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Research Article

Shifts in Bacterial Community Structure in the Process of Composting of Organic Wastes

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ABSTRACT

Using 454 pyrosequencing, changes in the community structure of composting bacteria were estimated over 270 days. The compost contained the organic fraction of municipal solid waste, sawdust polluted by oil, and sewage sludge. All of these wastes are typical for a Russian city and they were obtained in Kazan (Tatarstan Republic, Russia).

In the initial stage of composting, the taxa *Lactobacialles*, *Rhodospiralles*, *Burkholderiales*, and *Xanthmonadales* dominated in the compost. By the end of the thermophilic stage, the dominant species changed: typical compost inhabitants belonging to the taxa *Flavobacteriales*, *Chitinophagaceae*, and *Bacterioidetes*, as well as non-typical taxa *Ectothiorhodospiraceae* and *Parvibaculum* sp., were observed in the compost. The presence of the latter two taxa may be explained by the presence of oil-polluted sawdust in the composting mixture. In the later stage, the dominant taxa remained the same; however, their relative abundance declined.

Keywords: Bacterial community, compost, 454 pyrosequencing, biodiversity

1.INTRODUCTION

Composting is an aerobic process of decomposition of organic matter driven by microbes that is widely used as a waste-treatment tool [1]. Studying the composting processes allows researchers to obtain fundamental data that can be used in industrial biotechnology. According to the temperature changes that are caused by different biochemical processes occurring in compost, the composting process can be divided into four stages: mesophilic, thermophilic, cooling/curing, and maturation[2–5]. Bacterial and fungal communities of composts may differ significantly among the four stages [2,6,7]. Quite often, one type of organic waste may be not suitable for composting, while co-composting of different substrates with contributing characteristics can be more efficient in terms of duration of the process, need for additional fertilizers, or quality of the final product [8–10]. An analysis of microbial communities in different treated organic wastes may help in the understanding of the mechanisms

of microbial succession and may thus increase the effectiveness of composting [6,7,11,12].

The objective of this study was to analyze the changes occurring in the microbial community structure of a compost mixture containing three organic wastes typical for a Russian city: the organic fraction of municipal solid waste, sawdust polluted by oil, and sewage sludge.

2.MATERIALS AND METHODS

The compost mixture was prepared using the organic fraction of municipal solid waste, sawdust polluted by oil, and semi-wet sludge of mixed industrial and household waste water (1:1:3). The proportion of the components was calculated in order to reach the optimal moisture content (between 55% and 65%) and C:N ratio (from 25 to 30) [13,14]. Compost was incubated for 270 days at room temperature and aerated by mixing daily. The moisture content of the compost was maintained at 55% to 60%.

Total genomic DNA was extracted from the samples using the FastDNA®SPIN Kit for Soil (Bio101, Qbiogene, Heidelberg, Germany) according to the manufacturer's instructions. The DNA samples were stored at -20 °C or analyzed immediately.

Quantitative PCR (qPCR) was conducted in nine replicates of each compost mixture. 16S 984f and 1378r primers were used. PCRs were conducted with a 5 U μl^{-1} SynTaq Polymerase, 10x Buffer with SYBR Green, 10 mM each dNTP, 10 µM each primer and 1 µl of DNA template in the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Munich, Germany). The qPCR program consisted of initial denaturation at 95 °C for 5 min followed by 39 3-step cycles of 62 to 60 °C for 45 s, 95 °C for 15 s, and 72 °C for 30 s. The standard curves were generated for bacteria using serial dilutions of *Bacillus pumilus* DNA.

The bacterial V4 region of the 16S rRNA amplified with the 27f/533r primers (with barcodes and adaptors) using GS Junior Technology (Roche 454 Life Sciences, Branford, USA). For amplification, each 25 µl reaction mixture contained 1 µM template DNA, 5 U/µl HiFi Polymerase (GS Junior Titanium emPCR Kit (Lib-A)), FastStart 10x Buffer #2, 10 mM each dNTP, and 10 µM primer. Cycling conditions were: 95 °C for 3 min of initial denaturation and 35 cycles of 95 °C for 15 s of denaturation, 55 to 65 °C for 45 s of annealing, 72 °C for 1 min for each cycle extension and a final extension step at 72 °C for 8 min. Amplicons were purified and concentrations were measured on a Qubit 2.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA) using the Picogreen dsDNA reagent (Invitrogen Ltd, Paisley, UK), the libraries were pooled in equimolar concentrations to prepare for pyrosequencing. Emulsion PCR and sequencing of the pooled sample in a single 454 GS Junior run were carried out according to the manufacturers' instructions. The 454 pyrosequencing data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) platform, version 1.6.0. [15].

The taxonomic classification of each phylotype was determined in accordance with the Greengenes database. Operational taxonomical units (OTUs) were clustered with a similarity cutoff at 97%. The OTU data were used to calculate the richness and diversity indices of the bacterial community and the relative abundances of phylogenetic groups in the soils.

Statistical analyses were performed using Origin 8.0 (OriginLab, Northampton, USA) and R Statistical Software (R 3.0.0, R Foundation for Statistical Computing Version, Vienna, Austria).

3.RESULTS AND DISCUSSION

The mesophilic stage of composting lasted for 2 days, and the thermophilic one, for approximately 28 days. The cooling stage began on the 30th day of composing (data not shown). We estimated the structure of the bacterial community of the compost on the key days described above: on the 2nd and the 30th. We also estimated the bacterial

community structure of the immature and mature compost on the 150th and 270th days, respectively.

The number of 16S gene copies ranged from 4.4 × 10^6 (2nd day) to 4.9 × 10^7 (150th day) per gram of dry compost. These results are in accordance with those presented by other authors [16].

Using 454 pyrosequencing, we estimated the presence and abundance of bacterial OTUs in the compost. The maximal number of bacterial OTUs (157) was found on the 270th day of composting. No dependence of the number of OTUs on the duration of composing was observed. The highest number of OTUs belonged to the phyla *Proteobacteria* (mainly, *Alpha-* and *Gammaproteobacteria*) and to *Firmicutes* (Fig. 1). This is in line with previously published results [9,16–18]. The abundance of *Alphaproteobacteria* was constantly high during the whole process of composting and the abundance of *Gammaprotebacteria* rose with time.

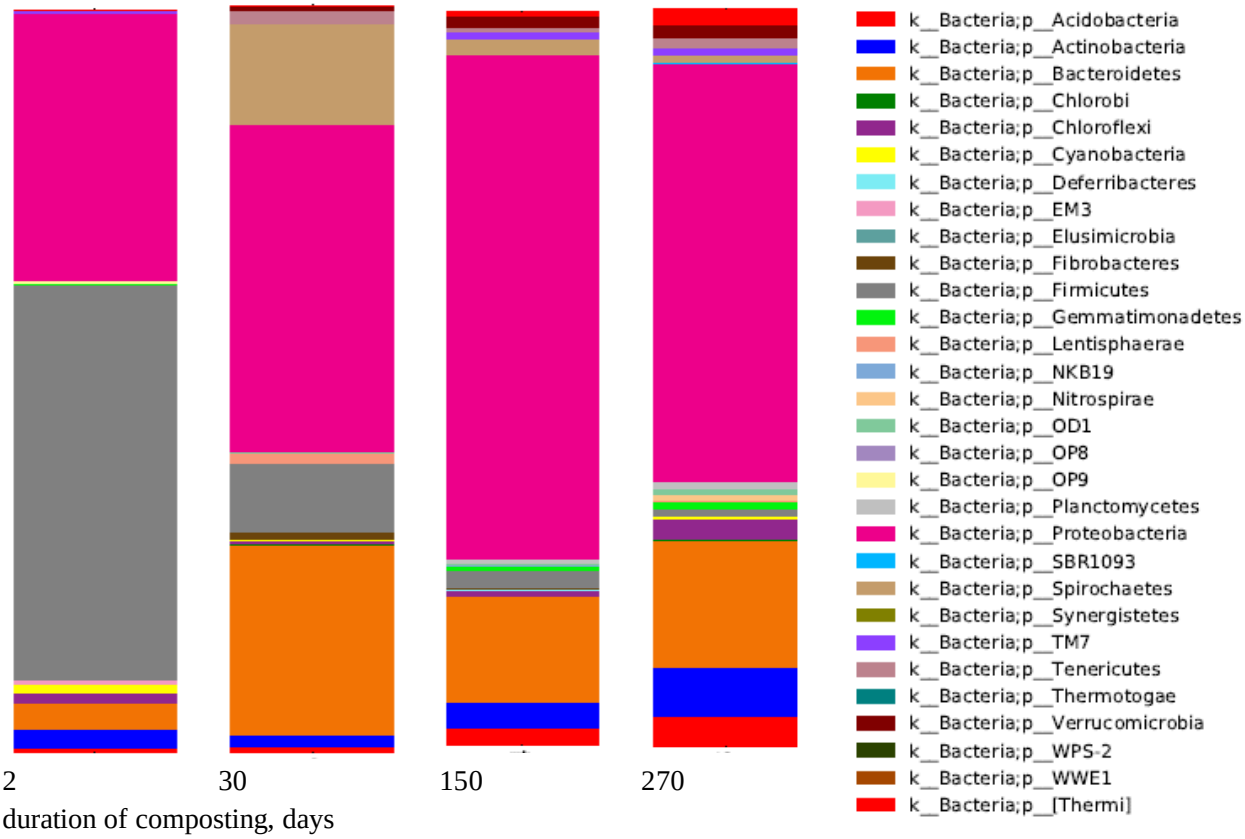

Fig. (1). – Bacterial community structure of composts of different age

In the initial stage of composting, the taxa *Lactobacialles, Rhodospiralles, Burkholderiales*, and *Xanthmonadales* dominated with 49.2%, 12.9%, 5.6%, and 4.9%, respectively. Among *Lactobacilliales*, one OTU predominated: it was, however, not identified to the species or genus level. In the literature, *Lactobacillus* spp. is described to be typical for the initial stages of composting [19]. Within the *Rhodospiralles* order, *Gluconobacter* and *Acetobacteraceae* families predominated. Both families are known as inhabitants of fruits and vegetable wastes, garden and baker's soil, etc. [20]. They may originate from a municipal solid waste contained in the composting mixture studied. Within the taxa *Burkholderiales*, representatives of *Comamonadaceae* dominated. Bacteria of this family have previously been described in sewage sludge [21,22]. The sub-dominating taxa *Xanthomodales* was represented mainly by the

species of the *Xanthomonadaceae* family. *Xanthomonadaceae* are aerobic, motile, catalase- and oxidase-positive bacteria[23] found in soil [24]. The *Pseudoxantomonas* genera with the maximal abundance on the 30th and 150th days of composting were previously observed in composts by de Gannes and co-authors [11]. One of the *Xanthomonadaceae* genera observed in our study belonged to *Stenotrophomonas*. This phytopathogenic genus [25] was abundant in the compost until the 150th day, but not in the final product.

On the 30th day of composting, the predominating OTUs changed. *Rhizobiales, Chromatiales,* and *Xanthomodales* were quite abundant, while *Bacteroidales* and *Sphaerochaetales* played a more important role, with 18.6% and 11.1%, respectively. In the later stages of composting, the abundance of some dominating OTUs rose and that of the others fell. As a result, in the final product, the bacterial community was represented by 10 dominating OTUs with abundances ranging from 3% to 9%. In the *Rhizobiales* and *Chromotiales* orders, two OTUs predominated: *Parvibaculum* sp. and the *Ectothiorhodospiraceae* family. These OTUs are not known to be typical of composts; however, they were previously found in the petroleum- contaminated sites [26]. We suggest that the presence of hydrocarbons in one of the compost components – sawdust polluted by oil – is a reason for the presence of these two hydrocarbon-degrading OTUs in the compost.

On the basis of the results obtained here, we estimated the alpha biodiversity in compost of varying age. The Shannon-Weaver index is expected to be higher in communities with higher amounts of OTUs found at similar frequencies [27–29]. The indexes calculated were equal to 3.27, 3.85, 3.81, and 4.19 for the communities on the 2nd, 30th, 150th, and 270th days, respectively. Thus, the bacterial community structure of the compost was less diverse in the initial stage as compared with the later stages. The alpha diversity increased significantly during the thermophilic stage. Later on, it remained high and even rose slightly in the final compost.

4.CONCLUSION

We can conclude that the bacterial community structure of compost depends on the initial wastes used for preparation of the compost mixture, and on the duration of composting. The most important changes in terms of OTU composition as well as in terms of bacterial diversity happened between the 2nd and the 30th days of composting, during the thermophilic stage.

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