INVESTIGATING THE DIVERSITY OF MICROBIOTA IN VERMICOMPOST APPLIED TO THE PRODUCTION OF ORGANIC FERTILIZERS CONTAINING MICROORGANISMS

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Abstract: *Vermicompost contains beneficial bacteria, vermicompost is a finely divided peat-like material with high porosity, good ventilation, drainage, water-holding capacity, microorganism activity, Excellent nutritional status and buffering capacity thus provide the physiological properties required for soil fertility and plant growth. Worm manure enhances soil biodiversity by promoting entry and enhances plant growth directly by producing plant growth-regulating hormones and enzymes. Due to its inborn biological, biochemical and physicochemical properties, vermicompost can be used to promote sustainable agriculture and also for the safe management of agricultural, industrial, and domestic wastes. In Vietnam, the production of vermi-compost still takes place with a small scale, the product of vermi-compost is still small and can only be used for ornamental plants and high-value crops. Research and production of vermi-compost to put into agricultural production is very necessary*

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1. INTRODUCTION

Earthworms affect the microbial community, the physical and chemical properties of the soil. They break down large soil particles and leaf carcasses and thus increase the availability of organic matter for microbial decomposition and turn organic waste into valuable vermi-compost by crushing and decomposing them with the help of aerobic and anaerobic microorganisms (Maboeta MS, 2003). Earthworms activity is found to enhance the beneficial microflora and suppress harmful pathogenic microbes (Lavelle P, 1992). Vermicomposting is an efficient nutrient recycling process that involves harnessing earthworms as versatile natural bioreactors for organic matter decomposition. Due to richness in nutrient availability and microbial activity vermicomposts increase soil fertility, enhance plant growth and suppress the population of plant pathogens and pests. This study we are investigating the diversity of microbiota in vermicompost applied to the production of organic fertilizers containing microorganism.

2. CLASSIFICATION OF WORMS

Earthworms, grouped under phylum annelida are long, narrow, cylindrical, bilaterally symmetrical, segmented soil dwelling invertebrates with a glistening dark brown body covered with delicate cuticle. They are hermaphrodites and weigh over 1,400–1,500 mg after 8–10 weeks. Their body contains 65% protein (70–80% high quality 'lysine rich protein' on a dry weight basis), 14% fats, 14% carbohydrates, and 3% ash. Their life span varies between 3–7 years depending upon the species and ecological situation. The gut of earthworm is a straight tube starting from mouth followed by a muscular pharynx, oesophagus, thin walled crop, muscular gizzard, foregut, midgut, hindgut, associated digestive glands, and ending with anus. The gut consisted of mucus containing protein and polysaccharides, organic and mineral matter, amino acids and microbial symbionts viz., bacteria, protozoa and microfungi. The increased organic carbon, total organic carbon and nitrogen and moisture content in the earthworm gut provide an optimal environment for the activation of dormant microbes and germination of endospores etc. A wide array of digestive enzymes such as amylase, cellulase, protease, lipase, chitinase and urease were reported from earthworm's alimentary canal. The gut microbes were found to be responsible for the cellulase and mannose activities (Munnoli PM, 2010).

Table 1. Types and ecology of earthworms, their characteristic features and beneficial traits

Species	**Ecological category**	**Ecological niche**	**Characteristic features**	**Beneficial trait**
Eisenia foetida, *Lumbricus rubellus,* *L. castaneus,* *L. festivus,*	Epigeics	Superficial soil layers, leaf litter, compost	Smaller in size, body uniformly pigmented, active gizzard, short life cycle, high reproduction rate and regeneration, tolerant to disturbance, phytophagous	Efficient bio-degraders and nutrient releasers, efficient compost producers, aids in litter comminution and early decomposition

Eiseniella tetraedra, Bimastus minusculus, B. eiseni, Dendrodrilus rubidus, Dendrobaena veneta, D. octaedra				
Aporrectodea caliginosa,	Endogeics	Topsoil or subsoil	Small to large sized worms, weakly pigmented, life cycle of medium duration, moderately tolerant to disturbance, geophagous	Brings about pronounced changes in soil physical structure, can efficiently utilize energy from poor soils hence can be used for soil improvements
A. trapezoides, A. rosea, Millsonia anomala,				
Octolasion cyaneum,	Polyhumic endogeic	Top soil (A1)	Small size, unpigmented, forms horizontal burrows, rich soil feeder	
O. lacteum,				
Pontoscolex corethrurus,	Mesohumic endogeic	A and B horizon	Medium size, unpigmented, forms extensive horizontal burrows, bulk (A_1) soil feeder	

Allolobophora chlorotica,				
Aminthas sp.	Oligohumic endogeic	B and C horizon	Very large in size, unpigmented, forms extensive horizontal burrows, feeds on poor, deep soils	
L. terrestris,	Anecics	Permanent deep burrows in soil	Large in size, dorsally pigmented, forms extensive, deep, vertical permanent burrows, low reproductive rate, sensitive to disturbance, phytogeophagous, nocturnal	Forms vertical burrows affecting air-water relationship and movement from deep layers to surface helps in efficient mixing of nutrients
L. polyphemus,				

The effects of vermi-compost on pH, conductivity (EC), C: N ratio and other nutrients have been noted. Earthworm activity reduces the pH and C: N ratio in the stool (Gandhi M, 1997). Chemical analysis showed vermicompost had a lower pH, EC, organic carbon (OC) (Nardi S, 1983), C:N ratio (Riffaldi R, 1983) nitrogen and potassium and higher amounts of total phosphorous and micronutrients compared to the parent material (Hashemimajd K, 2004). (Lazcano C, Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure, 2008) and concomitant production of CO2 (Elvira C, 1998). Vermicomposting of paper mill and dairy sludge resulted in 1.2–1.7 fold loss of organic carbon as CO2 (Elvira C, 1998), In contrast to the parent material used, vermicomposts contain higher humic acid substances (Albanell E, 1988), Humic acid substances occur naturally in mature animal manure, sewage sludge or paper-mill sludge, but vermicomposting drastically increases the rate of production and their amount from 40–60 percent compared to traditional composting. The enhancement in humification processes is by fragmentation and size reduction of organic matter, increased microbial activity within earthworm intestine and soil aeration by earthworm feeding and movement (Dominguez J, 2004). EC indicates the salinity of the organic amendment. Minor production of soluble metabolites such as ammonium and precipitation of dissolved salts during vermicomposting lead to lower EC values. Compared to the parent material used, vermicomposts contain less soluble salts and greater cation exchange capacity (Holtzclaw KM, 1979). C:N ratio is an indicator of the degree of decomposition. During the process of biooxidation, CO2 and N is lost and loss of N takes place at a comparatively lower rate. Comparison of compost and vermicompost showed that vermicompost had significantly less C:N ratios as they underwent intense decomposition .

3. MICROBIOTA AND RELATIONSHIP WITH EARTHWORMS

Interactions between earthworms and microorganisms seem to be complex. 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) (Sinha et al. 2010). This specific group of bacteria stimulates plant growth directly by solubilization of nutrients (Ayyadurai N, 2007), production of growth hormone, 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Correa JD, 2004), nitrogen fixation (Han J, 2005), 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 (Pizl V, 1993), 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 (Edwards CA, 1988). 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. An oxalate-degrading bacterium Pseudomonas oxalaticus was isolated from intestine of Pheretima species and an actinomycete Streptomyces lipmanii was identified in the gut of Eisenia lucens (E, 1980).

Table 2. *The biodiversity of vermicompost bacteria and their beneficial characteristics*

Vermicompost earthworm	Names of bacteria	Beneficial traits	References
Pheretima sp.	*Pseudomonas oxalaticus*	Oxalate degradation	Khambata and Bhat, 1953
Unspecified	*Rhizobium trifolii*	Nitrogen fixation and growth of leguminous plants	Buckalew et al. 1982

Vermicompost earthworm	**Names of bacteria**	**Beneficial traits**	**References**
Lumbricus rubellus	*R. japonicum*, *P. putida*	Plant growth promotion	Madsen and Alexander 1982
L. terrestris	*Bradyrhizobium japonicum*	Improved distribution of nodules on soybean roots	Rouelle, 1983
Aporrectodea trapezoids,	*P. corrugata 2*14OR	Suppress *Gaeumannomyces graminis* var. *Tritd* in wheat	Doube et al. 1994
A. rosea			
A. trapezoids,	*R. meliloti* L5-30R	Increased root nodulation and nitrogen fixation in legumes	Stephens et al. 1994b
Microscolex dubius			
Eisenia foetida	*Bacillus* spp., *B. megaterium,*	Antimicrobial activity against *Enterococcus faecalis*DSM 2570, *Staphylococcus aureus* DSM 1104	Vaz-Moreira et al. 2008
	B. pumilus, B. subtilis		
L. terrestris	Fluorescent pseudomonads,	Suppress *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* in asparagus, *Verticillium dahlia* in eggplant and *F. oxysporum* f. sp. *lycopersici* Race 1 in tomato	Elmer, 2009
	Filamentous actinomycetes		
Eudrilus sp.	Free-living N_2 fixers,	Plant growth promotion by nitrification, phosphate solubilisation	Gopal et al. 2009

Vermicompost earthworm	Names of bacteria	Beneficial traits	References
	Azospirillum, *Azotobacter*, Autotrophic *Nitrosomonas*, *Nitrobacter*, Ammonifying bacteria, Phosphate solubilizers, Fluorescent pseudomonads	and plant disease suppression	
E. foetida	Proteobacteria, Bacteroidetes, Verrucomicrobia, Actinobacteria, Firmicutes	Antifungal activity against *Colletotrichum coccodes, R. solani, P. ultimum, P. capsici* and *F. moliniforme*	Yasir et al. 2009a
Unspecified	*Eiseniicola composti* YC06271^T	Antagonistic activity against *F. moniliforme*	Yasir et al. 2009b

Earthworms harbor 'nitrogen-fixing' and 'decomposer microbes' in their gut and excrete them along with nutrients in their excreta (Singleton DR, 2003). Earthworms stimulate and accelerate microbial activity by increasing the soil microbial population, number of microorganisms and biomass, by improving aeration through burrowing operations. Worm manure has diversified the original microbial community of the waste. Actinobacteria and Gammaproteobacteria are abundant in vermi-compost, while conventional compost contains a lot of Alphaproteobacteria and Bacteriodetes, typical phylogenetic groups of non-cured compost (Vivas A, 2009). Total bacterial counts exceeded 10- 10/ g of vermicompost and it included nitrobacter, azotobacter, rhizobium, phosphate solubilizers and actinomycetes. 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.

4. ROLE OF VERMICOMPOST IN PLANT GROWTH PROMOTION

Vermicompost produced from different parent material such as food waste, cattle manure, pig manure, etc., when used as a media supplement, enhanced seedling growth and development, and increased productivity of a wide variety of crops (Edwards CA, 1988). Vermicompost addition to soil-less bedding plant media enhanced germination, growth, flowering and fruiting of a wide range of green house vegetables and ornamentals (Atiyeh et al. 2000a, b, c), marigolds (Atiyeh RM, 2001), pepper, strawberries and petunias (Chamani E, 2008). Vermicompost application in the ratio of 20:1 resulted in a significant and consistent increase in plant growth in both field and greenhouse conditions, thus providing a substantial evidence that biological growth promoting factors play a key role in seed germination and plant growth . Investigations revealed that plant hormones and plant-growth regulating substances (PGRs) such as auxins, gibberellins, cytokinins, ethylene and abscisic acid are produced by microorganisms.

Earthworms produce plant growth regulators. Since earthworms increase the microbial activity by several folds they are considered as important agents which enhance the production of plant growth regulators. Plant growth stimulating substances of microbial origin were isolated from tissues of Aporrectodea longa, L. terrestris and Dendrobaena rubidus. The use of vermi compost in plant breeding promotes rooting of the plant, increasing the number of roots and biomass. The hormone-like effects of worm fertilizers on plant metabolism, growth and development causing dwarfism, root stimulation, elongation, and early flowering are believed to be due to the presence of metabolites of microorganisms. (Tomati U, 1987). Earthworm casts stimulated growth of ornamental plants and carpophore formation in Agaricus bisporus when used as casing layer in mushroom cultivation.The intestinal microorganisms of earthworms enrich the worm feces with light-sensitive and water-soluble plant growth hormones that are absorbed into humic acids in the vermi compost. . They are extremely stable and help them survive longer in the soil thereby affecting plant growth. This was confirmed by the presence of the exchangeable auxin group in the macroscopic structure of extracting humic acid from vermi-compost. Application of vermi-compost increased plant spread (10.7%), leaf area (23.1%), dry matter (20.7%) and increased total strawberry yield (32.7%).

5. CONCLUSION

Vermicompost is a low-cost, environmentally friendly waste management technology that dominates both worms and related microorganisms and has many advantages over the preferred method of composting traditional heat. Worm fertilizers are an excellent source of bio-fertilizers and their addition improves the physiological and biological properties of agricultural soils. Worm manure amplifies the diversity and populations of beneficial microbial communities. Though there are a few reports that indicate that the bacteria are less harmful. Other plant growth-promoting beneficial bacteria in vermi-compost aggravate these harms. Vermicomposts with excellent physio-chemical properties and buffering ability, fortified with all nutrients in plant available forms, antagonistic and plant growth-promoting bacteria are fantabulous organic amendments that act as a panacea for soil reclamation, enhancement of soil fertility, plant growth, and control of pathogens, pests and nematodes for sustainable agriculture.

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KHẢO SÁT SỰ ĐA DẠNG CỦA HỆ VI SINH VẬT TRONG PHÂN TRÙN QUẾ DÙNG ĐỂ SẢN XUẤT PHÂN HỮU CƠ CÓ CHỨA VI SINH VẬT

Tóm tắt: *Phân trùn quế có chứa các vi khuẩn có lợi, phân trùn quế là một loại vật liệu dạng than bùn đã được chia mịn, có độ tơi xốp cao, thông gió tốt, thoát nước, khả năng giữ nước, hoạt động của vi sinh vật, tình trạng dinh dưỡng và khả năng đệm tuyệt vời do đó cung cấp các đặc tính sinh lý cần thiết cho độ phì nhiêu của đất và sự phát triển của cây trồng. Phân giun giúp tăng cường đa dạng sinh học của đất bằng cách thúc đẩy sự xâm nhập và tăng cường sự phát triển của thực vật trực tiếp bằng cách sản xuất các hormone và enzyme điều hòa sinh trưởng thực vật. Do các đặc tính sinh học, sinh hóa và lý hóa bẩm sinh của nó, phân trùn quế có thể được sử dụng để thúc đẩy nông nghiệp bền vững và cũng để quản lý an toàn chất thải nông nghiệp, công nghiệp và sinh hoạt. Ở Việt Nam, việc sản xuất phân vermi vẫn diễn ra với quy mô nhỏ lẻ, sản phẩm từ phân vermi còn ít và chỉ được sử dụng làm cây cảnh và các loại cây trồng có giá trị kinh tế cao. Việc nghiên cứu và sản xuất phân vermi-compost để đưa vào sản xuất nông nghiệp là rất cần thiết.*

Từ khóa: *Phân trùn quế, trùn quế, vi khuẩn có lợi, phân bón sinh học.*