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Evaluation of DNA Extraction and Purification Methods for Corn Straw Biogas Slurry

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Abstract. Corn straw biogas slurry always contains humic substances, which poses particular challenges in obtaining PCR-amplifiable DNA for analysis of microbial community. To establish an efficient and reliable DNA extraction method for straw biogas slurry, four approaches: i.e., direct SDS-based method, direct bead-based method, indirect SDS-based method, and indirect bead-based method were evaluated by comparing DNA yield, humic acid contamination, PCR amplifiability, and restriction fragment length polymorphisms (RFLP) of amplified 16S rRNA genes. Direct DNA extraction methods yielded 3-fold higher amounts of DNA than indirect procedures, but its DNA purity was lower. The A_{260}/A_{230} ratio of DNA from indirect methods (0.8-0.85) were higher than that of DNA from direct methods (0.5-0.6), indicating DNA from direct methods contained high levels of humate contamination. PCR amplification was successful with crude DNA from indirect methods, but not with crude DNA from direct methods. PCR products could also be obtained with purified DNA from direct bead-based method, whereas not direct SDS-based method. Among the four methods, direct bead-based method, indirect SDS-based method and indirect bead-based method could obtain high-quality DNA extracts from corn straw biogas slurry. RFLP analysis further demonstrated the restriction patterns of amplified 16S rRNA genes from three methods were relatively identical microbial diversity.

Introduction

Molecular techniques based on PCR amplification of 16S rRNA genes have been widely used to investigate microbial diversity in various natural and artificial ecosystems [1-3]. Crop straw is abundant and important renewable biomass, consisting of high levels of lignocellulose. Anaerobic degradation of lignocellulosic biomass to methane is a multi-step process mediated by varieties of microbial populations. In anaerobic corn straw-degrading microbial system, microbial cells remain tightly bound to straw and high levels of humic contamination produced during corn straw degradation, which poses particular challenges in obtaining PCR-amplifiable DNA for analysis of microbial community. Many studies focused on DNA extraction from soil or composting, however, DNA extraction approach for straw biogas microbial system is still limited.

The method used for DNA extraction can be classified as direct and indirect procedures. Direct DNA isolation is based on cell lysis within the sample matrix, whereas the indirect approach involves the extraction of cells from the environmental material prior to the lytic release of DNA. Indirect extraction methods are generally acknowledged to yield DNA of higher molecular mass and greater purity than direct lysis procedures. However, in many cases the amounts of DNA recovered by this strategy are significantly lower. Compared with indirect method, direct lysis protocols can readily yield the required amounts of DNA [4]. But some reports showed that greater DNA yield from direct methods does not always equal greater bacterial species richness and that sequence representation is strongly influenced by extraction method used [5,6]. To establish an efficient DNA extraction method for straw biogas slurry, four methods (direct SDS lysis, direct bead lysis, indirect SDS lysis, and indirect bead lysis) were investigated based on DNA yield, humic acid contamination, PCR amplifiability, and RFLP of amplified 16S rRNA genes.

Materials and Methods

Corn straw biogas slurry. Biogas slurry was taken from a lab-scale mesophilic (37°C), anaerobic completely stirred tank reactor (CSTR) with a volume of 4 liter. The reactor was fed with untreated corn straw as the sole feedstock for six months, and with cow manure as the original inoculum. The reactor was operated at an organic loading rate (OLR) of 2 g VS/L/d and hydraulic retention time (HRT) of 20 d. The biogas slurry was washed with phosphate buffer (10 mM, pH 7.2) and centrifuged at 5 000 rpm for 10 min, and the pellets were used for DNA extraction.

DNA extraction

Direct SDS-based method. Triplicate slurry samples (5 g) were homogenized by vortexing in 13.5 mL extraction buffer [100 mM Tris-HCl, 100 mM sodium EDTA, 1.5 M NaCl, 1% hexadecylmethylammonium bromide (CTAB), pH 8.0] and 100 μL of proteinase K (10 mg/L) were added before incubation at 37ºC for 30 min. After addition of 200 μL SDS (20%), mixtures were incubated at 65ºC for 2 h with gentle end-over-end inversions every 15 to 20 min. The supernatants were collected by centrifugation at 5000 rpm for 10 min and transferred into 50 mL centrifuge tube. The pellets were re-extracted twice by adding 4.5 mL of extractions buffer and 0.5 mL of 20% SDS, vortexing for 10 s, incubating at 65ºC for 10 min, and centrifuging as described above. Supernatants from the three cycles of extraction were combined and mixed with an equal volume of chloroform-isoamyl alcohol (24:1, vol/vol). The aqueous phase was recovered by centrifugation and precipitated with 0.6 volume of isopropanol at room temperature for 1 h. The pellet of crude nucleic acids was obtained by centrifugation at 12000 rpm for 20 min at 4ºC, washed with cold 70% ethanol, and resuspended in 200 μL sterile deionized water.

Direct bead-based method. Triplicate slurry samples (5 g) were homogenized in extraction buffer and supplied with proteinase K as described above. Additionally, 1 g silica beads (0.1 mm diameter) were added. After incubation at 37ºC for 30 min, the mixtures were agitated at top speed on a vortex mixer for 30 min, which corresponds to the vigor of a 1 min treatment in a mini bead beater. Subsequent steps, such as chloroform extraction and isopropanol DNA precipitation were carried out as described for the direct SDS-based method.

Indirect SDS-based method. Triplicate slurry samples (5 g) were dispersed in 30 mL 1×phosphate buffer and homogenized by vortexing for 30 min. Coarse particles were collected by low-speed centrifugation (3000 rpm for 10 min at room temperature), resuspended in 30 mL 1×phosphate buffer, and subjected to another two blending-centrifugation cycles as described above. Supernatants obtained during the three rounds of cell extraction were pooled. Supernatants were centrifuged at 12000 rpm for 30 min at 4ºC to collect the microbial cell fraction, which was subsequently washed in 50 mL of 0.1% sodium pyrophosphate. After a second wash in 50 mL Chrombach buffer (0.33 M Tris-HCl, 1 mM EDTA, pH 8.0), pellets were resuspended in 4 mL extraction buffer, 80 μL lysozyme (50 mg/mL), and 20 μL proteinase K (10 mg/mL) solution and incubated at 37ºC for 30 min. Lysis was completed chemically by adding 1 mL of 20% SDS and incubation for 2 h at 65ºC with rotary shaking (225 rpm). Chloroform extraction and isopropanol DNA precipitation were carried out as described for direct lysis methods. DNA pellets were dissolved in 200 μL sterile deionized water.

Inirect bead-based method. Triplicate slurry samples (5 g) were homogenized in 1×phosphate buffer by vortexing as described in indirect SDS-based method. After recovered microbial cells, subsequent steps were carried out as described for the direct bead-based method.

DNA purification. Crude DNA was purified using microElute DNA clean-up kit (OMEGA) following the manufacturer's instructions. Spectrophotometric A_{260}/A_{280} and A_{260}/A_{230} ratios were determined to evaluate levels of protein and humic acid impurities, respectively.

PCR amplification. Bacteria-specific primer EUB8F (5'-AGAGTTTGATCMTGGCTCAG-3'; positions 8 to 27 in the *Escherichia coli* gene), and the reverse primer UNIVI1492R (5'-TACGGYTACCTTGTTACGACTT-3'; positions 1492 to 1513 in *E. coli*) were used to amplify bacterial 16S rRNA genes [7].

RFLP analysis. The PCR products were purified with a TIAN quick MiDi purification Kit (Tiangen) and re-suspended in nuclease-free water. The purified PCR products were subjected to RFLP analysis with *Hae* III and *Hha* I restriction endonucleases at 37ºC for 4 h.

Results and Discussion

DNA extraction and purification from straw biogas slurry. To set up an efficient and reliable method for DNA extraction from straw biogas slurry, four methods, direct SDS-based, direct bead-based, indirect SDS-based, and indirect bead-based methods were investigated. The results showed all the four methods could recover genomic DNA from biogas slurry and the DNA fragments were all larger than 23 kb (Fig. 1a). Direct methods exhibited high extraction efficiencies, which yielded 3-fold higher amounts of DNA than indirect procedures. The amounts of DNA from two direct methods were 27.12 μg/g slurry and 16.78 g μg/g slurry, while the DNA yields from indirect methods were 6.76 μg/g slurry and 5.26 μg/g slurry, respectively (Table 1). Compared with direct methods, the crude DNA solutions from indirect methods were lighter in color and had higher A_{260}/A_{280} and A_{260}/A_{230} ratios, indicating DNA purity of indirect methods are higher than that of direct methods.

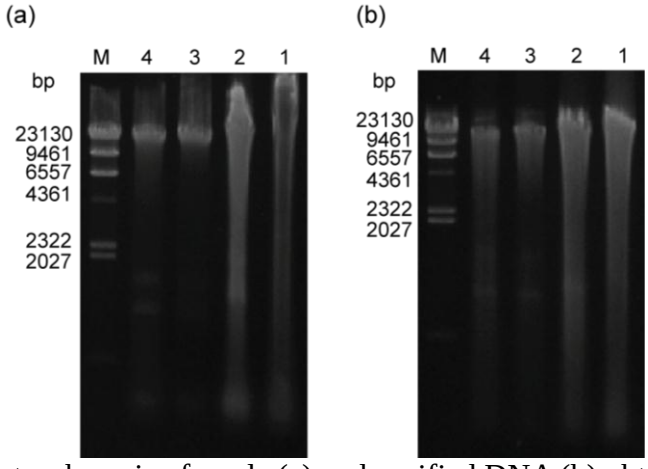

Fig. 1 Agarose gel electrophoresis of crude (a) and purified DNA (b) obtained from corn straw biogas slurry by four different approaches.
1-4: direct SDS lysis, direct bead lysis, indirect SDS lysis, indirect bead lysis; M: λ/*Hind* III DNA Marker

To eliminate the inhibition of humate contamination in DNA solutions, the crude extracts from biogas slurry were purified by a minicolumn method (Fig. 1b). The results indicated the minicolumn method could effectively remove the dark color from four crude DNA solutions. The A_{260}/A_{280} and A_{260}/A_{230} ratios of purified DNA were 1.7 to 1.8 and 1.6 to 1.7 respectively, indicating that the DNA was of good quality (Table 1). The minicolumn method can efficiently remove humate contamination in crude DNA, indicating the method was suitable for purification of DNA extracts from straw biogas slurry.

Table 1 Comparison of DNA yields, purity and recovery efficiencies from straw slurry by four methods

Extraction method	Direct DNA extraction		Indirect DNA extraction	
	SDS lysis	Bead lysis	SDS lysis	Bead lysis
Crude DNA yield (μg/g^{-1} slurry)	27.12±1.52	16.78±2.31	6.76±1.57	5.26±2.25
$A_{260/230}$ ratio	0.49±0.02	0.59±0.03	0.81±0.02	0.85±0.03
$A_{260/280}$ ratio	1.54±0.02	1.64±0.02	1.65±0.02	1.72±0.02
Final DNA yield (μg/g^{-1} slurry)	22.60±2.13	13.46±1.75	4.25±1.43	3.75±0.96
$A_{260/230}$ ratio	1.65±0.03	1.71±0.03	1.67±0.02	1.73±0.02
$A_{260/280}$ ratio	1.72±0.02	1.75±0.03	1.72±0.04	1.80±0.02
Crude DNA recovered (%)	83	80	63	71

PCR amplification. To investigate PCR amplifiability of the DNA extracts by four methods, the crude DNA and purified DNA were used as templates to amplify bacterial 16S rRNA genes with different dilution gradient (1, 1/10, 1/50, 1/100), respectively. Electrophoresis showed that no amplification products were detected when crude DNA from direct methods were used as templates (Fig. 2a), indicating crude DNA from direct methods could contain large amounts of humic acids, which might decrease its PCR amplifiability [8]. However, PCR products could be obtained with purified DNA from direct bead-based method, whereas not direct SDS-based method (Fig. 2b). Compared with direct methods, PCR amplification of 16S rRNA genes were successful with both crude and purified DNA from indirect methods.

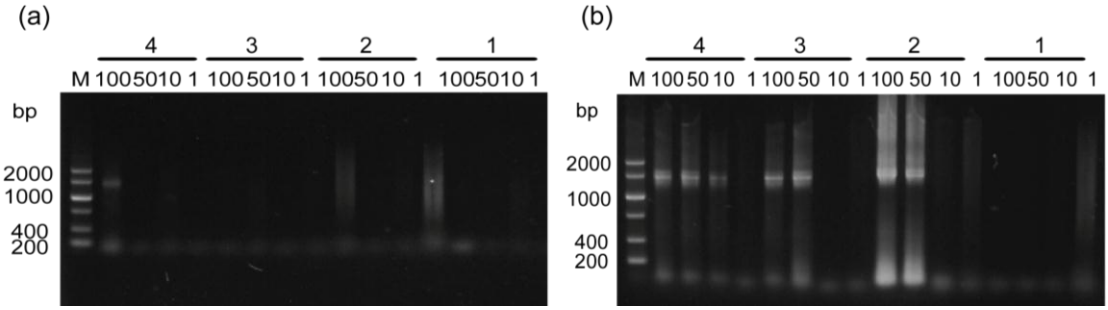

Fig. 2 Agarose gel electrophoresis of PCR products of crude (a) and purified DNA (b) from corn straw biogas slurry by four different approaches.

1-4: direct SDS lysis, direct bead lysis, indirect SDS lysis, indirect bead lysis; M: DNA Marker V; 1, 10, 50, 100: dilution ratio

PCR-RFLP analysis. RFLP was conducted to give further insights into the genetic diversity in DNA extracts from four DNA extraction approaches [9]. Different numbers of amplification cycles (15, 20, 25, 30, 35, 40, 45 cycles) were used to assess whether amplifications had been taken to saturation. The results showed the best PCR cycle for different methods were all 20 (Fig. 3a, e.g., indirect bead-based method). The bacterial 16S rRNA genes were amplified with 1/100×DNA template and 20 cycles. The RFLP map showed that DNA from indirect bead-based method, indirect SDS-based method and indirect bead-based method gave similar RFLP profiles, suggesting that DNA extracts from the three methods had the same genetic diversity (Fig. 3b).

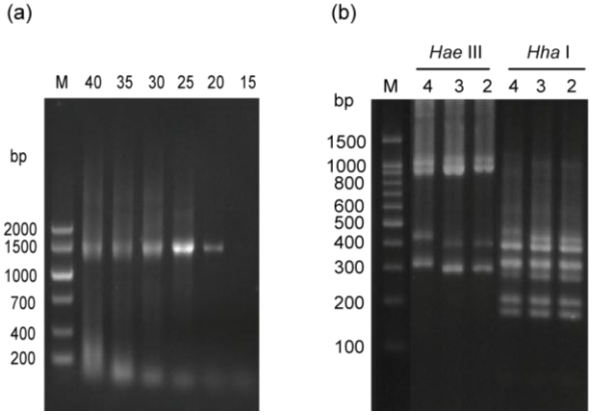

Fig. 3a PCR amplification of bacterial 16S rRNA with different cycles. M: Marker V
Fig. 3b RFLP patterns of bacterial 16S rRNA. 2-4: direct bead lysis, indirect SDS lysis, indirect bead lysis; M: 100 bp DNA Marker

Conclusions

In this study, four DNA extraction methods for corn straw biogas slurry, i.e., direct SDS lysis, direct bead lysis, indirect SDS lysis and indirect bead lysis were evaluated by comparing DNA yield, DNA purity, PCR amplifiability, and RFLP of amplified 16S rRNA genes. Direct methods yielded higher amounts of DNA than indirect procedures, but its DNA purity was lower. PCR amplification was successful with crude DNA from indirect methods, but not direct methods. However, PCR

products could be obtained with purified DNA from direct bead-based method. PCR-RFLP analysis indicated direct bead-based method, indirect SDS-based method and indirect bead-based method could obtain PCR-amplifiable DNA for molecular ecology.

Acknowledgements

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