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BIOLOGICAL FINGER PRINTING OF MICROBIAL DIVERSITY IN VERMICOMPOSTS OF DIFFERENT RAW MATERIALS

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ABSTRACT

There are number of techniques being used to convert the organic waste into the useful products like bio fuel, manure etc. Among them, the mesophilic process using earthworms, vermicomposting is the most effective and low cost process to convert large amount of organic waste in relatively shorter time.The significance of the process is not only to improve the fertility of the soil but also to improve the diversity of beneficial microorganisms in the soil.The process also eradicate harmful microbes from the ecosystem. The current study is to investigate the presence, range and diversity of microbial population in vermicast fed with different raw materials like cow dung, biomethanisation plant slurry, and press-mud). The vermicomposting unit at the Periyar Maniammai University, Vallam, Thanjavur, Tamil Nadu, India was the study area where open bed method is followed. Six samples were tested for this study, three for composts and three for vermicompost from the same raw materials.After inoculating earthworms, samples were collected on the 42nd day of the process. Samples from composting beds were collected at 90th day and proceeded to the metagenomic analysis.The sequence results were categorized from higher order phylum to lower order species level and the report indicates microbes like Bacteroidetes, Proteobacteria etc., were present in larger quantity in all the vermicompost samples compared to compost samples. The results indicate vermicompost obtained from the biomethanisation slurry did not have any pathogenic bacteria like *Salmonella spp*., *Escherichia coli*, Enterobacter spp., and *Enterococcus spp* despite night soil being one of the components used in the plant. In contrast, it had maximum number of bacteria species useful for the plant growth than other samples in our study.

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INTRODUCTION

When vermicomposting unit was introduced in the Periyar Maniammai University in 2002, cow dung and plant residues were used as raw materials. The unit was planned at minimum cost, hence the open bed system was chosen and the highly adaptable earthworm species Eisenia fetida was deployed for the vermicomposting process.Many combination of raw materials with cow dung as the major part were tried and the vermicomposts were tested for their physiochemical properties.The University introduced a multifeed biomethanisation unit in 2008 integrating nightsoil, human urine, cow dung and municipal solid waste(MSW) predominantly with food wastes.When the unit reached 45 days from its inauguration, it started expelling wet slurry. Department of biotechnology at PMU was to suggest a suitable mechanism to dispose the slurry and hence decided to use the spent slurry as a raw material in its vermicompost unit.However,the question of safety in using human feces and MSW arose, paving a new direction for research to study the impact of digestion process in the biomethanisation tank and vermicomposting on the microbial existence.Hence the authors decided to engage metagenomic analysis to find the microbial population and their diversity in all the vermicomposting processes developed by the department and comparing them with simple composting process adapted with same raw materials.

Fast and reliable molecular techniques based on rDNA amplified sequences analyses, have provided tools to determine microbial presence and diversity (Ranjard et al. 2000; Cardinale et al. 2004). Metagenomic surveys of microbial populations are often performed using the 16S ribosomal RNA (rRNA) gene, which contains conserved and variable regions that facilitate sequencing and phylogenetic classification. Here the complete Illumina workflow, 16S metagenomics studies with the MiSeq System was used to identify microbial populations up to species level, then the process was combined with the Illumina library preparation protocol, the MiSeq System, and simple analysis software.

The analysis led to characterising the taxonomic and phylogenetic composition of the bacterial communities present in vermicomposts by Eisenia fetida in three vermicompost samples and comparing each with respective composts.

MATERIALS AND METHODS

Methods:

To identify the presence of the various microorganisms in the compost, the genomes were sequenced. The complete genomic DNA from all the composts and vermicompost were sequenced using a Next Generation sequencing system. The study used the 16S Metagenomic Sequencing Library Preparation Guide to prepare sequencing libraries targeting the variable V3 and V4 regions of the 16S rRNA gene. Paired-end sequencing was performed on the MiSeq System and data were analyzed using the 16S Metagenomics App in the BaseSpace® analysis environment.

Composting and vermicomposting process:

The spent slurry from the biomethanisation(BM) plant was collected and shade dried and named as sample 1. The harvested vermicompost(30^{th} day) from the University unit which used the slurry collected from the BM plant on the same time and was named sample 2.Sample 3 contained cowdung compost prepared (90 days old) using the same raw materials which were used to prepare the vermicompost beds(sample 4 , harvested on 42^{nd} day).Sample 5 was the press mud compost(harvested on 90^{th} day) prepared by the farmer client of the University extension services and vermicompost prepared out of the same pressmud compost(harvested on 42^{nd} day)was taken as sample 6.All the compost and vermicompost manures were prepared using the open bed method. Earthworms were aggregated from the University units, gathered together, divided equally and used for inoculating beds from where samples 2, 4 and 6 were harvested for the study.

DNA Isolation:

Epicentre® DNA isolation kits deliver high-quality, inhibitor-free DNA from mixed samples of gram-positive and gram-negative bacteria derived from many environmental sources, including water, soil, fecal matter, and compost. In this study, DNA was isolated from there composts and three vermicomposts using the Meta-G-Nome TM DNA Isolation Kit2. Approximately 700 ng of DNA were extracted from each sample.

Library Preparation

The Illumina 16S Metagenomic Sequencing Library prepration protocol is optimized to target the V3 and V4 regions of the 16S rRNA gene, although it can be adapted to target other variable regions. It leads through each step of library preparation, from genomic DNA to sequencing-ready libraries. All necessary reagents, including the required primer sequences that target the V3 and V4 regions of the 16S rRNA gene were in the kit. These primers can also be modified to target different regions of the 16S gene, or altered for custom applications.All the six samples were prepared using the 16S library preparation protocol and the Nextera® XT DNA Index Kit6 for cost-effective sample multiplexing.

Sequencing

The MiSeq System can deliver 2 × 300 bp reads and up to 50 million paired-end reads, generating up to 15 Gb of data. The flexible system enables microbiologists to scale studies from one to hundreds of samples. Micro and nano flow cell options and accompanying reagents were available to support lower-throughput experiments by optimizing sample volume and coverage needs. Samples were loaded onto a MiSeq reagent cartridge and then onto the instrument. Automated cluster generation and a 2 × 300 bp paired-end sequencing run were performed. The resulting sequence reads were equally distributed across the samples, demonstrating uniform coverage.

Data Analysis

Illumina has removed much of the complexity from sequencing data analysis. Following the Illumina workflow,sequencing data generated on the Mi Seq System either on the instrument or in Base Space are analysed. Mi Seq Reporter software is able to analyze data on the sequencer or on a standalone computer. Alternatively, data can be transferred, analyzed, stored, and shared with collaborators in Base Space. Base Space can deliver analyzed sequences in as little as 12 hours following the 16S workflow, and Base Space applications (apps) provide access to a growing collection of analysis tools.

The samples were analyzed using the Base Space 16S Metagenomics App. The app delivers all phylogenetic data including coverage statistics and detected species in intuitive, easy-to-analyze reports. Sequencing reads are classified against the Greengenes7 database, achieving up to species-level sensitivity.

RESULTS

The NGS results shows the various microorganisms were found in the all the samples. Especially, vermicomposts had shown larger quantity of modified microorganisms which are beneficial to soil fertility and plant growth.At the phylum level the bacterial community showed the presence of *Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria, Chloroflexi, Candidatus Saccharibacteria, Cyanobacteria, Firmicutes, Ignavibacteriae* all the vermicomposts. At species level there more than 400 species present which belong to all the phylum mentioned above. The species level NGS statistics image was showed in the figure 1 which reveals all the samples had a unique composition of microorganisms (taxonomic marker) as well as modified microorganisms compared to respective composts. In addition to that, the result shows the vermicompost from the biomethanisation slurry had most valuable microorganisms compared to others which highly support plant growth. The colour below the figure indicates the microorganisms which are mentioned in the figure (taxonomic statistics at species level).

Actinobacteria, one of the predominant organisms in the samples analysed are known to produce non-antibiotic molecules that exhibit bioactivity, such as biopesticides and biosurfactants, and enzymes involved in the degradation of complex polymers (Manivasagan et al., 2013). This versatility in secondary metabolite production makes them important tools for pharmaceutical, medical, and biotechnological applications such as bioremediation.Key difference between the composts and vermicomposts was the presence of Acidobacteria in the latter, and this phylum showed increase in their population during vermicomposting. Several studies also found that bacteria from the phylum Acidobacteria are exclusively detected in vermicompost or vermifilter (Vivas et al. 2009; Fernandez-Gomez et al. 2012). Soil-associated bacterial communities comprise members of the *Bacteroidetes* phylum. Environmental *Bacteroidetes* are thought to be specialized in the degradation of complex organic matter in the biosphere, especially in the form of polysaccharides and proteins (Church, 2008)

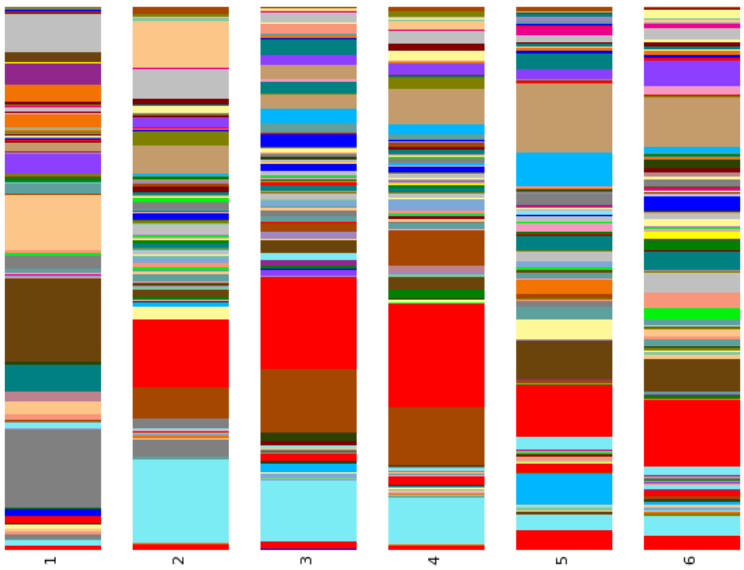

Fig: 1 Taxonomic statistics at species level.

CONCLUSIONS

We concluded all types of composts and their respective vermicompost had different types of microorganisms that useful for soil fertility and plant griwth. In addition, vermicompost from the slurry had shown absence of human pathogenic bacteria which are commonly seen in human feces. It is an important finding since human feces and municipal solid wastes are used in the biomethanisation unit and the spent slurry is used in the agriculture fields after vermicomposting. Hence we recommend vermicomposting as one of the cost effective and safest method of handling such wastes and convert the huge organic wastes into valuable manures and the nutrients are reused.

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