

Amplification of Plant Beneficial Microbial Communities During Conversion of Coconut Leaf Substrate to Vermicompost by *Eudrilus* sp.

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Received: 22 October 2008 / Accepted: 16 February 2009 / Published online: 11 March 2009
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Abstract The population densities of 15 microbial communities in the coconut leaves + cow manure mixture (10:1 ratio, w/w) and pure cow manure, gut contents of the earthworm, *Eudrilus* sp., reared on the above substrates and vermicompost produced by the worm were studied. The enumeration was done by dilution plate and most probable number method using several selective and semi-selective microbial media. In the vermicompost produced from coconut leaves + cow manure (CLV) mixture, 9 out of 15 microbial communities, particularly the plant beneficial ones, were amplified whereas five communities were amplified in case of pure cow manure (CMV). The CLV contained significantly high population of fungi, free-living nitrogen fixers, phosphate solubilizers, fluorescent pseudomonads, and silicate solubilizers. The CMV was preponderant with aerobic heterotrophic bacteria, actinomycetes, and *Trichoderma* spp. Spore formers were present in similar numbers in both the vermicomposts. Presence of *Azotobacter* was detected only in CMV. The results obtained in this study suggest coconut leaf litter to be a good alternative for cow manure for the production of vermicompost, especially in the areas where coconut is grown in plenty.

Introduction

Vermicomposting is a non-thermophilic biodegradation of organic material through interaction between earthworms

and microorganisms resulting in production of vermicompost [11]. Vermicomposts not only increase the soil fertility through the addition of plant growth hormones and increased levels of soil enzymes [6], they are also responsible for the dissemination of important microorganisms as they are rich in microbial diversity, population, and activity [18, 30]. However, the potential impact of the feeding habits of the earthworms on the microbiota of the ingested matter during gut passage and consequently on the microbial potential and activities of soil is less known [9]. There are a few publications on bacterial populations in different food resources, gut, and cast of *L. terrestris* [12, 28], but none of them address the plant beneficial microbial communities present in the vermicompost, which are very important for the improvement of soil health and fertility.

In this paper, we report the investigation on the populations of general and plant beneficial microbial communities present in coconut leaves + cow manure mixture (10:1 ratio, w/w) and pure cow manure, gut contents of the earthworm, *Eudrilus* sp., during the digestion of the substrates, and the vermicompost produced by the worm.

Materials and Methods

Substrates

The senescent leaves shed by the adult coconut palms (West Coast Tall variety) were collected from Central Plantation Crops Research Institute farm. The leaves were cut into half and mixed with fresh cow manure (obtained from Agrostology unit of the Institute) in the ratio of 10:1 on weight basis and heaped into a mound. Water was sprinkled to maintain 40–50% moisture level of the substrate, and was allowed to undergo preliminary microbial

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decomposition for 20 days. Three replicates of this substrate weighing 1000 kg each were kept in cement tanks of dimensions $7.5 \times 2.0 \times 1.0$ m (length \times width \times depth, respectively).

Fresh cow manure produced by hybrid cows (Holstein Friesian X) maintained for milking purpose at the Institute was heaped into a mound and allowed to decompose for 15 days. Three to five replicates of about 10 kg material were maintained. Three heaps that had decomposed without getting contaminated with maggots were then selected for further studies.

From these pre-decomposed substrates, 100 g sub-samples were drawn, homogenized, and analyzed for general and plant beneficial microbial communities.

Collection of Gut Contents of the Earthworms

Eudrilus sp. worms were inoculated into both the substrates after the preliminary microbial decomposition and allowed to initiate vermicomposting process [26]. Sixty days later, adult *Eudrilus* sp. weighing in the range of 1.4–1.6 g were collected, washed thrice with sterile physiological saline (0.1% NaCl) containing 0.01% Tween 80, and rinsed with 50 ml of sterile plain water. The earthworm body surface was then sterilized by brief flaming with ethanol. This protocol ensured the destruction of the microflora present on the body surface which was confirmed by plate count tests. The surface cleaned earthworms were then pinned to sterile thermocole with the ventral side up. Using a fine scalpel, the worms were dissected from the clitellum portion toward the anterior end. The midgut and hindgut portion stretching after the gizzard were dissected out. The guts were then slit and about 10 g of gut contents were collected for microflora analysis.

Vermicompost Collection

Active earthworms were collected from the vermicomposting material. The body surface of the worms was cleaned with physiological saline and sterile plain water as mentioned above, followed by their placement into plastic basins lined with three layers of moistened filter paper. The basins were covered with lid and kept in dark for an hour. The fresh coconut leaf vermicompost (CLV) and cow manure vermicompost (CMV) excreted by the worms were collected for the microflora analysis. Ten-grams of vermicompost was collected from each replication of both the substrates for microbial analysis.

Microbial Characterization

Samples of 10 g (fresh weight) of substrates/earthworm gut contents/vermicomposts were serially diluted in 90 ml

Ringers solution up to 10^{-7} dilution and 1 ml of aliquot was pour plated in different media. Three sets of samples were drawn from each treatment. Five replications for each group of microorganisms were maintained. Aerobic heterotrophic bacteria were counted on nutrient agar [1], spore forming bacteria on Luria agar after pasteurizing the materials (substrate, earthworm gut content and vermicompost) at 80°C for 10 min before plating, for fluorescent pseudomonads producing green or greenish blue fluorescence, King's B agar [19] was used and the colonies were counted under UV light, actinomycetes on Ken Knights and Munaier's agar [1], fungi on Martin's rose Bengal agar [21], free-living nitrogen fixing bacteria counted on N-free media [3], and *Trichoderma* spp. on modified TSM agar [5] distinguished by its greenish color of the spores and growth characteristics. Phosphate solubilizers, cellulose degraders, and silicate solubilizers were counted by locating the clear halo formed around the colonies on Pikovskaya agar [25], cellulose-czapek dox agar [16], and Bunt and Rovira agar media [4].

In addition, *Azospirillum* and the nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*, were estimated by Most Probable Number method [2]. The population of ammonifying bacteria was qualitatively determined using Nessler's reagent test. The results of the microbial analysis were given as CFU g^{-1} fresh weight of samples. Each CFU value was the average of three sample replicates and five plate replications per sample replicates.

Statistical analysis of the microbial populations was carried out through analysis of variance (ANOVA) [13].

Results

Population of Microbial Communities During Process of Vermicompost Production

The populations of aerobic heterotrophic bacteria, filamentous actinomycetes, fungi and spore forming bacteria were significantly higher in cow manure substrate, gut contents of *Eudrilus* sp. feeding on it when compared to same stages of vermicompost production from coconut leaf litter + cow manure substrate. The population of fungi was more in the coconut leaf vermicompost (17×10^4 cfu g^{-1} CLV compared to 6×10^4 cfu g^{-1} in CMV) while the population of spore formers was observed to increase in the gut contents of earthworm (8×10^4 cfu g^{-1} in coconut substrate and 35×10^4 cfu g^{-1} in cow manure) and then decrease in numbers in the vermicomposts (Table 1).

Among the plant beneficial microorganisms, the population of free-living nitrogen fixers, *Azotobacter* and *Azospirillum* were high in cow manure substrate and gut contents of earthworm decomposing the same. The

Table 1 Population of general microflora in different substrates, gut content of earthworms reared on these substrates, and the vermicomposts produced by the *Eudrilus* sp. (results are an average of 15 replicates and are expressed in cfu g⁻¹ of fresh sample)

Treatments	Aerobic, heterotrophic bacteria (×10 ⁶)	Filamentous actinomycetes (×10 ⁴)	Fungi (×10 ⁴)	Spore forming bacteria (×10 ⁴)
Substrate A	2.0 c	11.0 b	10.0 b	1.0 c
Gut contents A	11.0 b	4.0 c	3.0 c	8.0 b
Vermicompost A	3.0 c	4.0 c	17.0 a	1.0 c
Substrate B	21.0 a	12.0 ab	17.0 a	8.0 b
Gut contents B	19.0 a	5.0 c	12.0 b	35.0 a
Vermicompost B	10.0 b	15.0 a	6.0 c	2.0 c
CD (<i>P</i> = 0.05)	2.5	3.1	3.6	4.3

Means followed by the same letter are not significantly different at *P* = 0.05 using analysis of variance and mean separation (LSD)

Substrate A = coconut leaf + cow manure (10:1, w/w); Substrate B = cow manure; Gut A = gut content of earthworms composting substrate A; Gut B = gut content of earthworms composting substrate B; Vermicompost A = casts excreted by *Eudrilus* sp. fed on substrate A; Vermicompost B = casts excreted by *Eudrilus* sp. fed on substrate B

numbers of free-living nitrogen fixers significantly increased in the vermicompost produced from coconut leaves + cow manure mixture (15 × 10⁴ cfu g⁻¹), whereas, the *Azospirillum* count remained on par as compared to vermicompost produced from cow manure alone. The population of *Azotobacter* could not be detected in the coconut leaf vermicompost. The *Nitrosomonas* and *Nitrobacter* numbers were higher in vermicompost produced from cow manure substrate than coconut leaf + cow manure mixture. Qualitative analysis of ammonifying bacteria revealed that their activity increased during the digestion of the substrate in the gut contents and in vermicompost (Table 2).

The counts of fluorescent pseudomonads at 10 × 10⁴ cfu g⁻¹ and phosphate solubilizers at 2 × 10⁴ cfu g⁻¹ were significantly high in CLV than in CMV. However, *Trichoderma* population was present in higher numbers in CMV (6 × 10³ cfu g⁻¹) and absent in CLV. The numbers of silicate solubilizers, though quite high in CLV, was not statistically significant. Cellulose degraders were present in similar numbers in both the vermicomposts (Table 2).

Discussions

The profile and populations of the general and plant beneficial microorganisms in the vermicompost (CLV) produced by *Eudrilus* sp. reared on coconut leaf litter + cow manure substrate (10:1 ratio, w/w) was observed to

differ from that of vermicompost (CMV) produced from cow manure substrate. The microbial diversity was marginally more in CMV than in CLV. Křišťůfek et al. [20] reported that the number of bacteria, microfungi, and micromycetes increased in the guts of *Lumbricus rubellus* while Edwards and Burrows [10] reported that vermicompost was richer in fungi, bacteria, and actinomycetes compared to the soil. We noticed that the number of spore forming bacteria, mostly of *Bacillus* spp., had increased in the gut contents followed by decrease in the vermicomposts when the *Eudrilus* sp. was reared on both the substrates. This could be attributed to the fact, as reported by Pedersen and Hendriksen [24], that in the guts, there could have been an increase in the number of vegetative cells as well as germination of the spores of this bacterial community due to an increase in nutrient availability by the release of nutrients from ingested material. However, *Nitrosomonas*, P-solubilizers, and silicate solubilizers showed low count in gut contents than in vermicomposts. A plausible explanation for this could be that once the ingested material was expelled as cast from the earthworm body, an increase in the aerobic microflora would have taken place because of the increased O₂ concentrations compared to the anoxic environment in the earthworm gut.

The important groups of microorganisms beneficial for soil health, fertility and plant growth were present in abundance in the CLV. Schmidt et al. [27] reported that when food was tested as carrier and inoculum source for *Pseudomonas corrugata*, a biocontrol agent, high numbers (10⁵–10⁷ cfu g⁻¹ fresh weight) of the bacteria were recovered from fresh casts of three earthworm species. Similar results were obtained in our earlier work, where we inoculated *Metarhizium anisopliae* along with coconut leaves for the control of *Oryctes rhinoceros*, a pest which breeds in decomposing coconut leaf substrate during vermicompost production [14]. Both these reports support our observation of increased densities of fluorescent pseudomonads and other beneficial microorganisms during the production of cast from the coconut leaf substrate. However, *Azotobacter* and *Trichoderma* were not detected in the CLV but they were present in CMV. The absence of these two important plant beneficial microorganisms in CLV could be due to the negative influence of the earthworm activity. During conversion of substrate to CLV in the earthworm gut, chemical transformations take place. It is possible that *Azotobacter* and *Trichoderma* were sensitive to the chemicals produced as a result of these transformations. The population of free-living nitrogen fixers and *Azospirillum* increased in CLV to that of the substrate whereas it declined in CMV to that observed in its respective substrate. A possible reason for this could be the presence of higher concentrations of readily available nutrients in CLV than in CMV. The substrate for production

Table 2 Population of plant beneficial microbiota in different substrates, gut contents of earthworms reared on these substrates and the vermicomposts produced by the *Eudrilus* sp. (results are an average of 15 replicates and are expressed in cfu g⁻¹ of fresh sample)

Treatments	Free-living N ₂ fixers (×10 ⁴)	<i>Azotobacter</i> (×10 ³)	<i>Azospirillum</i> (×10 ⁴)	Autotrophic <i>Nitrosomonas</i> (×10 ³)	Autotrophic <i>Nitrobacter</i> (×10 ³)	Ammonifying bacteria [#]	Fluorescent pseudomonads (×10 ⁴)	Phosphate solubilizers (×10 ³)	Cellulose degraders (×10 ⁴)	Silicate solubilizers (×10 ³)	<i>Trichoderma</i> spp. (×10 ⁵)
Substrate A	7.0 bc	4.0 c	2.0 c	0.02 c	0.13 c	+	6.0 b	6.0 c	4.0 b	10.0	1.0 b
Gut contents A	3.0 c	8.0 b	1.0 c	0.26 c	0.5 c	+++	1.0 c	4.0 c	8.0 a	6.0	2.0 b
Vermicompost A	15.0 a	0.0 d	11.0 b	17.0 b	1.9 b	+++	10.0 a	20.0 b	1.0 b	16.0	0.0
Substrate B	10.0 ab	9.0 b	16.0 a	1.40 c	0.02 c	++	1.0 c	46.0 a	11.0 a	10.0	1.0 b
Gut contents B	11.0 ab	16.0 a	1.0 c	0.43 c	0.2 c	+++	1.0 c	4.0 c	1.0 b	6.0	4.0 ab
Vermicompost B	4.0 c	6.0 b	8.0 b	23.0 a	2.8 a	+++	1.0 c	4.0 c	3.0 b	8.0	6.0 a
CD (<i>P</i> = 0.05)	5.7	3.9	4.2	3.51	0.55	—	2.2	8.3	3.8	NS	3.6

[#] Qualitative estimation of ammonifying bacteria based on ammonification test using Nessler's reagent (+ indicates low, ++ indicates medium and +++ indicates high ammonification, which in turn is indicative of the extent of ammonifiers)

Means followed by the same letter are not significantly different at *P* = 0.05 using analysis of variance and mean separation (LSD)

Substrate A = coconut leaf + cow manure (10:1, w/w); Gut contents A = gut contents of earthworms composting substrate A; Vermicompost A = vermicompost excreted by *Eudrilus* sp. reared on substrate A; Substrate B = cow manure; Gut contents B = gut contents of earthworms composting substrate B; Vermicompost B = vermicompost excreted by *Eudrilus* sp. reared on substrate B

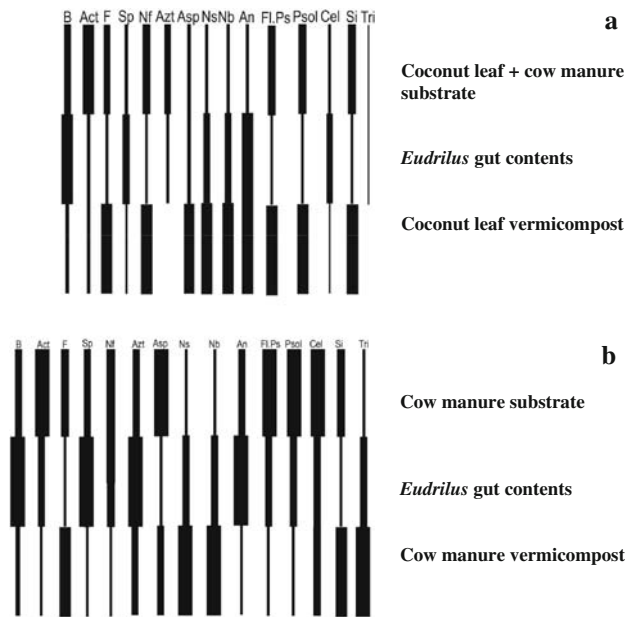


Fig. 1 Qualitative bar coding microbial dynamics during the production of coconut leaf + cow manure vermicompost (CLV) (a) and cow manure vermicompost (CMV) (b) from substrate to earthworm gut contents and in vermicompost (The acronym details: B–bacteria, Act–actinomycetes, F–fungi, Sp–spore formers, Nf–free-living N-fixers, Azt–*Azotobacter*, Asp–*Azospirillum*, Ns–*Nitrosomonas*, Nb–*Nitrobacter*, An–Ammonifiers, Fl Ps–fluorescent pseudomonads, Cel–cellulose degraders, Si–silicate solubilizers, Tri–*Trichoderma*)

of CMV was cow manure. This substrate was already a digested product from the ruminant cattle. It again went through digestion in earthworm's gut before coming out as CMV. This double digestion perhaps led to higher depletion of nutrients. As compared to this, the substrate for production of CLV was coconut leaves amended with cow manure in 10:1 (w/w) ratio. The bulk of this substrate underwent digestion only once, i.e., in earthworm's gut, thereby, retaining more nutrients than CMV. The predominance of microflora involved in N-cycle, chiefly free-living nitrogen fixers, microaerophilic *Azospirillum* sp., and ammonifying bacteria would have added to the ammonium content in the fresh CLV through their di-nitrogen fixing and organic nitrogen mineralization activity. On the other hand, existence of strong population of autotrophic nitrifiers (*Nitrosomonas* and *Nitrobacter*) cause rapid nitrification which results in stable levels of both nitrogen forms (NH_4^+ and NO_3^-) due to organic matter protection in dry casts [7]. The increased occurrence of phosphate solubilizers, fluorescent pseudomonads, cellulose degraders, and silicate solubilizers in CLV could reflect on the greater dissipation of available phosphorus and other nutrients [8] and plant growth promoting substances [31] in vermicomposts. Thus, out of 15 groups of microbial communities that were enumerated, the populations of nine were amplified in the CLV when compared to their populations in the coconut

leaf + cow manure substrate. However, *Azotobacter* and *Trichoderma* were not detected in CLV (Fig. 1a). When cow manure was the substrate, population of five out of 15 groups of microorganisms increased in the CMV and all the 15 groups were present (Fig. 1b). The microbial dynamics from the substrate to the earthworm gut and finally in the vermicompost differ because of specific nutritional interactions and requirement of the earthworms that feed on the microorganisms [15], feed quality [29], differential microbial survival in gut depending on their availability to use or withstand the various substances and substrate found in the gut [22, 23], and the gut load and gut transit time [17]. Though we observed that as many as six groups of microorganisms in coconut leaf + cow manure substrate and 10 out of 15 in plain cow manure substrate were in high populations, these microorganisms would have undergone a dissimilar fate while transiting through the gut of *Eudrilus* sp., giving rise to differences in microbial profiles of the CLV and CMV. This is because the food sources differ not only in their chemical composition but also in their microbiota, which influence the microbial communities in the gut contents of earthworms feeding on those, and eventually that of the earthworm cast. Similar results have been reported in many of the earlier studies [32].

Conclusions

Through the present study, we conclude that there was an amplification of general and plant beneficial microorganisms when coconut leaf litter [amended with 10% (w/w) cow manure] was converted to vermicompost by the indigenous strain of *Eudrilus* sp. The microbial profile and densities were modulated while transiting through the gut of *Eudrilus* sp., which in turn was influenced by the microorganisms present in the substrates.

Acknowledgments The authors are grateful to the anonymous reviewers for their constructive comments on the ms.

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