Evaluation of Microbiological Quality of Vermicompost Prepared from Different Types of Organic Wastes using *Eisenia fetida*

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**ABSTRACT**

**Background:** Vermicomposting is the agricultural technique of conversion of organic wastes to a fertile product, which can result in better crop growth and production. However, even though earthworms are the main organisms participating in the process, the microbes associated with it also have an important role to play. These microbes degrade the waste products biochemically and are responsible of the conversion processes. Few studies are carried out on microbial diversity and related enzymes activities in the vermicompost prepared from different organic waste materials.

**Methods:** In this paper, we isolated both bacteria and fungi from seven different types of vermicompost, using different selective media. We also studied the activity of hydrolytic enzymes that are associated with the isolated microbes.

**Result:** It was observed that bacteria like *Bacillus sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Staphylococcus aureus*, *Streptococcus*, *Micrococcus*, *Actinomyces*, Pigment producing *Streptomyces*, Azotobactor and fungi like *Penicillium purpurogenum*, *Aspergillus sp.*, *Alternaria alternata*, *Fusarium solani*, *Rhizopus sp.*, *Mucor hiemalis*, *Myrothecium verrucaria* etc. were present in our vermicompost preparations. The presence of nitrogen fixing bacteria, phosphate solubilizing microorganisms and PGPR indicated the good fertilizer value of the vermicompost samples. It was also observed that the diversity of microbes present supported significant levels of CMCase Exoglucanase, Xylanase, β-Glucosidase, Phosphatase and Urease activities.

**Key words:** Earthworms, Enzymes, Microbial diversity, Organic wastes, Symbiosis, Vermicomposting.

**INTRODUCTION**

Vermiculture is the technology of producing vermicompost using earthworms from the agro-wastes. It is simple and useful and thus has been proposed to be one of the front runner technologies of the second green revolution, as it ensures sustainability of agriculture (Sinha et al, 2010; Chaudhary et al, 2004). Vermicomposting refers to the biooxidation and stabilization of organic matter, involving the synergistic actions of earthworms and microorganisms, thereby turning wastes into a valuable soil amendment called vermicompost. Earthworms are directly or indirectly involved in biodegradation of organic wastes, in association with bacteria, actinomycetes, fungi, yeasts, etc., which give rise to humus formation (Jeevanrao and Ramalakshmi, 2002; Lavelle and Spain 2001; Munnoli 2007). While earthworms fragment and condition the substrate, increase the surface area for microbial growth and alter its biological activity, the microorganisms are known for the biochemical degradation of organic waste matter (Dominguez et al, 2010).

Vermicomposting converts the original waste materials to give a final product free of pollutants and of greater economic value (Lakshmi et al, 2014; Munnoli, 2015). Studies on the microbes associated with earthworms show that these microbes are directly or indirectly involved in the decomposition of organic matter and soil stabilization. These processes are associated based on symbiotic relationships between the microorganisms and earthworms in the earthworm gut, burrows, casts and pasture land. It is known that microbial biomass is greater in casts of earthworms than in parent soil (Airà et al, 2003; Munnoli, 2007; Tiunov and Scheu, 2000; Agarwal and Arora, 2011).

High numbers of microbes were seen in plots treated with vermicompost than in untreated plots, such as nitrogen-fixing bacteria. Higher microbial load was also observed in vermicompost treated paddy fields (Kale et al, 1992; Barik et al, 2010). An increase in the microbial load with potato waste was recorded using leaves of the tree *Paulownia elongata* and press mud waste using earthworms like *Megacolex megascoleus*, *Eisenia fetida* and *E. eugeniae* on comparison with the surrounding soil (Munnoli, 2007; Sarma et al, 2012).

The different enzyme activities present in vermicompost that play a major role in the composting process are cellulases, phosphatas and ureases. These enzymes help...
Microbes to utilize different carbon and nitrogen sources from biodegradable waste, while converting them to vermicompost by earthworms. It has also been reported that the enzyme activity and microbial counts in vermicompost samples are related (Singh et al, 2013). However little information is available on microbial diversity and its relation with the enzyme activities in the vermicompost prepared from different organic wastes (Gilanjali, 2007).

In this paper, our objectives are: 1. To study the microbial quality of the different vermicompost samples prepared from different organic wastes, by plating them on various nutrient media and 2. To study the activities of different microbial hydrolytic enzymes such as Carboxymethyl Cellulase (CMCase), Exoglanucanase, Xylanase, β-Glucosidase, Phosphatase and Urease present in these vermicompost samples.

**Materials and Methods**

All the vermicompost samples produced using different substrates (rice straw, grass, orange peel, sugarcane bagasse, neem leaves, wheat straw and sawdust) in the small scale experiment in pots on trial basis and then grown in tanks. Then, the samples were analyzed for their microbial composition and their hydrolytic enzymes activities. The vermicompost was prepared from late June to mid September, 2018, during monsoon season in the premises of Institute of Home Economics, Hauz Khas, New Delhi, India (Mean temp 30-35°C, humidity 70-75%). The steps were as follows:

**Collection of organic wastes**

The locally available organic wastes selected for this study were rice straw, grass, saw dust, wheat straw, sugarcane bagasse, orange peel and neem leaves.

**Preparation of tank for rearing Eisenia fetida**

The tank was prepared in the dimensions of 100x40x40cm in length, width and height using concrete, cement, stones as materials for construction on sides but not on floors. The vermicomposting set up prepared for 15 days by sprinkling water every 2-3 days. After adding 5 kg substrate it was covered with big dried leaves and wire mesh. The composting mixture was allowed to stand for 15 days by sprinkling water every 2-3 days. After turning, further incubation is allowed till another 15 days. A set up of organic wastes without earthworms was taken as control.

**Experimental Setup for the preparation of Vermicompost**

In this, cemented pots of sizes 16 inch height x 14 inch diameter, each of capacity 10kg, with small holes at the bottom were used. Small pebbles were placed in these pots followed by spreading a shallow layer of sand of approximately 1 cm. On this layer, 1 kg cow dung was added and then 40 earthworms were introduced in each pot. After adding 5 kg substrate it was covered with big dried leaves and wire mesh. The composting mixture was allowed to stand for 15 days by sprinkling water every 2-3 days. After turning, further incubation is allowed till another 15 days. A set up of organic wastes without earthworms was taken as control.

**2. Pit experiment**

Pits of sizes 1m length x 80cm width x 20cm depth, made of bricks, concrete and cement on four sides but not on floors were constructed. The vermicomposting set up prepared by putting one inch layer of pebble at the bottom and then spreading a shallow layer of sand and 4 kg cow dung layer, earthworm 2 kg over cow dung layer and finally 20 kg substrate. The seven substrates were used in duplicates namely grass, wheat straw, sawdust, neem leaves, sugarcane bagasse, orange peel and rice straw. The contents were covered with a framed wire mesh. Turning was done after 2 weeks and water was added regularly to avoid drying up of the biomass.

**Harvesting compost**

Vermicompost was ready after sixty days. The worms were harvested and vermicompost samples were allowed to dry for three days. The samples were then collected, sieved and carefully packed in air tight plastic bags. These were stored in a refrigerator and samples were used for microbial and enzymatic analysis.

**Analysis of microbiological quality of vermicompost samples**

The media used to determine the microbiological quality of samples were Nutrient Agar, Yeast Mannitol Agar, Potato Dextrose agar, King’s Media (base B), Jensen Media, Pikovyskaya’s Media, Cellulose Agar Media, Starch Casein Agar, Azosprillum Media. The most commonly isolated fungal strains were sent for identification at Indian Type Culture Collection, IARI.

**Analysis of enzyme activity**

The vermicompost samples were analyzed for the activities of Endoglucanase (Carboxymethyl Cellulase or CMC assay) Exoglanucanase (Filter paper assay), Xylanase, β-Glucosidase, Phosphatase and Urease. Enzyme activity of the samples was estimated using live, heat killed enzyme, buffer solutions and respective substrates.

**Results and Discussion**

Analysis of microbial quality of vermicompost samples

Various kinds of microorganisms were found in the different samples, as shown in Fig 1-5. Some of the microbes which were identified include:

**Bacterial colonies**

*Bacillus sp.*, *Pseudomonas sp.*, *Klebsiella sp.*, *Staphylococcus aureus*, *Streptococcus*, *Micrococcus*, *Actinomycetes*, *Pigment* producing *Actinomycetes*, *Streptomyces* and *Azotobacter*. The presence of nitrogen fixers like *Azotobacter*, *Klebsiella* and phosphate solubilizers like *Bacillus sp* and PGPR like *Pseudomonas sp.* confirmed the good nutritious quality of the vermicompost samples prepared from the different organic wastes and therefore may be successfully used to increase soil fertility.
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The commonly isolated fungi from vermicompost samples were identified at Indian type culture collection center IARI New Delhi, as *Penicillium purpurogenum*, *Aspergillus niger*, *Alternaria alternata*, *Fusarium solani*, *Rhizopus sp.*, *Mucor hiemalis*, *Myrothecium verrucaria* etc.

Microbial variation in the vermicompost was 10 to 20 times higher than in control substrate samples without earthworms. Similar reports on increase in microbial load in soil treated with vermicompost were given by other investigators (Ghilarov, 1963; Munnoli, 1998, Aira et al., 2003). The presence of bacteria like *Bacillus sp.*, *Azotobacter sp* and *Klebsiella sp* and fungi such as *Aspergillus sp* and *Penicillium sp* were also reported by other researchers (Ilanjiam et al., 2019).

**Fungal colonies**

Microbial variation in the vermicompost was 10 to 20 times higher than in control substrate samples without earthworms. Similar reports on increase in microbial load in soil treated with vermicompost were given by other investigators (Ghilarov, 1963; Munnoli, 1998, Aira et al., 2003). The presence of bacteria like *Bacillus sp.*, *Azotobacter sp* and *Klebsiella sp* and fungi such as *Aspergillus sp* and *Penicillium sp* were also reported by other researchers (Ilanjiam et al., 2019).

**Analysis of enzyme activity**

The vermicompost sample prepared from rice straw showed
maximum CMCase activity (5.1µmol/g/h) followed by orange peels (3.4µmol/g/h), grass (2.8µmol/g/h), sawdust (2.6µmol/g/h), neem leaves (2.2µmol/g/h), wheat straw (2µmol/g/h) and sugarcane bagasse (1.6µmol/g/h).

The Exogluccanase activity was measured highest in vermicompost of grass (2.7µmol/g/h), followed by orange peel (1.4µmol/g/h), wheat straw (1.2µmol/g/h), sawdust (0.8µmol/g/h), rice straw (0.6µmol/g/h) and neem leaves (0.4µmol/g/h).

Xylanase activity was recorded maximum in rice straw vermicompost (8.6µmol/g/h), followed by those made from sawdust (3.3µmol/g/h), grass (2.9µmol/g/h), orange peels (2.7µmol/g/h), wheat straw (1.6µmol/g/h), neem leaves (1.6µmol/g/h) and sugarcane bagasse (1.3µmol/g/h).

β-Glucosidase activity was highest in vermicompost obtained from rice straw (0.2µ mol/g/h), followed by that obtained from orange peel (0.1µ mol/g/h), neem leaves (0.08µ mol/g/h), wheat straw (0.06µmol/g/h) and lowest in sugarcane bagasse (0.02µmol/g/h).

In all cases, the Control organic waste sample showed negligible enzyme activity. The results on cellulase and xylanase activities show that the microbes obtained from vermicompost are able to hydrolyze plant based substrates through these enzymes. Similar results were reported for vermicompost samples by other researchers (Karthika et al., 2020; Chatterjee et al., 2020). Table 1 shows the activity of different hydrolytic enzymes present in vermicompost prepared from different organic wastes.

Vermicompost obtained from orange peel (0.6µmol/g/h) showed highest Phosphatase activity and the overall Phosphatase activity among the seven samples ranged from 0.2 to 0.6 µmol/g/h. This indicates the presence of phosphate solubilizers in the vermicompost samples. This is advantageous, as these microbes solubilize inorganic phosphates present in the soil and make them readily available for plants (Balachandar et al., 2020). The presence of phosphate solubilizers was also confirmed by microbial colonies on phosphate containing media. Presence of phosphatase activity in vermicompost was also confirmed by other researchers (Biruntha et al., 2020; Balachandhar et al., 2020; Karmegam et al., 2019). Urease activity obtained was in the range of 0.3 to 1.0 µmol/g/h, with the maximum activity shown by rice straw vermicompost. The presence of Urease activity indicates the presence of microbes associated with the Nitrogen cycle, allowing more Nitrogen to be available to growing plants from the soil. Urease activity in vermicompost was shown by other investigators also (Karmegam et al, 2019; Sudkolai and Nourbakhsh, 2017).

**CONCLUSION**

Several reports state that during vermicomposting there is an increase in the microbial count, but few studies have been done on the presence of microbial diversity and related enzyme activities in the vermicompost samples obtained from different organic wastes. In this study, we isolated and identified certain bacteria, like Bacillus sp., Pseudomonas sp., Klebsiella sp., Staphylococcus aureus, Streptococcus, Micrococcus, Actinomycetes, Pigment producing Actinomycetes, Streptomyces, Azotobactor, as well as fungi like Penicillium purpurogenum, Aspergillus sp., Alternaria alternata, Fusarium solani, Rhizopus sp., Mucor hiemalis, Myrothecium verrucaria etc. These microbes were isolated from different samples of vermicompost prepared from organic wastes viz. sawdust, grass, sugarcane bagasse, wheat straw, neem leaves, rice straw and orange peels. The related high levels of enzymes were seen in the vermicompost samples prepared from different organic wastes in our study. The rich microbial diversity and high levels of related enzyme activities in the vermicompost samples establishes their usefulness as green manure and a safe method of organic waste disposal.

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