

Bacterial diversity in the alimentary canal of earthworms

Abstract

The soil bears infinite life that promotes diverse microflora. Soil bacteria viz., *Bacillus*, *Pseudomonas* and *Streptomyces* etc., are prolific producers of secondary metabolites which act against numerous co-existing phytopathogenic fungi and human pathogenic bacteria. Microbial communities also support a large number of soil invertebrates, which in turn have an important regulatory effect on the microbial populations. Decomposition of organic material is assumed to be mainly mediated by microorganisms. The rate and extent of the decomposition depends on the chemical composition of the material, environmental factors, and on the microbial community. The activity of the decomposing microorganisms is accelerated by the activity of the soil fauna. The microorganisms show a high degree of specialization and display a large number of enzymes for the breakdown of organic matter. It is certainly proven that the growth of earthworms is dependent on microbial associations. In fact, microorganisms are largely responsible for the decomposition of the materials ingested by earthworms and in turn earthworm regulates modifications in microbial communities thus sharing a mutualistic relationship.

Keywords: gut of earthworm, gut transit time, gut associated microbes, gut symbiosis, vermicasts

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Introduction

Earthworm's ability to increase plant nutrient availability is likely to be dependent on the activity of earthworm gut microflora. Earthworms indirectly influence the dynamics of soil chemical processes, by comminuting the litter and affecting the activity of the soil microflora.¹⁻³ Interactions between earthworms and microorganisms seem to be complex. Earthworms ingest plant growth-promoting rhizospheric bacteria such as *Pseudomonas*, *Rhizobium*, *Bacillus*, *Azospirillum*, *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).⁴ This specific group of bacteria stimulates plant growth directly by solubilization of nutrients,^{5,6} production of growth hormone, 1-aminocyclopropane-1-carboxylate (ACC) deaminase,⁷ nitrogen fixation,⁸ and indirectly by suppressing fungal pathogens. Antibiotics, fluorescent pigments, siderophores and fungal cell-wall degrading enzymes namely chitinases and glucanases^{6,8-12} produced by bacteria mediate the fungal growth-suppression. Earthworms are reported to have association with such free living soil bacteria and constitute the drilosphere.¹³ Earthworm microbes mineralize the organic matter and also facilitate the chelation of metal ions.^{14,15} Gut of earthworms *L. terrestris*, *Allolobophora caliginosa* and *Allolobophora terrestris* were reported to contain higher number of aerobes compared to soil.¹⁶ Earthworms increased the number of microorganisms in soil as much as five times¹⁷ and the number of bacteria and 'actinomycetes' contained in the ingested material increased upto 1,000 fold while passing through their gut.¹⁸ Similar increase was observed in plate counts of total bacteria, proteolytic bacteria and actinomycetes by passage through earthworms gut.^{16,19-21} Similarly microbial biomass either decreased,^{3,21} or increased²² or remained unchanged¹⁹ after passage through the earthworm gut. An oxalate-degrading bacterium *Pseudomonas oxalaticus* was isolated from intestine of *Pheretima* species²³ and an actinomycete *Streptomyces*

lipmanii was identified in the gut of *Eisenia lucens*.²⁴ Scanning electron micrographs provided evidence for endogenous microflora in guts of earthworms, *L. terrestris* and *Octolasion cyaneum*.²⁵ Gut of *E. foetida* contained various anaerobic N₂-fixing bacteria such as *Clostridium butyricum*, *C. beijerinckii* and *C. paraputrificum*.²⁶ Alimentary canal of *Lumbricus rubellus* and *Octolasion lacteum* were found to contain more numbers of aerobes and anaerobes²⁷ and culturable denitrifiers. Earthworms harbor 'nitrogen-fixing' and 'decomposer microbes' in their gut and excrete them along with nutrients in their excreta.²⁸ Earthworms stimulate and accelerate microbial activities by increasing the population of soil microorganisms,²⁹ microbial numbers and biomass,³ by improving aeration through burrowing actions. Vermicomposting modified the original microbial community of the waste in a diverse way. Actinobacteria and Gamma proteobacteria were abundant in vermicompost, while conventional compost contained more Alpha proteobacteria and Bacteroidetes, the bacterial phylogenetic groups typical of non-cured compost.³⁰ Total bacterial counts exceeded 10-10⁹/g of vermicompost and it included *nitrobacter*, *azotobacter*, *rhizobium*, phosphate solubilizers and actinomycetes.³¹ Molecular and culture-dependent analyses of bacterial community of vermicompost showed the presence of α -Proteobacteria, β -Proteobacteria, γ -Proteobacteria, Actinobacteria, Planctomycetes, Firmicutes and Bacteroidetes. Several findings showed considerable increase in total viable counts of actinomycetes and bacteria in the worm treated compost.^{32,33} The increase of microbial population may be due to the congenial condition for the growth of microbes within the digestive tract of earthworm and by the ingestion of nutrient rich organic wastes which provide energy and also act as a substrate for the growth of microorganisms.³⁴ The differences in microbial species, numbers and activity between the earthworm alimentary canal or burrow and bulk soil indirectly support the hypothesis that the bacterial community structures of these habitats are different from those of the soil. Specific phylogenetic groups of bacteria such as *Aeromonas hydrophila* in *E. foetida*,³⁵ fluorescent pseudomonads in

L. terrestris,³⁶ and Actinobacteria in *L. rubellus*³⁷ have been found in higher numbers in earthworm guts, casts, or burrows.

Enzymatic activity characterization and quantification has a direct correlation with type and population of microbes and reflects the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations and provide information about the maturity of the compost.³⁸ Wormcasts contain higher activities of cellulase, amylase, invertase, protease, peroxidase, urease, phosphatase and dehydrogenase.^{3,39} Dehydrogenase is an intracellular enzyme related to the oxidative phosphorylation process⁴⁰ and is an indicator of microbial activity in soil and other biological ecosystems.⁴¹ The maximum enzyme activities (cellulase, amylase, invertase, protease and urease) were observed during 21–35 days in vermicomposting and on 42–49 days in conventional composting. Also, microbial numbers and their extracellular enzyme profiles were more abundant in vermicompost produced from fruitpulp, vegetable waste, groundnut husk and cowdung compared to the normal compost of the same parental origin.³³ *Pseudomonas*, *Paenibacillus*, *Azoarcus*, *Burkholderia*, *Spiroplasm*, *Acaligenes*, and *Acidobacterium*, the potential degraders of several categories of organics are seen associated with the earthworm's intestine and vermicast.²⁸ Firmicutes viz., *Bacillus benzoovorans*, *B. cereus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, *B. macroides*; Actinobacteria namely *Cellulosimicrobium cellulans*, *Microbacterium spp.*, *M. oxydans*; Proteobacteria such as *Pseudomonas spp.*, *P. libaniensis*; ungrouped genotypes *Sphingomonas sp.*, *Kocuria palustris* and yeasts namely *Geotrichum spp.* and *Williopsis californica* were reported from vermicomposts.⁴² Pinel et al.⁴³ reported the presence of a novel nephridial symbiont, Verminephrobacter eiseniae from *E. foetida*. *Ochrobactrum sp.*, *Massilia sp.*, *Leifsonia sp.* and bacteria belonging to families *Aeromonadaceae*, *Comamonadaceae*, *Enterobacteriaceae*, *Flavobacteriaceae*, *Moraxellaceae*, *Pseudomonadaceae*, *Sphingobacteriaceae*, *Actinobacteria* and *Microbacteriaceae* were reported to occur in earthworms alimentary canal. The microbial flora of earthworm gut and cast are potentially active and can digest a wide range of organic materials and polysaccharides including cellulose, sugars, chitin, lignin, starch and polylactic acids.^{30,44,45} Single-strand conformation polymorphism (SSCP) profiles on the diversity of eight bacterial groups viz., *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Verrucomicrobia*, *Planctomycetes*, and Firmicutes from fresh soil, gut, and casts of the earthworms *L. terrestris* and *Aporrectodea caliginosa* showed the presence of Bacteroidetes, *Alphaproteobacteria*, *Betaproteobacteria* and representatives of classes Flavobacteria, Sphingobacteria (Bacteroidetes) and *Pseudomonas spp.* in the worm casts in addition to unclassified *Sphingomonadaceae* (*Alphaproteobacteria*) and *Alcaligenes spp.* (*Betaproteobacteria*).^{46–49}

Conclusively enough, the activity of the decomposing microorganisms is accelerated by the activity of the soil fauna. The microorganisms show a high degree of specialization and display a large number of enzymes for the breakdown of organic matter. It is certainly proven that the growth of earthworms is dependent on microbial associations. In fact, microorganisms are largely responsible for the decomposition of the materials ingested by earthworms and in turn earthworm regulates modifications in microbial communities thus sharing a mutualistic relationship.

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Conflict of interest

Authors declare there is no conflict of interest.

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