate counts of total bacteria, proteolytic bacteria and actinomycetes by passage through earthworms gut. Similarly microbial biomass either decreased, or increased or remained unchanged after passage through the earthworm gut.

Keywords: Gut; Pheretima; *Allolobophora caliginosa*; rhizospheric soil

INTRODUCTION

In the 21th century, also why we concentrate on earthworms the answer is today earthworms widely accepted as ecosystem engineer. Earthworms increase microbial activities by providing in their gut mucus consisting of energetic and easily metabolizable compounds Martin, *et al* (1987) and considerable physico-chemical conditions: neutral pH, high moisture and ideal temperature conditions Barois and Lavelle (1986). Earthworms seem to have poor proper enzymatic systems and they appear to rely upon the ingested soil microorganisms to degrade soil organic matter. A major part of earthworm activity on soil properties is contributed to interactions with soil vicinity microorganisms (Edwards and Fletcher, 1988). However, these interactions are still not clearly understood, including the effect of gut passage on the community structure of ingested soil microorganisms. Interactions between earthworms and soil microorganisms are key character for soil processes such as decomposition and transformation of plant residue, humus formation and the formation of the pool of nutrient elements and microbial communities. The wide spectrum of these interactions makes it possible to speak about a close relationship between earthworms and soil microorganisms. Soil fungi, bacteria etc are assumed to be the main source of food for earthworms (Byzov, 2003). While feeding, earthworms regulate the growth of soil microorganisms by eating some microbial populations and providing ideal conditions for the growth of others in their digestive tract and in casts (Tiunov and Scheu, 2000). The gut of many soil organisms (microinvertebrates and macroinvertebrates) contains microbial communities that usually helpful in the digestion. These microbial-animal relationships create mutualisms. Earthworms are also having a mutualistic relationship with soil microorganisms (Bacteria, Fungi, etc) passing through their digestive tract, but the nature and role of the microbiota inhabiting their gut are virtually unknown (Lavelle and Spain, 2001). However, little is understood on the interactions between earthworm and soil microorganisms, including the gut microbial community. The goal of the present review work was to list out the gut microflora of different species of earthworms.

EFFIECIENCIES OF EARTHWORMS

Earthworms are worldwide considered as an excellent bioindicators of the relative health of soil vicinity and possess a number of qualities that predispose them for use in monitoring terrestrial ecosystems (Vandecasteele, *et al*, 2004). Earthworms are decomposers, aerators, crushers, mixers, chemically degraders and biologically stimulators in the soil vicinity. They effectively harness the beneficial soil microflora, destroy soil pathogens and convert organic wastes (Domestic waste, Industrial waste, Hospital waste, etc) into vitamins, enzymes, antibiotics, growth hormones and protein rich casts. Earthworms are sensitive for the detection and assessment of soil contamination. Therefore earthworms can be used as bio-indicators to measure soil contamination. According to Mostafaii, *et al* (2016), the addition of organic matter, due to the high propensity of earthworms to consume it, and the non-use of contaminated soil reduce the ability of earthworms for bioremediation. The use of earthworms for soil bioremediation is a biological method, so that the pollutant concentrations in the soil are reduced through bioaccumulation mechanisms in the body of the earthworms (Matscheko *et al*., 2002, Slizovskiy and Kelsey, 2010). These organisms can accumulate high concentrations of heavy metals in their body (Li *et al*., 2010). Because they are the main components of biomass, earthworms are the most important food source for other organisms higher in the food pyramid (Nahmani *et al*., 2007). Accumulation of toxic substances such as metals and pesticides in their bodies cause the animals that eat them to be directly affected (Garcia *et al*., 2008). Previous studies have shown that the presence of excess heavy metals in the soil, is leading to increased mortality of the worms (Spurgeon and Hopkin, 1995, Haghparast *et al*., 2013, Jamshidi and Golchin, 2013). The study of Darling and Thomas (2005) showed that the concentration of soluble lead compounds in earthworm bodies are more than of low soluble compounds. During their study, Avila *et al*. (2009) identified that increasing the organic material can reduce the toxicity of heavy metals in the body of earthworms. Irizar *et al*. (2015) concluded during their study that, if the organic material in the soil is low, earthworms are not able to digest the soil and, as a result, the toxicity of cadmium increases in them, and the mortality and disorder in reproduction rise. Haghparast *et al*. (2013) showed that organic material is a source of energy for *Eisnia Fetida* earthworms and increases the percentage of their survival.

ALIMENTARY CANAL OF EARTHWORM

The alimentary canal in earthworm is a straight tube. It starts from the mouth. The mouth is located at the anterior end of the peristomium. It leads into the buccal cavity. The buccal cavity is located in the first two segments. The buccal cavity leads into a thick walled muscular chamber called pharynx. It lies in the segments three and four. The pharynx leads into a short tube called oesophagus. It lies in the 5"«segment. The oesophagus leads into a hard thick walled muscular sac called gizzard. It is located in the e segment. The gizzard is followed by the intes-tine. It is long and straight and it extends up to the anal segment. The dorsal wall of the intestine has a fold which projects into the lumen of the intestine. It is called typhlosole. It increases the area of absorption. The intestine opens to the outside by the anus located at the posterior end of the anal segment. Earthworm guts considered as ideal habitats for microorganisms (bacteria, etc), because several studies showed increased microbial numbers in the guts versus the soil, in which earthworms were living (Wolter and Scheu, 1999). Some previous studies proposed that the earthworm gut microbial community is qualitatively not much different from the microbial community in the surrounding soil vicinity (Parle, 1963), later studies found significant differences for selected phylogenetic groups or functional guilds of microorganisms, eg: Proteobacteria (Schonholzer, 2002), Actinobacteria, denitrifiers or cellobiose utilizers (Kritufek, *et al*, 1993; Ihssen, *et al*, 2003). Earthworms gut performes a unique vicinity subsystem of soil vicinity. The earthworm gut has stable conditions different from surrounding vicinity. Earthworm gut is a straight tube bioreactor, which maintains a stable temperature through novel temperature regulatory mechanisms, thus accelerating the rates of the bioprocesses and preventing enzyme inactivation caused by high temperatures. Earthworm's gizzard is a novel colloidal mill in which the feed is ground into particles smaller than 2 mm giving, thereby, enhancing surface area for microbial processing (Karthikeyan, *et al*, 2004).

Earthworm gut is basically an effective tubular structure extending from mouth to the anus; its different regions are the muscular pharynx, oesophagous, intestine and associated digestive glands. The gut contents usually comprise mucus, organic and mineral matter. An analysis of gut contents in earthworm revealed the occurrence of different kinds of symbiont like microfungi, bacteria, protozoa, etc; most microfungal species are present in the foregut, gradually decreased in number in the mid and hindgut with fewest in freshly laid casts (Dash, *et al*, 1980). It is well established that the earthworm gut provides ideal conditions for the development of microorganisms (bacterial & fungi colonies) since earthworm casts contain significantly higher counts of bacteria than in the surrounding soil (Edwards and Lofty, 1977; Kale, *et al*, 1992; Munnoli, 1998; Bhattacharyya, *et al*, 2000; Munnoli, 2007). The rate of food transit along the digestive tract of earthworms is very high. Therefore, it appears that earthworms that earthworms quickly kill and digest the soil microorganisms consumed. It has previously been shown that the digestion of microorganisms in the gut of invertebrates begins with the quick death of certain microbial cells caused by specific killing agents of nonprotein nature (Byzov, *et al*, 1998). Microorganisms may constitute a very important part of the diet of earthworms, which can feed on then selectively (Moody, *et al*, 1995; Edwards, 2004). The gut environment is anoxic, pH 6.9 with about 50% water content. The gut bacteria are enriched in total carbon, organic carbon and total nitrogen with a carbon to nitrogen ratio of 7 (Horn, *et al*, 1996). The bacterial counts in guts/vermicompost were higher than the surrounding soil ecosystem (Edwards and Bohlen, 1996; Suthar, 2008) and as the organic matter ingested passes through the gut, it undergoes biochemical changes effected by gut-inhibiting bacteria. Therefore there is a greater role played by the DNA of microorganisms and earthworm species. The genetic makeup of the strains and environment has a profound influence on the efficiency of earthworms in the bioconversion process (Giraddi, *et al,* 2009) and in the assessment of diversity and community composition (Thakuria, *et al*, 2010).

The earthworm gut could be used as an environment and nutrition by microorganisms and gut derived enzymes could affect the microbes on the soils particles (Brown, 1995). On the other hand, the earthworm is migrating in the soil and thus soil flows through earthworm gut. In the gut of the earthworms (*Eisenia* *fetida*) the microbial composition was changed towards the increasing numbers of non-spore. Forming bacteria and decreasing numbers of spore. Forming bacteria, this also resulted in enhanced levels of nitrogen fixation (Tereshchenko and Naplekova, 2002). A number of novel N_2O producing species of bacteria fromgerieva dechloromonas (Bata Proteo Bacteria), fromgerieva dechloromonas (Cytophaga Flara bacteria group Bacteroidetes). Earthworm activity does not only mediate macroaggregate formation, but also microaggregate formation (Barois, *et al*, 1993). Based on thin sections of the earthworm gut, casts and control soil from earthworm microcosms, several studies have shown that during gut transit organic materials are intimately mixed and become encrusted with the mucus to create new nuclei for microaggregate formation (Shipitalo and Protz, 1993). The digestive system of earthworm consists of a phar Shipitalo MJ and Protz ynx, oesophagus and gizzard (reception zone) followed by an anterior intestine that secretes enzymes and a posterior intestine that absorbs nutrients. Earthworm activity does not only mediate macroaggregate formation, but also microaggregate formation. Based on thin sections of the earthworm gut, casts and control soil from earthworm microcosms, several studies have shown that during gut transit organic materials are intimately mixed and become encrusted with the mucus to create new nuclei for microaggregate formation.

BACTERIA IN THE ALIMENTARY CANAL OF EARTHWORMS

The diversity of 8 bacterial groups from fresh soil and gut of the earthworms *L. terrestris* and *Aporrectodea caliginosa* were studied by single strand conformation polymorphism (SSCP) analysis using both newly designed 16SrRNA gene specific primer sets targeting Alphaproteo bacteria, Betaproteo bacteria, Gamma-proteo bacteria, Deltaproteo bacteria, Bacteroids, Verrucomicrobia, Planctomycetes and Firmicutes as a conventional universal primer set for SSCP, with RNA and DNA as templates. Whereas using fluorescence in situ hybridization Bacteroidates, Alphaproteo bacteria and Betaproteo bacteria were predominant in communities from the soil and worm cast samples, some specific bacterial taxonomic groups maintain their diversity and even increase their relative numbers during transit through the gastrointestinal tract of earthworms Nechitaylo, *et al*, 2010).

Lumbricus terrestris, *L. friend*, *Aporrectodea caliginosa* and *A. longa* contain ecological group specific gut wall associated bacterial communities. The abundance of specific gut wall associated bacteria, including proteobacteria, firmicutes and an actinobacterium was dependent on the ecological group. Bacteria are of minor importance in the diet, algae are of moderate importance; protozoa and fungi are major sources of nutrients. Worms, produced under sterile conditions, could live on individual cultures of certain bacteria, fungi and protozoa, but grew best on various mixtures of microorganisms. Symbiotic interactions between earthworms and microorganisms break down and fragment organic matter progressively, finally incorporating it into water stable aggregates. Valle Molinares *et al* 2007 identified 7 species of bacteria from the genus Bacillusm (*B. insolitus, B. megaterium, B. breris, B. pasteurii, B. sphaericus, B. thuringiensis and B. pabuli*) within the intestine of *Onychochaeta borincana*. All these species are typical soil bacteria. In addition, it was found that the microbial weight of the intestinal region decreased from the anterior to posterior section. For studies on bacterial within the intestine of earthworms, diverse methods and techniques have been used which have helped in identifying species of the genus Bacillus, Pseudomonas, Klebsiella, Azotobactor, Serratia, Aeromonas and Enterobacter (Byzov, *et al*, 2007; Singleton, *et al*, 2003). Earthworm gut environment may act as specific filter as well as a fermenter for some soil bacteria and fungi with those bacterial cells that survive passage through the midgut can even multiply in the hindgut. Spores of some fungi that survived in the midgut vicinity of earthworms. Researchers have attempted to study earthworm gut microbes using direct culture methods (Kritufek, et al, 1992; Karsten and Drake, 1995) and electron microscopy (Jolly, *et al*, 1993). 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 the gut content (Singleton, *et al*, 2004). These finding suggest that the earthworm gut favourable for the growth and activity of certain bacterial species. Karsten and Drake (1995) reported the first Scanning Electron Microscopy (SEM) study on the digestive tube of earthworms. They find the cocoid bodies from the foregut of *O. cyaneum.* In *O. borincana* they were only seen in the hindgut. Earthworm revealed the presence of cocci, rod-shaped bacteria and filamentous microorganisms in *Lumbricus terrestris* L., 1758 and *Octolasion cyaneum*. The key role of the microorganisms in the gut of earthworms is not clearly understand: indigenous bacteria are not generally found in earthworms while, if present they may aid digestive processes. Seven different species of *Bacillus* have been identified from the gut of *O. Borincana* (Santiago, 1995). Apparently, the presence of these microorganisms in the intestinal tube may be a result of soil ingestion and do not seem to assist in its digestion. Although rod-shaped bacteria were observed associated to the intestinal wall of *O. borincana.* The profiles of bacteria are very closely similar in soil and in the gut contents of many earthworm species, suggesting an absence in the gut of any indigenous bacterial groups. In the time of scanning of the gut surface of these two species (*Lumbricus terrestris* and *Octolasion cyaneum*), only two rod shaped organisms were found. In contrast, the Transmission Electron Microscopy (TEM) investigation of *L. terrestris* hindgut showed several rod-shaped bacteria over a relatively small area. Earthworms are ubiquitous soil invertebrates that ingest large amounts of mineral soil and organic matter containing a variety of microorganisms (Satchell 1967). Hendriksen (1991) concluded that a field population of 75gm-2 per ha. The effects on bacteria of the passage through the earthworm intestinal tract may have a major impact on the composition of the soil bacterial community and so gut passage should be taken into consideration when assessing the risk of releasing non-indigeous, eg genetically engineered, bacteria into terrestrial ecosystems. Denitrification in the earthworm gut is involved in the in vivo emission of N2O by earthworms, cultured denitrifiers occur in high numbers in the earthworm gut (Matthies, *et al*, 1999; Elliott, *et al*, 1991; Svensson, *et al*, 1986). Earthworms can directly regulate microbial population (Bacteria, Fungi) by consuming large amount of soil. This leads to elimination of some microorganisms and proliferation of others in the digestive tract of earthworm (Bonkowski, *et al*, 2000). Microorganisms are a vital food component of soil invertebrates including earthworms. The importance to fungi and bacteria as important sources of food is indicated by several phenomena. The animals in soil avoid consuming fresh leaf litter. which could be toxic, the animals themselves have no intrinsic capacity to digest cellulose and they depend on microorganisms as sources of essential amino acids (Mindermann and Daniels, 1967; Pokarzhevskii, *et al*, 1984). It was proposed that earthworms derive more of its energy and nutrients from gut specific microbiota than from microbiota already present in the ingested soil (Sampedro, *et al*, 2006). According to Kulinska (1961), the total number of microorganisms (bacteria, fungi) in a alimentary tract or in the fresh excrements of earthworms were from half to 6 times higher in comparison with the surrounding soil.

FUNGAL FLORA

Microfungi in the casts of earthworms, *Perionyx millardi*, *Eudrilus eugeniae*, *Lampito mauritii* and certain other earthworm species are available. But, the scientific knowledge available on the fungal flora in the casts of the earthworm, *Perionyx ceylanensis* Mich. *Aspergillus fumigates*, *Mucor circinelloides*, *F. circinelloides* and *Penicillium expansum* were dominant in the intestine of earthworms (Vaclav and Alena Novakova, 2003).

INTERACTION OF MICROBES AND EARTHWORM IN THE PROCESS OF VERMICOMPOSTING:

Earthworms promote the growth of beneficial decomposer microbes (bacteria, actinomycetes and fungi) in waste biomass (Binet, *et al*, 1998). They hosts millions of decomposer microbes in their gut which is described as little bacterial factory. They devour on microbes and excrete them out (many time more in number than they ingest) in soil along with nutrients nitrogen (N) and phosphorus (P) in their excreta. The nutrients N and P are further used by the microbes for multiplication and vigorous action. Earthworms and microbes act symbiotically and synergistically to accelerate and enhance the decomposition of the organic matter in the waste. It is the microorganisms that break down the cellulose in the food waste, grass clippings and the leaves from garden wastes (Morgan and Burrows, 1982).

CONCLUSION

The earthworm gut is an ideal vicinity for microorganisms such as bacteria, fungi etc. Earthworms ingest plant growth-promoting rhizospheric bacteria such as *Pseudomonas, Rhizobium, Bacillus, Azosprillium, Azotobacter*, etc. along with rhizospheric soil, and they might get activated or increased due to the ideal micro-environment of the gut. Therefore earthworm activity increases the population of plant growth-promoting rhizobacteria (PGPR). This specific group of bacteria stimulates plant growth directly by solubilization of nutrients, production of growth hormone, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, nitrogen fixation, and indirectly by suppressing fungal pathogens. Antibiotics, fluorescent pigments, siderophores and fungal cell-wall degrading enzymes namely chitinases and glucanases produced by bacteria mediate the fungal growth-suppression. Earthworms are reported to have association with such free living soil bacteria and constitute the drilosphere. Earthworm microbes mineralize the organic matter and also facilitate the chelation of metal ions. Gut of earthworms *L. terrestris, Allolobophora caliginosa* and *Allolobophora terrestris* were reported to contain higher number of aerobes compared to soil. Earthworms increased the number of microorganisms in soi1 as much as five times and the number of bacteria and 'actinomycetes' contained in the ingested material increased upto 1,000 fold while passing through their gut. Similar increase was observed in plate counts of total bacteria, proteolytic bacteria and actinomycetes by passage through earthworms gut. Similarly microbial biomass either decreased, or increased or remained unchanged after passage through the earthworm gut.

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