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Discovery and Microbiological Analyses of a Novel Biorecycling System of Garbage that Proceeds with Participation of Lactic-acid Bacteria as Predominant Species

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Research aims

We found a novel system of biorecycling (composting) of garbage that is sustained under thermoacidophilic conditions (at pH 3.5–6.5 and 50–70°C) for prolonged periods of time (more than 2 years). This is in striking contrast with the nature of known accelerated high-temperature composting processes, which generally proceed under slightly alkaline conditions (pH 7.5–9). This process was also found to emit only low levels of odor, in contrast to other known composting processes. Thus, due to its sustainability and low level of odor emission, the system may be a low-maintenance process that could overcome the problems associated with conventional accelerated-composting processes. In the present study, we analyzed microflora of the process by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and fluorescent *in situ* hybridization (FISH) to show the participation of lactic acid bacteria during this novel composting process.

Methods

The composting apparatus used in this study had a maximum capacity of 5 kg (wet wt) of garbage a day. Prior to the first addition of the substrate (garbage), starter microorganisms (0.5 g, wet wt.) that had been maintained as a mixed culture of thermoacidophilic and thermoacido-tolerant soil bacteria and base material (cedar-wood saw dust, 32 liters) were mixed in a vessel by running the machine for 1 h without any substrate. Excess water in the fresh garbage was drained using a basket, and a specific quantity of the substrate was added daily to the vessel of the composting system. Typical contents of added substrates were food refuse and raw residual materials generated during cooking. Basically, all edible materials, fruit rinds, animal skins, fish bones, and eggshells are good substrates for the composting system. However, large animal bones, shells, and wood materials were not used as substrates for composting. Through repeated additions and composting of the substrate, the volume of the product compost in the vessel gradually increased. At appropriate times, the excess compost was removed to maintain the volume of the compost in the vessel at less than 27 liters. PCR-DGGE analysis of the compost microflora was carried out using PCR primers 520F (GTG CCA GCM GCC GCG G), 1400R (ACG GGC GGT GTG TRC), and GC1400R (CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAC GGG CGG TGT GTR C). FISH analysis of the compost microflora were carried out using the fluorescence-labeled oligonucleotide probes EUB338-FITC [5′-(fluo)GCT GCC TCC CGT AGG AGT-3′] and LAB-Cy3 [5′-(Cy3)GGT ATT AGC AYC TGT TTC CA-3′]. Organic acid analysis of the compost samples were done using a Photal CAPI-3200 microcapillary electrophoresis system (Otsuka Eectronics).

Results

The pH and temperature of the compost were in the ranges of 5.5–5.6 and 60–65°C, respectively, during the process throughout the composting process monitored, indicating that the composting proceeds under thermoacidophilic conditions. Figure 1 shows the results of PCR-DGGE analysis of the compost microflora using universal primers designed for specific amplification of 16S (or 18S) rDNA sequences. Several major bands (1204A-D, Fig. 1) were amplified and were analyzed for their nucleotide sequences. The results showed that all of these DNAs have 16S rDNA sequences of the genus *Lactobacillus*, suggesting that lactobacilli should predominantly occur in the compost to play an important role for biodegradation of organic wastes even under thermoacidophilic conditions. To more quantitatively evaluate the participation of lactic acid bacteria during the composting, FISH analysis of the compost microflora was also carried out. The results showed that the compost contained 4.6×10^7 microbial cells per g compost, 44–54% of which were specifically hybridized with the lactic acid-specific probe LAB-Cy3. These results showed that lactic acid bacteria were indeed predominant species of the compost ecosystem. The organic acid analysis showed the occurrence of high levels (up to 850 ppm) of lactic acid and acetic acid in the compost, suggesting that lactic fermentation should take place during the process. The compost extract inhibited the growth of *Escherichia coli* (not shown), implying the existence of bacteriocin-like substances in the compost.

Conclusion

The present composting process showed a stable microbial ecosystem with lactic-acid bacteria being predomi-

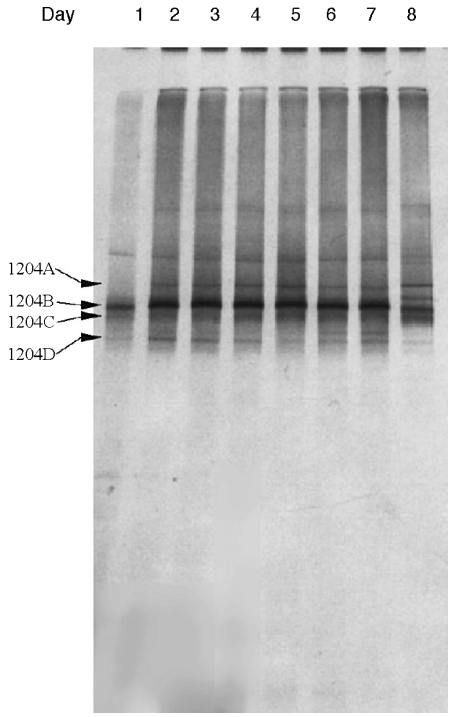

Fig. 1. PCR-DGGE analysis of the composting process.

nant species. These lactic-acid bacteria should probably be thermotolerant or thermophilic species that have originally been present in garbage and have survived under thermoacidophilic conditions of the process. It is likely that the growth of microorganisms that may hamper the compost process is severely inhibited by acidic conditions due to lactic fermentation, high-temperature conditions, and bacteriocin produced by the habitat bacteria, and this may account for the observed stability of the process.