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Diversity of a mesophilic lignocellulolytic microbial consortium which is useful for enhancement of biogas production

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ABSTRACT

A mesophilic lignocellulolytic microbial consortium BYND-5, established by successive subcultivation, was applied to enhance the biogas production. The degradation efficiency of BYND-5 for rice straw was more than 49.0 ± 1.8% after 7 days of cultivation at 30 °C. Various organic compounds, including acetic acid, propionic acid, butyric acid and glycerin were detected during biodegradation. The diversity analysis of BYND-5 was conducted by ARDRA (Amplified ribosomal DNA restriction analysis) of the 16S rDNA clone library. Results indicated that bacterial groups represented in the clone library were the *Firmicutes* (5.96%), the *Bacteroidetes* (40.0%), *Deferribacteres* (8.94%), *Proteobacteria* (16.17%), *Lentisphaerae* (2.13%), *Fibrobacteraceae* (1.7%), and uncultured bacterium (25.1%). Additionally, the enhancement of biogas yield and methane content was directly related to the pretreatment with BYND-5. The microbial community identified herein is potential candidate consortium for the degradation of waste lignocellulose and enhancement of biogas production under the mesophilic temperature conditions.

1. Introduction

Nowadays, energy crisis and environment pollution are world widely concerned, the need to develop alternative energy sources, including lignocellulosic biomass. Rice straw, one of the important renewable lignocellulosic biomass, is the most abundant agricultural residue in China with an annual production rate of about 180 million tons (Ma et al., 2009). Lignocellulose is the main component in rice straw, which is a compact structure of cellulose (35–45%) and hemicelluloses (25–40%) in close association with lignin (5–25%) (Klinke et al., 2003). However, these lignocellulosic materials are underutilized currently, most of which are usually burned or incinerated, buried on site causing environment pollution problems with waste of resources (Kheng et al., 2006).

Presently, bioconversion of lignocellulosic biomass into energy, fuels and chemicals are attracting much interest throughout the world. The rate-limiting step in the bioconversion of straw is the degradation of lignocellulose. It has been reported that many pure cultures such as anaerobic bacteria, fungi, and actinomycetes were used to degrade lignocellulosic materials (Desvaux et al., 2000; Xu and Goodell, 2001). Naturally, lignocellulose is degraded with the cooperation of many microorganisms (Wongwilaiwalin et al., 2010). In recent years, the research on biodegradation of cellulosic biomass has been focused on microbial co-cultures or complex communities (Wongwilaiwalin et al., 2010). Haruta and colleagues constructed a stable thermophilic microbial community with a high activity on filter paper, absorbent cotton, wheat straw, and sawdust (Haruta et al., 2002). Wongwilaiwalin et al. (2010) screened a thermophilic lignocellulolytic microbial consortium and investigated the structure and dynamics of the microbial community together with proteomic characterization of its multi-species lignocellulolytic enzyme system. Other lignocellulose-degrading thermophilic consortia have been described (Liu et al., 2009; Wang et al., 2011), but mesophilic consortia which can degrade lignocellulosic biomass efficiently would be desirable.

Here, a mesophilic lignocellulolytic microbial consortium which is useful for the enhancement of biogas production was constructed by enrichment method. The structure and diversity of the microbial community were investigated by 16S rRNA clone library and ARDRA analysis. The effect of hemicellulolytic mesophilic microbial community on biogas formation was also studied. Analysis of microbial components provides valuable information for further study on multi-microbial species cooperation, and the mechanisms involved in the lignocellulose degradation and the enhancement of biogas production.

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2. Methods

2.1. Cellulosic materials

Raw rice straw was cut into about 10 cm in length, soaked with 1% NaOH for 24 h, washed with distilled water until neutrality and dried at 60 °C. Then the rice straw was autoclaved at 121 °C for 15 min before use. Filter paper was purchased from Xinhua paper Corporation (Hangzhou, China). All chemicals and reagents used in this work were of analytical or molecular biology grade.

2.2. Cultivation and enrichment

Ten milliliters of slurry samples collected from a biogas-producing digester under mesophilic temperature (30 °C) which fed mainly on liquid cow manure were selected as the microbial source, and were cultured in PCS (peptone cellulose solution) medium (0.1% yeast extract, 0.5% peptone, 0.2% $CaCO_3$, 0.5% NaCl, 0.5% filter paper, pH 7.0) supplemented with 1.0 g rice straw, and with a filter paper strip (0.3 g) as an indicator for cellulase activity (Wongwilaiwalin et al., 2010). Enrichment of lignocellulolytic microbial community was conducted following the procedure reported by Wang et al. (2011). The enrichment temperature was about 30 °C. The degradation capacity of the enriched microbial consortium was investigated by measuring the degradation ratios of rice straw and its cellulose, hemicellulose, and lignin every day during the period of incubation. The amount of cellulose, hemicellulose, and lignin of rice straw before and after degradation were determined by an Ankom 220 Fiber Analyzer (Ankom Technology, Fairport, NY). Degradation ratio was calculated following the procedure reported by Wang et al. (2011).

2.3. Production analysis during rice straw degradation

Ten milliliters of mesophilic microbial community culture were inoculated in 90 mL PCS medium containing 1.0 g rice straw at 30 °C under static condition for 7 days. Every day 200 μL of cultures were filtered through a 0.2 μm filter and the filtrate was analyzed for volatile products content.

Separation and identification of volatile compounds were performed by GC–MS (model QP-5050, Shimadzu, Japan). The injection volume for all measurements was 1 μL with splitless mode. A CP-Chirasil-Dex CB, 25 m × 0.25 mm i.d. capillary column was used. The analysis conditions were: initial oven temperature 50 °C, hold for 2 min, then programmed to 100 °C at 5 °C/min, then 5 min at 100 °C, then at 15 °C/min to 190 °C and held for 2 min. The injector and detector temperatures were 190 and 230 °C, respectively. Qualitative analysis was then carried out by target testing the spectra of the components suspected to be in the mixture.

2.4. Amplification and cloning of 16S rDNA

Benzyl chloride method was used for total genomic DNA extraction from the microbial consortium (Zhu et al., 1993). The16S rDNA gene was performed in AmpliTaq Gold™ system (Perkin Elmer, Applied Biosystems, NJ, USA) by using the forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and the reverse primer 1492R (5′-GGTTACCTTGTTACGACTT-3′). The 50 μL reaction mixture consists of 1.0 μL template DNA, 4.0 μL 25 mM $MgCl_2$, 5.0 μL 2 mM GeneAmp dNTP® Mix, 5.0 μL 10× PCR Gold Buffer, 0.2 μL AmpliTaqGold™, 1.0 μL of forward and reverse primer, respectively (45 pM), 33.6 μL ddH_2O. PCR (polymerase chain reaction) was performed with an initial denaturation at 95 °C for 10 min, 25 amplification cycles (60 s at 93 °C, 60 s at 50 °C, and 90 s at 72 °C), and a final elongation at 72 °C for 5 min.

PCR products were purified with a QIAquick PCR purification Kit (Qiagen, UK), ligated into the pGEM-T easy vector (Promega, Madison, WI), according to the manufactures' instruction, and transformed into *Escherichia coli* TOP10. The 16S rDNA gene inserts in the *E. coli* transformants were amplified using vector universal primers M13-47 (5′-CGC CAG GGT TTT CCC AGT CAC GAC-3′) and RV-M (5′-GAG CGG ATA ACA ATT TCA CAC AGG-3′) targeting the flanking regions of the multi-cloning site of pGEM-T easy vector. The thermocycle program for PCR was the same as described above.

2.5. ARDRA, sequencing, and phylogenetic analysis

PCR products were analyzed by restriction digest with *Hae* III and *Hinf* I. Restriction fragments were separated on 2.5% Metaphor agarose gels (FMC, Rockland, ME, USA) and grouped according to DNA fingerprinting.

The representative cloned fragments were sequenced with primers 1492R and 27F as well as the vector primers M13-47 and RV-M at Shanghai Sangon Biotechnology Company. Sequence identification was initially estimated by using of the BLASTN facility of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/). Multiple alignments of sequences were performed using the CLUSTALX program and the trees were constructed the neighbor-joining method with MEGAR 4.0 software. The robustness of the phylogeny was tested by bootstrap analysis with 1000 iterations. The nucleotide sequences determined in this study have been deposited in the Genbank under accession numbers JF834120–JF834134.

2.6. Batch-culture and biogas fermentation experiment

Laboratory batch experiments were carried out following the guidelines reported by Weiß et al. (2010). Flasks (1000 mL) were used as seeding tank. The fermentation mixture contained 720 mL PCS medium, 40 g straw, and 80 mL cultures of mesophilic microbial community. Controls were charged with PCS medium. After static fermentation at 30 °C for 10 days, the mixture cultures were inoculated into a biogas fermentation tank (6000 mL). The biogas fermentation tank contained 3700 mL water, 250 g dry matures, and 500 mL biogas slurry which collected from mesophilic anaerobic fermentation. Biogas fermentation was performed at a constant mesophilic temperature (30 °C) for 30 days. Drainage method was used for measuring biogas production, the volume of water was measured with the measuring cylinder at the same time every day. CH_4 concentrations were measured using a GC-17A gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector.

3. Results and discussion

3.1. Degradation properties of the microbial community

After enrichment for half a year, a mesophilic microbial community with capacity of degrading lignocellulose effectively, designated BYND-5 was constructed. It can be found that this consortium can be degraded more than 49.0 ± 1.8% of rice straw within 7 days at 30 °C. The degradation ratios for cellulose, hemicellulose, and lignin were 40.7 ± 1.6%, 53.6 ± 2.3% and 55.5 ± 2.4%, respectively (Fig. 1). Previous study suggested that the degradation rate of the easily assimilable cellulose was higher than that of the complex lignin in presence of mixed microbial community (Guo et al., 2008). However, in this experiment, more hemicellulose and lignin were decomposed compared with cellulose. This discrepancy might be due to the enzymatic hydrolysis of more