Extraction method of soil microbial DNA for molecular ecology research

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Abstract

The effective of three soil DNA extraction methods which based on different lytic principles for isolation of the total farmland microbial DNA were compared and comprehensive evaluated for the yields and purity. The results show that the chemical-enzymatic-metchanical method got the highest total DNA yield, but with the highest humic acid contamination which will strongly restrain the following PCR and DGGE analysis. The chemical-enzymatic method obtained the best DNA with the highest molecular weight and purity and was more propitious to molecular ecology study. [Life Science Journal. 2007;4(1):85-88] (ISSN: 1097-8135).

Keywords: molecular ecology; soil microbial DNA; extraction method

1 Introduction

Soil is the most important habitat for microbes. Any change of the environment can influence the composition of the microbial community, and a certain environment has its unique microbial composition. So the diversity of the microbial community in soil is an important issue in modern soil microbiology[1]. Routine methods for detection of bacteria in soil usually use methods which are based on the culture method, by which only 0.1% - 3% of the total bacterial population can grow under laboratory condition with artificial media[2]. So the result can't truly reflect the original community composition of the soil. In order to solve this problem, many molecular strategies were introduced to the soil ecology study[3]. Molecular methods which analyse DNA directly extracted from the soil samples can study microbial diversity in soil samples without cultivation[4,5]. Now the soil DNA analysis has replaced the soil bacterial culture to evaluate the microbial diversity in the ecology study[6]. The molecular techniques based on total community DNA extracted from soil have been widely applied[7]. These include community DNA hybridization[8], single-strand-conformation polymorphism (SSCP) analysis of PCR products of 16S rDNA[9], denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE)[7], and so on. However, extraction of DNA is not simple, as the soil contains a number of compounds such as humic acids, phenolic compound and heavy metal[10]. So the critical step is separation of DNA from humic substance since these are acid macromolecules. That will interfere with the following PCR amplify and other molecular analysis[4]. Thus, the application of a proper DNA extraction protocol is critical[10]. As PCR is critical for the following molecular analysis, it's important to identify the DNA function by PCR analysis.

In this study, we compared three DNA isolation methods which stand for three different techniques based on their lytic principle. They are the typical delegate of chemical-enzymatic method, chemical-mechanical method and chemical-enzymatic-mechanical method. The three way of soil DNA extraction will be valued with same samples. Attention was paid to the efficiency, the quality (the fragment size) and the yield of the different extraction method, and the DNA function will be compared by PCR with 2 primers.

2 Materials and Methods

2.1 Soil sample

The soil was sampled under the ground at the deep of 0 - 10 cm and 20 - 30 cm respectively on the farmland, located at Gouzhao of Zhengzhou, China, which was rotated maize and wheat for more than 10 years. The samples were stored at -20 ℃ during the experiment period.

2.2 DNA isolation

Three DNA extraction methods were applied in parallel, 5 g sample was used for each method. The key differences of the three methods were listed in Table 1.

Method 1 was the chemical-enzymatic method, in

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which the nucleic acids were extracted by the modified protocol[11]: 5 g of soil samples, 13.5 ml of DNA extraction buffer (100 mM Tris pH 8.0; 100 mM sodium EDTA, pH 8.0; 100 mM sodium phosphate, pH 8.0; 1.5 M NaCl; 1% CTAB) and 100 μl Proteinase K (10 mg/ml) in a oakridge tube. Those were mixed with by horizontal shaking with 225 rpm at 37 ℃ for 30 minutes to get the sample mixture. Add 1.5 ml of 20% SDS to the sample mixture and to be incubated for 2 hours at 65 ℃ water bath with gentle inversion each 15—20 minutes. To be extracted by the centrifuge with 6000 rpm for 10 minutes at room temperature and transfer the supernatant to a 50 ml centrifuge tube. Continue to be extracted two times by adding 4.5 ml of the extraction buffer and 0.5 ml of 20% SDS with 10 seconds. Vortex, incubating at 65 ℃ for 10 minutes and centrifuge too. Transfer and combine the three times extraction supernatant to a 50 ml centrifuge tube.

Method 2 was the chemical-mechanical method which was derived from Kuske's[12] method. 10 ml of TENS buffer (50 mM Tris, pH 8.0; 20 mM disodium EDTA; 100 mM NaCl; 1% sodium dodecyl sulfate) was added to 5 g soil samples, then to be mixed thoroughly by vortex and to be incubated in a 70 ℃ water bath for 1 hour. The samples were mixed well at 15-minute intervals during the incubation, and then were centrifuged at 6000 rpm for 10 minutes, and the supernatant was collected. The soil pellet were washed with 5 ml of TEN buffer (TENS buffer without sodium dodecyl sulfate) and centrifuged again. Drop the supernatant and then the soil pellet was re-suspended in 7.5 ml of TEN buffers and exposed to three sets of thermal shocks by immersion of the tubes at − 20 ℃ for 10 minutes. Followed by rapid thawing in a 65 ℃ water bath. After centrifugation at 6000 rpm, the supernatants were collected.

Method 3 was the chemical-enzymatic-mechanical method invented by Tsai[13]. 5 g soil samples were mixed with 10 ml of 120 mM sodium phosphate buffer (pH 8.0) by shaking at 150 rpm for 15 minutes. The slurry was palletized by centrifugation at 6000 rpm for 10 minutes. The pellet was washed again with phosphate buffer, re-suspended in 10 ml of lyses solution I (0.15 M NaCl; 0.1 M disodium EDTA, pH 8.0) containing 15 mg of lysozyme/ml, and incubated in a 37 ℃ water bath for 2 hours with agitation at 20 to 30 minutes intervals, and then 10 ml of lyses solution Ⅱ (0.1 M NaCl; 0.5 M Tris-HCl, pH 8.0; 10% sodium dodecyl sulfate) was added. Three cycles of freezing in −20 ℃ and thawing in a 65 ℃ water bath were conducted to release DNA from the microbial cells in the soil, and then centrifuged at 6000 rpm for 15 minutes to get the supernatants.

Each supernatant which got from the different methods was mixed with an equal volume of chloroformisoamyl 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 hour. The pellet of crude nucleic acids was obtained by centrifugation at 12000 rpm for 20 minutes at room temperature, washed with cold 70% ethanol, and re-suspended in sterile deionized water, and to give a final volume of 500 μl.

Table 1. Treatments for soil sample lyses in different methods

Method	Lyses treatment		
	Mechanical	Chemical	Enzymatic
1	SDS CTAB	Proteinase K	
2	SDS		Freezing and thawing
3	SDS	Lysozyme	Freezing and thawing

The DNA quality and quantity were compared and estimated by agarose gel electrophoresis and spectrophotometry.

Samples of extracted DNA were analyzed in 0.8% agarose gel containing 1 μg of ethidium bromide per ml. To determine the quality of extracted DNA, the concentration of DNA in the crude extraction was determined spectrophotometrically at 260 nm. Spectrophotometric A260/A280 and A260/A230 ratios were determined to evaluate levels of protein and humic acid impurities, respectively[11].

2.3 PCR analysis

The DNA was purified with the TaKaRa agarose gel purification kit. And then we used two different universal primers to amplify the 16s rDNA and the V3 fragments. F338gc and R518 were used to amplify the V3 fragments sized about 260 bp[7], BR8 and BL1541 were used to amplify the 16s rDNA fragments sized about 1500 bp[13], and the PCR reaction system and cycling condition have been described previously[7,11]. The PCR products were analysed by electrophoresis in 1.5% agarose gels and ethidium bormide staining.

3 Results

Figure 1 showed that the quality of the DNA extracted from the two soil samples with the different method have notable difference. DNA isolated from method 1 yielded fragments of larger molecular size than the other method. From the electrophoresis result in the agarose gel we can see that, the amounts of DNA extracted from the two samples differed greatly. With the first method we got high molecular weight and few small DNA fragment. The fragment is larger than 20 bp. The DNA got by the other two methods was not very good, as the fragments of the DNA are not identical and yield

broad spectra. And the second method is better than the third one, because it yield lighter broad spectra and higher molecular size. We can also learn that each method has a good reproducibility.

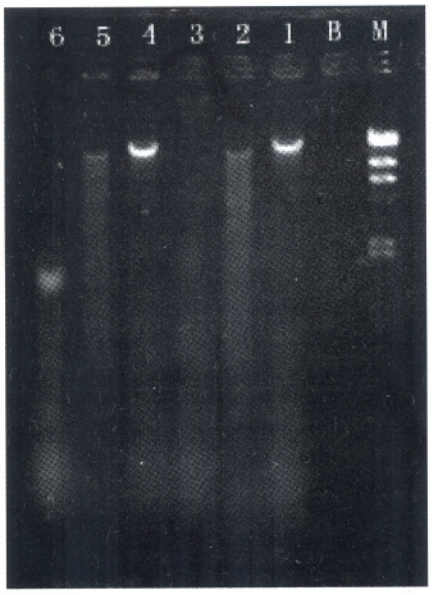

Figure 1. DNA extracted with different method. M: Marker digested by λ-*Hind* Ⅲ; B: Blank; Lane 1 and Lane 4: DNA extracted by method 1; Lane 2 and Lane 5: DNA extracted by method 2; Lane 3 and lane 6: DNA extracted by method 3.

The effect of three different methods on DNA yield and purity is apparent in the Table 2. It shows that the quality and purity of the DNA got from three different methods are discrepant. The absorbency under 260 nm stands for the concentration of the DNA, and the absorbency under 280 nm and 230 nm show us the content of the protein and humic substance. The purity of DNA was assessed spectrophotometrically by calculating A260/A230 and A260/A280 ratios for humic acid contamination and protein impurities respectively. Though the quality of the DNA yield from the first method is lower than the other two methods, the purity is higher. So the first method which is based on chemical-enzymatic method is reproducible and has high efficiency.

Table 2. The OD of the DNA extracted by different methods

Method	Deep (cm)	OD				
		230 nm	260 nm	280 nm	A260/A280	A260/A230
1	0—10	0.319	0.267	0.230	1.161	0.837
	10—20	0.267	0.240	0.189	1.270	0.899
2	0—10	0.464	0.361	0.312	1.157	0.780
	10—20	0.340	0.239	0.209	1.144	0.703
3	0—10	0.842	0.339	0.409	0.829	0.403
	10—20	0.653	0.410	0.380	1.079	0.628

DNA isolated using different methods were amplified with universal bacterial primers in PCR reactions, and the result was shown in Figure 2 and Figure 3. PCR amplification of the 16S rDNA and the V3 fragments were successful by method 1 and method 2, but failed to get any PCR fragment by method 3. And the quality and yield of the product got by method 1 are best. This result also indicated that the chemical-enzymatic method is more suitable for the microbial molecular ecology research.

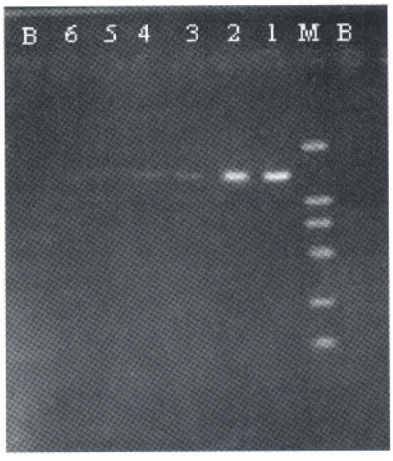

Figure 2. Agarose gel electroctiophoresis of 16s rDNA amplified by different DNA. M: DL2000; B: Blank; Lane 1 and Lane 2: DNA extracted by method 1; Lane 3 and Lane 4: DNA extracted by method 2; Lane5 and Lane 6: DNA extracted by method 3.

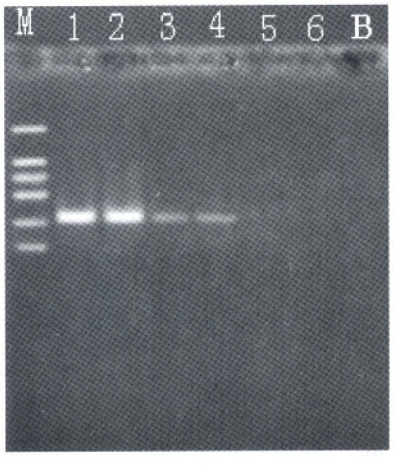

Figure 3. Agarose gel electroctiophoresis of the V3 fragments amplified by different DNA. M: DL2000; B: Blank; Lane 1 and Lane 2: DNA extracted by method 1; Lane 3 and Lane 4: DNA extracted by method 2; Lane5 and Lane 6: DNA extracted by method 3.

4 Discussion

A large number of methods have been published for the extraction of total microbial community DNA from

soils[4,11,12,13]. We can classified them into three kinds of method by their lytic principles: chemical-enzymatic, chemical-mechanical and chemical-enzymatic-mechanical method. Our result shows that with the chemical-enzymatic method, we can get the best DNA with high molecular weight and purity. The help of enzyme and the chemical substance such as SDS and CTAB can make the cell lyses more efficiency. But with the freezing and thawing method, when the cell was destroyed, the DNA fragments were also broken. So the DNA got with the other two methods is smeared. So the DNA extracted with the enzyme and chemistry substance is more efficient. It's more suitable for the further molecular analysis.

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