ISSN - 2250-1991 | IF : 5.215 | IC Value : 77.65

Original Research Paper Agriculutural Science

A Study on Microbial Fingerprinting of Different Organic Samples in Vermicompost

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ABSTRACT

Environmental degradation is a major threat confronting the world, and the rampant use of chemical fertilizers contributes largely to the deterioration of the environment through depletion of fossil fuels, generation of carbon dioxide and contamination of water resources. It leads to loss of soil fertility due to imbalanced use of fertilizers that has adversely impacted agricultural productivity and causes soil degradation. Now there is a growing realization that the adoption of ecological and sustainable farming practices can only reverse the declining trend in the global productivity and environment protection. In nature's laboratory there are a number of organisms that have the ability to convert organic waste into valuable resources containing plant nutrients and organic matter, which are critical for maintaining soil productivity. Microorganisms and earthworms are important biological organisms helping nature to maintain nutrient flows from one system to another and also minimize environmental degradation. This clearly indicates that earthworm population decreases with soil degradation and thus can be used as a sensitive indicator of soil degradation.

The present study intended to isolate and identify the bacteria present in four different samples such as cowdung, cowdung compost, slurry, slurry compost. The physiochemical parameters of all the samples and the surface morphology of them were also analysed.

KEYWORDS Fossil fuel, fertility, earth worms, cow dung, slurry

INTRODUCTION

Solid wastes management is the most chaotic challenge today. Because, every year nearly 55 million metric tonnes of solid wastes are being generated in Indian cities. The industries also add larger volume of both solid and liquid wastes annually. The volume and weight of these solid wastes are increasing rapidly. Most of the wastes that are created are dumped into land without prior treatment. These wastes emit greenhouse gases like methane and carbon dioxide thus add global warming. However any organic wastes can be degraded resulting in usable organic manure. For that the generated wastes ought to be classified and suitable method of decomposition need to be suggested. The simplest and quickest method is needed to make compost out of organic and biodegradable wastes. All the wastes are required to be segregated and treated effectively for conversion. Economical consideration and value added reuse are the key components in deciding the sustenance of such disposal procedures.

Vermicompost is an age old practice naturally evolved as evident from the presence of earthworms in soils where in higher amount of organic and plant wastes are added. Vermicomposting is a suitable system for studying microbe earthworm interactions. Earthworms are popularly known as the farmers' friend or natures' plowman. They influences microbial community, physical and chemical properties of soil. The primary decomposers of organic matter are microorganisms. Microbial activity in the earthworms gut, cast and soil is very essential for the breakdown and release of nutrients in available form to plants. The microorganisms and earthworms act symbiotically to accelerate and enhance the decomposition of organic matter.

Vermicomposting is totally a bioconversion process, requiring no additional inputs except the earthworms and little of water to keep the source of materials in moist condition. It has been proved effective for converting various wastes including animal, plant, pharmaceutical, food waste and sewage. It normally takes 30 to 90 days depending upon the type and stage if these wastes. The products out of vermicomposting are vermicompost and vermiwash. They are rich in nitrogen, phosphorus and potassium. Vermicomposting methods range from a bin for household wastes to large sized beds or tanks for accommodating tons of organic materials on a continuous basis. In general ***Eisenia fetida*** (red earthworm) ***Eudrilus eugeniae*** (African) and ***Perionyx exavatus*** are used largely. Earthworms convert the smelly organic matter into a dark, odourless, homogenous material called castings or vermicast. Microbial activity is stimulated by favorable conditions like moisture content, pH and high concentration of mucus in the anterior part of the gut, in the midgut this enhanced microbial activity results in the digestion of soil organic matter and the digestion are partially absorbed in the posterior part of the gut. Epigeic species which consume considerable amounts of raw organic matter have a range of enzymatic activities, probably mainly originating from ingested microflora. For instance the presence of fungal endophytes substantially increased the nutritional value of grass leaves for ***E. fetida*** (Humphries et al. 2001). Soil, is the soul of infinite life that promotes diverse microflora. Soil bacteria viz., ***Bacillus, Pseudomonas*** and ***Streptomyces*** etc., are prolific producers of secondary metabolites which act against numerous coexisting phytopathogeic fungi and human pathogenic bacteria (Pathma et al. 2011). Soil, the major reservoir of microbes, meets the food requirement of earthworms and this has necessitated the establishment of different kinds of relationship between earthworms and microbes.

They are:

- microbes form a part of food for earthworm,
- microbes are proliferated in the gut and vermicomposts,
- earthworm help in the distribution of microbes and
- together with earthworm microbes mineralise, humifies organic matter etc facilitates chelation (Lavelle et al. 1998; Parthasarathi and Ranganathan, 1998; Canellas et al. 2002).

Thanjavur districts administration has initiated the task of solid waste management with the active participation of various Government agencies, educational institutions, business houses, volunteers and the scheme named as "**Clean green and hygienic Thanjavur Movement**" They are performing more attempts to segregate the solid wastes at the site as a policy and scientific disposal of degradable and organic wastes through biochemical processes compiled with making of vermicomposts. The main objective of the present study was to assess the microorganisms of substrates that the earthworms ingest. The present study aims at the profiling the above mentioned changes in vermicomposts produced at one of the treatment areas at thanjavur, with respect to specific methods of treatments namely biomethanisation and vermicomposting. The study area was chosen at Periyar Maniammai University, Thanjavur and different types of vermicomposts generation were taken for microbial profiling with the following objectives,

- Collection of samples from cow dung, cow dung compost, slurry and slurry compost.
- Isolation and identification of microorganisms.
- Organic analysis of soil samples.
- Scanning Electron Microscope analysis of sample.

METHODS AND METERIALS

Study area

This present was conducted at Periyar Manniammai University, Thanjavur, where vermicomposting project are going on very effectively.

Preparation of vermicompost

The digestion of organic wastes into compost by using earthworms it known as vermicomposting. Pits were made in shade. Bricks, stones and tiles were piled at the bottom of pit to a height of 5cms. Earthworms were inoculated into the stone bed. The slurry was made in fruits, vegetable waste, human waste, and bathroom wastes. Then fresh cow dung and slurry was separately pited. It was piled on the stone bed to a height of 5cms and dried leaves were spread on the cow dung. The dry leaves were then wetted by sprinkling enough water. After 4 weeks wet organic wastes were spread on the first pile to about 5cms height. Then it was repeated many times at regular intervals of 4 days still it reached the ground level. The pile was covered with coconut leaves.

Isolation of bacterial isolates

Glass wares were soaked in chromic acid solution (10% potassium dichromate in 25% sulfuric acid) for few hours and washed thoroughly followed by detergent solution. Glass wares were again washed with tap water and dried in the hot air oven. 1gm of soil samples from vermi pits were taken and mixed in 10 ml distilled water in test tubes. Then serially diluted and were spread on a nutrient agar medium (peptone-5g,sodium chloride-5g, yeast extract -1.5g ,beef extract-1.5g, agar-15g/L pH-7.2) and incubated at 37^{0}C for 2 days.

Identification and characterization of marine bacterial isolates

The tests were undertaken to identify and characterize bacterial isolates according to procedure described and the biochemical test were also analyzed. Morphological analyses of the colony of bacterial isolates were studied based on the color, shape, size and margin of the colonies. Morphological tests were done by the standard procedures given by Barthalomew and Mittewer (1950).

Gram staining

The colonies were stained by staining method, in order to identify the morphology and gram's reaction of the bacterium. A thin smear was prepared on a clean slide using the isolated individual colony. The smear was heated fixed and cooled. The dried smear was then flooded with the primary stain crystal violet solution and allowed to stand for 1 minute. The slide was then washed with water and decolorized with 75% ethanol for few seconds and washed gently with running tap water then the slide was flooded with a counter stain safranin for 1 minutes. After drying, the stained smear was observed under the microscope to identify the organism.

Biochemical tests

Biochemical tests were carried out as the method given by Cappuccino and Sherman (1992) with 24 hr. old cultures.

Physico-chemical analysis of organic soil samples

The physico-chemical analysis of organic soil samples such as cow dung, cow dung compost, slurry, and slurry compost was done. The pH of the suspension was read using pH meter (Systronics, India), to find out the soil pH. Electrical conductivity of soil was determined in the filtrate of the water extract using Conductivity Bridge and Cation exchange capacity (CEC) of the soil was determined by using 1 N ammonium acetate solution as described by Jackson (1973).Organic carbon content was determined by adopting chromic acid wet digestion method as described by Walkley and Black (1934).

Available nitrogen was estimated by alkaline permanganate method as described by Subbiah and Asija (1956) and available phosphorus by Brayl method as described by Bray and Kutz (1945). Available potassium was extracted from soil with neutral 1 N ammonium acetate (1:5) and the potassium content in the extract was determined by using flame photometer, calcium (Neutral 1 N NH_4 OAC extractable 1:5) was extracted with neutral 1 N ammonium acetate and the available calcium in the extract was determined by Versenate method (Jackson, 1973).

Other nutrient based parameters i.e. available phosphate and total nitrogen were estimated using standard methods of APHA (1987). Available micronutrients such as Zn, Cu and Mn were determined in the diethylene triamine pentaacetic extract of soil using Perkin-Elmer model 2280 Atomic Absorption Spectrophotometer (Lindsay and Norvell, 1978). Other nutrients such as magnesium, sodium and available iron were analyzed following the method of Muthuvel and Udayasoorian (1999). The reagents used for the analysis were AR grade and double distilled water was used for the preparation of solutions.

Analysis of vermicompost samples by Scanning Electron Microscope

The scanning electron microscope analysis of vermicompost samples were carried out to identify the surface morphology. The samples were dried overnight at 80^{o}c in hot air over. The dried samples were grinded into fine particles and it was observed under scanning electron microscope.

RESULTS AND DISCUSSION

Preparation of vermicompost pit

In the present investigation**,** the prepared vermin pit showed 2 -5 folds increased body weight as they were treated with feed daily Fig.1,2,3&4 They released their excreta in the waste. The excreta was observed readily soluble in water. As a result a good quality vermicompost was formed within 25 - 45 days.

Fig.1: Sample 1- Cow dung and Earthworms

Fig.2 Sample 2- Cow dung slurry and Earthworms

Fig.3 Sample 3- Slurry and Earthworms

Fig.4 Sample 4- Slurry compost and Earthworms

Isolation and identification of bacterial isolates

In the present study, effort was made to isolate distinct bacterial isolates from different samples mentioned above. Pure colonies were obtained by sub culturing for more than three times. The pure culture plates and staining results of all 4 bacterial isolates were studied. Determination of bacterial diversity showed the predominance of 4 bacterial isolates in all samples.

Biochemical tests

The gram-staining has been carried out to classify the isolated strains into two basic groups as gram positive and gram negative. Based on microscopic and biochemical observations with the reference on Bergeys manual, 4 bacteria were isolated and identified [Table 1]

Table 1: Observation of Bacterial isolates in the samples

S.No.	samples				Name of the bacterial Isolate
1	C1	C2	S1	S2	
2	N	N	N	P	E.Coli
3	N	N	N	P	E.Coli
4	P	P	P	P	Enterobacter aerogenes Klebsiella pnemoniae
5	P	P	P	P	Enterobacter aerogenes Klebsiella pnemoniae Pseudomonas fluorescence

MR : Methyl red ; VP: Vogus proskaures ; += Positive; - = Negative

Based on the nutrients and moisture presence of each bacterial isolates, ***Escherichia coli***, ***Klebsiella pneumonia***, ***Enterobacter aerogenes,*** and ***Pseudomonas fluorescence*** were deliberated to be common as they were existing in all samples.

Coliforms are present in large numbers in the intestinal flora of most warm-blooded animals, and therefore their presence in the environment is associated with sources of fecal contamination. Because of this, they are used as indicators of the potential presence of enteropathogens in water and soil environments (Sidhu J. P. S., Toze S. G, 2009). Although coliforms may also be found in the soil environment as part of the native microflora (Byappanahalli et al. 1998), their screening is of special relevance in vermicompost produced from animal manures.

In the present investigation the effect of ***E. coli*** & agar on the survival of earthworms, the counts of earthworms taken at regular intervals were analyzed. Highest number of earthworms was observed in the system with ***E coli*** as observed by Kalpita mulye et al. (2015). Thus, ***E coli*** culture is not having any detrimental effect on the growth of earthworms. Thus, vermicomposting can be used as an effective environment friendly strategy for disposal of agar waste containing ***E. coli*** and enterobactericeae family.

Physico-chemical analysis of samples

The result of the physico-chemical analysis of the four samples for pH, electric conductivity, organic matter contents, macronutrients and micronutrients values was shown in Table 2. Among the soil physico-chemical parameters pH for all samples were same i.e alkaline in nature, Electrical conductivity was found more in cow dung than other samples. EC are usually found to have greater exchangeable K, calcium (Ca), and magnesium (Mg) contents than bulk soil (Edwards et al. 1996; Mariani et al. 2007). This was also confirmed by Teng et al. (2012) who examined the physical, chemical and biological properties of composts produced by endogeic species ***Metaphire tschiliensis tschiliensis*** in clay soil incubated in the dark for two weeks.

The pH of the compost is important, as the application of compost to the soil can alter the soil pH, which in turn affects the availability of nutrients to the plants. Worms can survive in a pH of 5-9. Vermicompost worked by both worms tolerated a pH range of 6-7, and both species was comfortable to breed in the wastes. Earthworms by passing through the soil and organic matter gradually make acid soil less acidic and alkaline soil less alkaline (Ndegwa, P.M., & Thompson, S.A.2000). Electrical conductivity represents the amount of soluble metals ions present in the vermicompost, can be utilized by the plants.

The organic carbon presence was more in slurry and slurry compost. It was observed that the percentage of organic matter was found to be more in slurry and comparatively same for all other samples. In 1881, Darwin was one of the first scientists who noted that the topsoil consisted mostly of earthworm castings, thus highlighting the importance of earthworms in pedogenesis processes (soil organo-mineral complex). For example, the earthworm population builds galleries and ingests large quantities of organic and mineral matter, thus modifying the porosity and aggregation of the soil. This earthworm bioturbation may subsequently be reflected in soil profiles (Zhang et al. 1995), for example: soil profile disturbance, soil structure modification, and vertical and horizontal redistribution of soil and organic matter. This redistribution of organic matter depends on the earthworm ecological groups. Endogeic earthworms keep moving inside the soil to feed on soil organic matter while anecic ones feed on plant litter and organic residues at the soil surface and tend to stay in the same burrow (Lavelle et al.1997).

In case of availability of macro nutrients, nitrogen content was more in slurry followed by cow dung and available phosphorus was observed high in cow dung, in case of potassium it was observed more in cow dung but more or less same to all samples. Cortez et al. (2000) reported that the presence of earthworms whatever the ecological category, increased the quantity of inorganic N in the organic samples. This was caused by enhanced mineralization of N forms, both of a N-labelled residue and that of the soil organic matter. Earthworms can impact plant growth by promoting N-availability (Li et al. 2002; Ortiz-Ceballos et al. 2007). Vermicasts have higher waste exchange capacity, total exchangeable potassium, manganese and calcium. This increase may be due to the breeding activity of earthworms and the added effect of the cattle manure that adds to the nutrient mineral content of the soil.

The study of available micro nutrients revealed that the presence of zinc was high in cow dung followed by slurry compost and almost same for all the samples. In cow dung slurry, copper was found to be high. Likewise it was same for the availability of iron. i.e more in cow dung and cow dung slurry. The presence of nickel, cobalt, zinc, manganese and cadmium were found to be relatively same for all the samples. Copper was high in cow dung slurry. Earthworms play an important role in the process of soil formation and soil aggregation, mainly through the production of casts.

Edwards and Bohlen (1996) reported that earthworm cast contains more water stable aggregate than the surrounding soil. Earlier studies have shown that vermicast appears to be enriched with polysaccharides which act in the soil as cementing substances causing aggregate stability, contributing to create and maintain the soil structure and causing better aeration, water retention, drainage and aerobic conditions, very useful for root development and nutrient availability to plants (Tomati and Galli, 1995).In the present study, it was observed that microbial population and the earth worm population were highly influenced by physio-chemical parameters of organic soil [Table 2].

Table 2. Analytical Report of Organic Samples

S. No.	Name of the parameter	ORGANIC SAMPLES			
		S1	S2	S3	S4
1.	pH	7.45	7.36	7.77	7.62
2.	Electrical conductivity (dsm^{-1})	1.72	0.91	0.44	0.83
3.	Organic Carbon (%)	7.54	7.69	8.26	8.10
4.	Organic Matter (%)	15.08	15.38	16.52	15.48
5.	Total Nitrogen (%)	1.69	1.54	1.87	1.55
6.	Total Phosphorus (%)	0.52	0.48	0.49	0.42
7.	Total Potassium (%)	2.35	2.65	2.34	2.48
8.	Total Sodium (%)	0.45	0.54	0.52	0.42
9.	Total Calcium (%)	3.19	3.16	2.95	3.08
10.	Total Magnesium (%)	2.03	2.16	2.05	2.08
11.	Total Sulphur (%)	0.26	0.24	0.21	0.25
12.	Total Zinc (ppm)	2.64	2.54	2.59	2.61
13.	Total Copper (ppm)	1.09	1.24	1.08	1.16
14.	Total Iron (ppm)	18.64	18.35	7.98	17.69
15.	Total Manganese (ppm)	4.59	4.51	4.67	4.48
16.	Total Chromium (ppm)	0.59	0.54	0.64	0.62
17.	Total Nickel (ppm)	0.05	0.06	0.08	0.06
18.	Total Cobalt (ppm)	0.32	0.28	0.28	0.29
19.	Total Cadmium (ppm)	0.13	0.12	0.16	0.14

Surface analysis by Scanning Electron Microscope

Vermicompost was produced from four different wastes inoculated with the earthworm *Eisenia fetida*. Scanning electron microscopy analysis was used to investigate the changes in surface morphology. SEM analysis of all four samples shows more fragment and pores. Fig.5& 6

Fig.5 SEM analysis of samples C1& C2

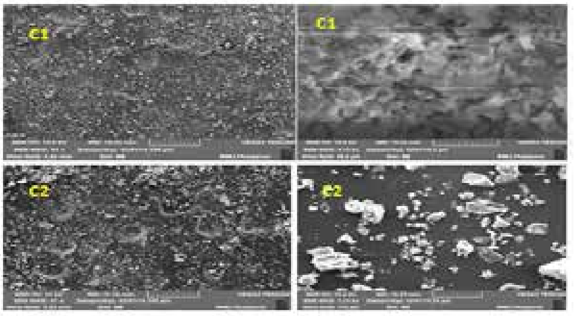
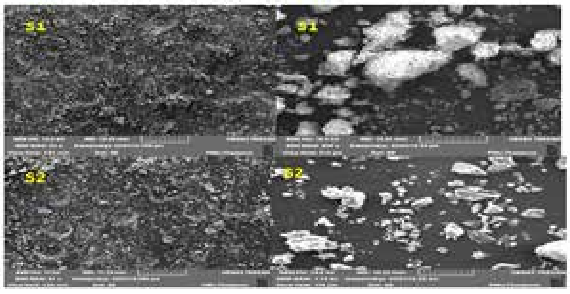


Fig.6 SEM analysis of samples S1 & S2



CONCLUSION

The production of degradable organic waste and its safe disposal becomes the current global problem. Meanwhile the rejuvenation of degraded soils by protecting topsoil and sustainability of productive soils is a major concern at the international level. Provision of a sustainable environment in the soil by amending with good quality organic soil additives enhances the water holding capacity and nutrient supplying capacity of soil and also the development of resistance in plants to pests and diseases. By reducing the time of humification process and by evolving the methods to minimize the loss of nutrients during the course of decomposition, the fantasy becomes fact. Earthworms can serve as tools to facilitate these functions. They serve as "nature's plowman" and form nature's gift to produce good humus, which is the most precious material to fulfill the nutritional needs of crops. So, it is a requisite to isolate and identify diversified microbial population in various combinations of organic wastes to improve the quality of soil and quantity of crop yield as organic farming today. The utilization of vermicompost results in several benefits to farmers, industries, environment and overall national economy.

To farmers:

- Less reliance on purchased inputs of nutrients leading to lower cost of production
- Increased soil productivity through improved soil quality
- Better quantity and quality of crops
- For landless people provides additional source of income generation

To environment:

- Wastes create no pollution, as they become valuable raw materials for enhancing soil fertility

To national economy:

- Boost to rural economy
- Savings in purchased inputs
- Less wasteland formation

Acknowledgement:

We thank TBI of Periyar Manniammai University, Thanjavur, Tamil Nadu to carry out this project successfully.

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