INVESTIGATION OF PREVALENCE OF ANTIBIOTIC RESISTANCE GENES IN THE COMPOST MADE FROM COW MANURE

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Introduction. Due to the misuse of antimicrobials like antibiotics, multidrug resistance in bacterial pathogens has become a global concern. Clinical pathogens have spread across the globe and acquired genes for antibiotic resistance, which has decreased the number of available viable treatments for resistant diseases. Exposure of livestock to antimicrobials can lead to resistance microbes in feces which are then composted and added to the soil for agricultural use. Although composting is a common method for lowering the danger of infections, some drug-resistant genes continue to exist and may even become more prevalent when added to soil. It is crucial to investigate the compost's microbiome to comprehend the presence and persistence of drug-resistant genes linked to that microbiome to lower the risk to the public's health.

The objective of the work is to determine the presence of antibiotic resistance genes in the microbiome of compost and to investigate how composting affects antibiotic resistance genes in the microbial population of cow manure.

Methodology. Cow manure samples was collected from Altamira, Tlaxcala, Mexico. DNA was extracted from the cow manure on day 0 and day 56 of composting. Libraries were constructed from the DNA extracts and Shotgun sequenced using Illumina NovaSeq 6000 (161 PE). The obtained raw sequences were preprocessed (host DNA and contamination removal, cut adapters and trimming of low-quality reads) and then cleaned reads were assembled into contigs of high quality using metagenomic assemblers. Different programs and algorithms were used for detection of antibiotic resistance genes in both the reads and assembled contigs. The results from both types of inputs (read and contig) were compared statistically and interpreted for designing an efficient workflow to investigate the prevalence of resistance genes during composting.

Results. Sequencing of DNA from the compost yielded 22,447,492 reads (Day 0) and 55,055,314 reads (Day 56) and of average quality 36. After preprocessing by cleaning and trimming, nearly 98% of raw reads were retained. In terms of the first objective, to determine the presence of antibiotic resistance genes in the compost, the cleaned reads, obtained after the series of filtration and trim, were analyzed by using AMR++ pipeline that uses MEGARes database (Table 1).

Table 1. Number of genes found in the sample – Day 0 Vs Day 56

Type	Number of genes in sample	
	Day 0	Day 56
Biocides	2893	8275
Drugs	153762	154485
Metals	18711	21645
Multi-compound	24403	42417

Among the antibiotics that are associated with the resistance genes in the compost, the top three groups were MLS (Macrolide-Lincosamide-Streptogramin), Rifampin and Aminoglycosides and moreover the abundance of these genes were sustained during the process of composting.

Conclusions. The biocide category of resistance genes has increased many folds from day 0 which is contrary to the concept of composting. More studies need to be conducted for understanding the proliferation of resistance genes during composting. The research will be continued with the assembly of reads and detection of antibiotic resistance genes in the contigs.

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