Bacterial diversity of decomposing oil palm empty fruit bunches based on PCR and denaturing gradient gel electrophoresis of 16S rRNA gene fragments

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Abstract

The microbial community that inhabit the thermophilic stage of composting process consist of complex thermophile communities that interact with each other to biodegrade lignocellulosic materials. The diversity of this community during oil palm empty fruit bunches (OPEFB) composting was investigated using PCR and denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments. Compost samples were collected during the thermophilic phase with a temperature of between 60°C-62°C. Based on DGGE profile of 16S rDNA amplicons, four discrete bands were observed. Sequence analysis of the excised bands showed that sequences of these bands are closely related to those found in uncultured bacteria from other compost environments. The results suggest that the composting process of OPEFB during the thermophilic phase were dominated by yet-uncultured bacteria species.

Introduction

Composting of oil palm empty fruit bunches (OPEFB) is a common way of treating this abundant bio-solid waste from the palm oil industry. Composting is a self-heating process where organic matter is degraded into smaller substrates by the action of microbes under aerobic condition (Dees and Ghiorse, 2001). The compost temperature may reach more than 50°C during the composting process (Beffa *et al.*, 1996). During the thermophilic phase, most of the successive microbes are expected to be thermophiles or thermo-tolerant microbial groups. The application of molecular genetic technique can reveal more than 99% of microbial species that cultivation techniques failed to detect (Hugenholtz *et al.*, 1998). Few studies have been performed on hot compost of different organic materials based on molecular genetic techniques (Blanc *et al.*, 1999; Takaku *et al.*, 2006). In this study, denaturing gradient gel electrophoresis (DGGE) analysis based on V3 region of 16S rRNA gene was employed to detect dominant microbes involved during the thermophilic phase of OPEFB composting.

Methods And Materials

Sampling and total genomic DNA extraction
Compost samples were collected from the core of a 4-days old compost pile at a temperature range of 60°C-62°C. Total genomic DNAs were extracted from the compost sample with the PowerSoil™ DNA isolation kit (MO Bio laboratories, USA) as instructed by the manufacturer's manual.

PCR amplification

16S rDNA fragments with a size of approximately 1.5 kb were PCR amplified using published bacteria-specific primer pair (Lane, 1991) according to methods previously described (Goh *et al.*, 1996). For DGGE analysis, nested PCR were performed using primers 341fGC (341f with GC-rich sequence attached at 5'-end) and 534r to generate hypervariable V3 regions of approximately 200 bp, from the 16S rDNA genes. A 100-µl reaction mixture for PCR consisted of purified 16S rDNA amplicon (150 ng), 5 U of *TaKaRa LA Taq*™ (TaKaRa Bio Inc, Japan), 1X GC buffer I, 0.4 mM of dNTP mixture, and 0.25 mM of each primer (341fGC and 534r). The mixture was subjected to touchdown PCR in a thermocycler (Bio-Rad, USA). The incubation condition started with 95°C for 10 min; 20 cycles of 95°C for 1 min, 65°C for 45 sec (temperature dropped 0.5°C for each cycle), 72°C for 1.5 min; 30 cycles of 95°C for 1 min, 55°C for 45 sec, 72°C for 1 min; and with a final extension at 72°C for 20 min. The PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide and photographed using a gel documentation system (Syngene, UK).

DGGE analysis

PCR products were separated using a DCode universal mutation detection system (Bio-Rad, USA). PCR products were electrophoresed through a 10 % polyacylamide gel with a linear denaturing gradient ranged from 30% to 60% in 1X TAE buffer at 130 volts for 5 hours at 60°C. A 100% denaturant is defined as 7 M urea and 40% (vol/vol) formamide. The gel was stained with ethidium bromide and photographed with a gel documentation system (Syngene, UK) under UV light.

Sequencing analysis

The intense bands were excised from the gel and re-amplified using the primer pair 341f and 534r. After the amplicons were cloned and screened, sequencing reactions were carried out with DYEnamic ET terminator cycle sequencing kit (GE Healthcare, UK). Sequencing was done by commercial sequencing facilities. Comparison of the obtained sequences to the GenBank database was conducted using BLASTn.

Results And Discussion

Total genomic DNA of the hot compost sample was extracted. PCR-DGGE analysis based on hypervariable V3 region of the hot compost bacterial community formed a unique banding pattern as shown in Fig.1. Four intense bands as shown in Fig.1 were excised and purified for sequencing analysis. BLASTn analysis results of the band sequences are shown in Table 1. The results revealed that all of the predominant sequences are most closely related to several species of yet-uncultured bacteria found in different compost samples. This observation suggests that similar bacterial communities may be present in composting processes involving different starting materials.

Band A and C are closely related to uncultured bacterium clone library Niitsu which were recovered from garbage compost as reported by Takaku *et al.* (2006). Both affiliated sequences were obtained from day 24 and day 31 of composting (Takaku *et al.*, 2006). The nearest sequence match of band C to the culture sequence collection is from members of phyla Deinococcus-Thermus (*Meiothemus timidus* from family *Thermaceae*) which is isolated from hot spring. Band B was most closely affiliated to

uncultured compost clone 5-33, and closely related to *Bacillus* sp. GB02-25 from the culture sequences database. Band D most closely related to uncultured compost bacterium 2B06, and distantly related to culture sequence of *Thermoanaerobacter* sp. ToBE (genera *Clostridium*). Both of them belong to phyla Firmicutes isolated from high temperature environments of hydrothermal sediment and subterranean vein, respectively. It is expected that members from the phyla Firmicutes are the predominant bactera present in the thermogenic compost (Dees and Ghiorse, 2001).

Table 1 : Phylogenetic affiliation of excised DGGE bands

Band	Closest relative (accession #)	Similarity (%)	Source	Closest culture relatives (accession #)	Similarity (%)	Source
A	Uncultured bacterium clone Niitsu31-15 (AB187980)	99	compost	*Desulfuromonas alkaliphilus* strain Z-0531 (DQ39326)	85	Soda lake
B	Uncultured bacterium clone 5-33 (AB034720)	98	compost	*Bacillus* sp. GB02-25 (DQ079010)	98	Hydrothermal Sediments
C	Uncultured bacterium clone Niitsu24-5 (AB187910)	100	compost	*Meiothermus timidus* (AJ871170)	85	Hot spring water
D	Uncultured compost bacterium clone 2B06 (DQ346478)	93	compost	*Thermoanaerobacter* sp.ToBE (AB062280)	91	Subterranean vein

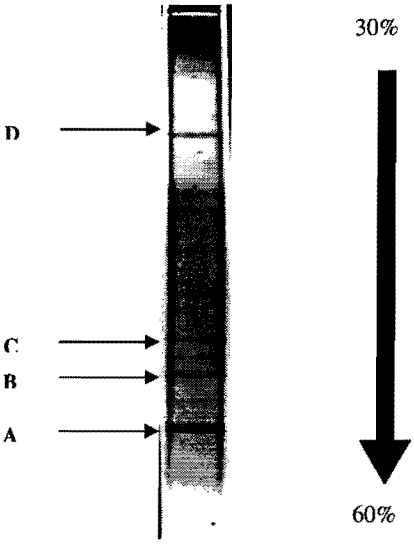

Fig.1 DGGE pattern of PCR amplified hypervariable V3 region of 16S rRNA gene of the compost sample. The labeled bands were excised for subsequent analysis. The bolded vertical arrow indicates the percentage of linear denaturing gradient.

Conclusion

The results of our work indicated that the prominent members during the thermophilic stage of OPEFB compost were mainly yet uncultured and unidentified bacteria as reported in other compost environments.

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