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Metagenomic DNA extraction from soil and sediments by silica-based method

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ABSTRACT

A new method is described for extraction of metagenomic DNA from soil and sediments which is based on DNA adsorption to silica without the use of Alchols, precipitation or a cesium chloride gradient. High-quality DNA was obtained, and PCR inhibition was overcome by adding bovine serum and adjusting magnesium low concentration. By using PCR-DGGE with Firmicutes and lactic acid bacteria-specific primers the extracted metagenomic DNA was shown to contain a mixture of bacterial genomes. This method can be used for screening bacterial diversity in soil and sediment samples.

Keywords: Metagenomic DNA - Microbial community - Sediments - Soils - DGGE

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INTRODUCTION

The study of the microbiological structure and composition of complex ecosystems such as soil and sediments is important for a better understanding of bacterial community physiology, for the development of new approaches in bioremediation and recycling, and for discovering new biotechnology applications. Research in this area has been encouraged by the development of culture-independent approaches [1–3] such as PCR-DGGE (denaturing gradient gel electrophoresis), which can provide an early overview of species richness in a particular sample/ecosystem and offers clues on the nature of resident microorganisms. PCR analysis of metagenomic DNA is a sensitive, specific method for detection and monitoring of microorganisms in environmental samples. However, successful detection and characterization of microbial DNA in the environment requires efficient extraction of metagenomic DNA ($_{mg}$DNA) and its adequate purification to remove the contaminants that may inhibit Taq polymerase and other enzymes used in molecular studies [4].

Different methods exist for direct isolation of DNA from soil and sediments and they mostly vary in the procedures of sample preparation and the removal of contaminants that may affect the quality of DNA [5–10]. Most of them involve the removal of humic material through polyvinylpolypyrrolidone (PVPP) and size exclusion chromatography. The extraction buffer, the highly degraded DNA, and other contaminants (such as enzyme inhibitors) are commonly eliminated either by DNA precipitation with isopropanol, ethanol, or polyethylene glycol [11] or by column/membrane filtration [12], thus increasing cost and sample manipulation. Commercial soil DNA extraction kits are expensive and thus impractical for the large-scale sampling studies. Therefore, the aim of this study was to develop a simple, effective method for the recovery of high molecular weight, metagenomic DNA from sediment and soil samples that could be suitable for PCR-based methods and restriction enzyme analysis.

MATERIALS AND METHODS

Sediment and Soil Samples

Using a 5-cm diameter PVC pipe, sediment samples were taken up to 20-cm depth from a coastal marsh (‘Laguna Rosada’) on the north coast at the Yucatan Peninsula, Mexico. Soil samples were collected with a sterilized spoon, from a poultry farm and an orange orchard in the state of Yucatan, Mexico. All samples were placed in sterile nylon bags, manually homogenized and frozen at −20°C until further analysis.

Preparation of 4% Silica Suspension

An aliquot of 2 g of SiO_2 (Sigma S-5631) was placed into a 50-ml centrifuge tube and thoroughly resuspended in 15 ml of distilled water. The suspension was then centrifuged for 2 min at 1,000g at room temperature (RT) and supernatant was discarded. After a second wash

under the same conditions, the resulting pellet was resuspended in 50 ml of distilled water and stored at RT.

DNA Extraction

The initial DNA extraction was assayed according to the method proposed by Zhou et al. [13] with samples taken from poultry farm and coastal marsh sediments. The method was scaled down to start with 0.5 g of sample and followed as described but column purification step was not assayed.

For DNA extraction by the silica-based method, 0.5 g of each sample was poured into a 1.7-ml microcentrifuge tube with the addition of 1 ml of TEN buffer (100 mM Tris–HCl, 50 mM EDTA, 500 mM NaCl, pH 8.0) and vortexed for 1 min. After centrifugation for 10 min at 10,000g at RT, the pellet was washed second time under the same condition and finally resuspended in an 1 ml of TEN buffer with 0.2 mg of added lysozyme and incubated for 1 h at 37°C under gentle agitation. After incubation, three cycles of freezing/defrosting were done by incubation 10 min in an ice/alcohol bath and then 5 min at 65°C. An aliquot of 100 µl of 20% SDS was added and vortexed for 1 min and incubated for 30 min at RT. After centrifugation for 10 min at 10,000g at RT, the supernatant was transferred into a new microcentrifuge tube and 500 µl of 5 M potassium acetate was added and then incubated at 65°C for 5 min. Tubes were placed in an ice bath for 20 min and centrifuged at 20,000g for 30 min at 4°C. The supernatant was transferred into a new microcentrifuge tube and 200 µl of silica suspension was added and incubated at RT for 2 min with gentle agitation. Silica-DNA complex was recovered by centrifugation at 16,000g for 2 min at RT and the pellet was washed by adding 1 ml of 70% ethanol. DNA was eluted from silica with 50 µl of sterile distilled water and incubating at 55°C for 5 min and recovered by centrifugation at 16,000g for 5 min at RT.

PCR Amplification and DGGE

Two fragments of 16S rRNA of each extracted metagenomic DNA were PCR amplified by using primer pairs 16SS/16SR (universal) (1,300 bp) and FirF:369/FirR:1244 (Fimicutes) (850 bp) (Table 1). An aliquot of 50 µg of DNA was used as template in a reaction mixture containing 200 mM Tris–HCl, pH 8.4, 500 mM KCl, 200 µM deoxynucleoside triphosphates, 0.2 µM each primer, 2.5 U of Taq DNA polymerase (Invitrogen), and bovine serum albumin (BSA, Sigma Cat. #05491) at the concentration indicated in the legends of Figs. 1 and 2. $MgCl_2$ was standardized for each primer combination. Amplification was done in a thermal cycler My Cycler (BIO-RAD, Hercules, CA) with an initial denaturation of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 45 s at 45°C (for primers 16SS/16SR) or 63°C (for primers FirF:369/FirR:1244) and 1 min at 72°C with a final extension of 5 min at 72°C. An aliquot of 1 µl of products obtained with primers FirF:369/FirR:1244 was re-amplified with the nested primer gcPedio2:644 and using FirR:1244 as reverse (Table 1), in a reaction mixture essentially the same as described above but $MgCl_2$ was kept at 1.5 mM and no BSA was added. Amplification was done with an initial denaturation of 5 min at 94°C, followed by 25 cycles of 1 min at 94°C, 45 s at 65°C and 1 min at

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72°C with a final extension of 5 min at 72°C. Amplicons were checked by electrophoresis on 1% agarose gel in TBE buffer (89 mM Tris–HCl, 89 mM boric acid, 2 mM EDTA, pH 8.0). For DGGE analysis, a denaturing gradient between 10 and 35% was used in an 8% (100% denaturing solution is 8 M urea and 40% formamide) polyacrylamide gel. Samples were electrophoresed at 60°C, and 80 v, for 16 h in TAE buffer (40 mM Tris–HCl, 20 mM acetic acid, 1 mM EDTA, pH 8.0). Gels were stained with SYBR Gold (Invitrogene).

Table 1 Primers used for the amplification of $_{mg}$DNA

Primer	Sequence (5′ →3′)	References
16SS	AGAGTTTGATCCTGGCTC	[17]
16SR	CGGGAACGTATTCACCG	
FirF:669 (Forward)	GGAGGCAGCAGTAGGNAATCTTC	This study
FirR:1244 (Reverse)	TAGCCCArGTCATAAGGGGCATG	
gcPedio2:644[a] (Forward)	GATTTATTGGGCGTAAAGCGA	

Degenerated position in primers: R = A or G; N = A or G or C or T
[a]gc, GC clamp as previously described [3]

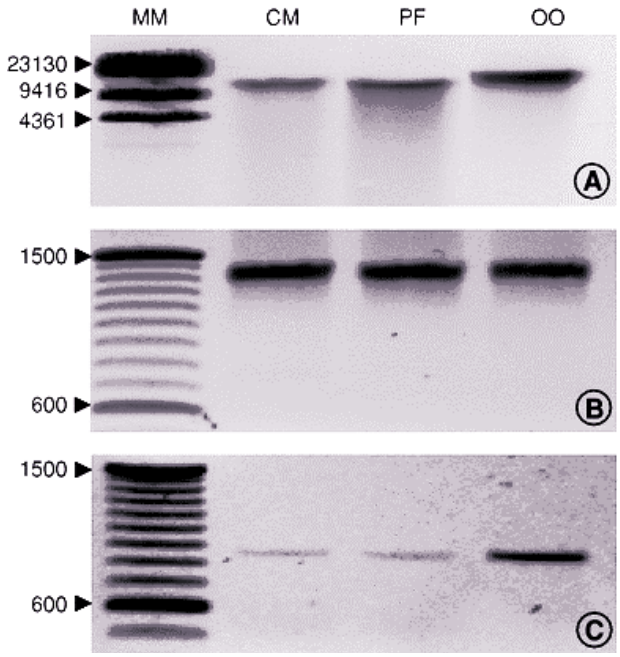

Fig. 1 (a) DNA obtained from poultry farm soil (PF) and coastal sediment (CM) samples by using the method reported by Zhou et al. [13]. (b) Amplification of the resulting DNA with universal primers 16SS and 16SR and using BSA as a facilitator. MM, molecular markers; λ/Hind III fragments (a) and 100 bp ladder (b)

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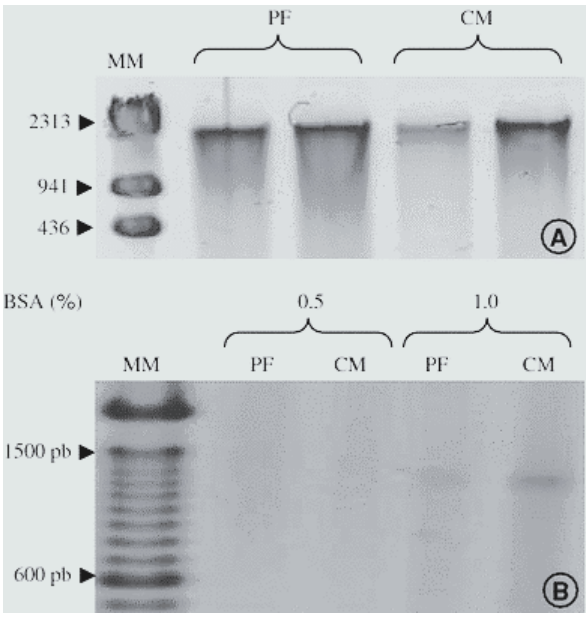

Fig. 2 (a) DNA obtained from sediment and soil samples by the silica method. (b) PCR products amplified with universal primers 16SS and 16SR, using 0.1% BSA and 7 mM $MgCl_2$. (c) PCR products amplified with phylum-specific primers FirF:369 and FirR:1244 using 0.5% BSA and 6 mM $MgCl_2$. MM, molecular markers, λ/Hind III fragments (a) and 100 bp ladder (b, c). CM, sediments from coastal marsh. PF, soil from poultry farm. OO, soil from orange orchard. Samples were electrophoresed on 1% agarose gel in 1× TBE buffer

RESULTS AND DISCUSSION

Samples used in this work diverge in their content of organic matter and ionic load. Those coming from poultry farm are mainly composed of a mixture of soil, feces, and decomposing rice husk and/or wood shaving used for chicken breeding, while samples from sediments contain the highest concentration of salt. These two samples were initially used to assay the protocol described by Zhou et al. [13] without column purification, since it might be a restrictive step when a large set of samples need to be processed. Resulting DNA from two independent extractions are shown in Fig. 1a and quantitative data from three extractions are listed in Table 2. Although DNA of high molecular weight was obtained, quantitative data showed high levels of contamination with phenol derivatives, reflected in a low $A_{260/230}$ ratio. Besides, amplification was only successful when BSA was increased up to 1% in the reaction mix and still a very faint band was obtained for DNA extracted from poultry farm soil. Moreover, high concentration of BSA makes difficult to recover PCR products due to the coagulation of the protein caused by continuous heating/cooling cycling.

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Table 2 Quantitative data from three independent extractions by the silica-based method and as described by Zhou et al. [13]

Sample	DNA yield (µg/g of sample)		$A_{260/280}$		$A_{260/230}$	
	Silica	Zhou et al.	Silica	Zhou et al.	Silica	Zhou et al.
Coastal marsh sediments	14.9 ± 1.0	9.7 ± 0.7	1.60 ± 0.17	1.45 ± 0.1	0.50 ± 0.02	0.32 ± 0.01
Poultry farm soil	6.5 ± 0.3	8.3 ± 0.5	1.57 ± 0.04	1.32 ± 0.08	0.65 ± 0.05	0.29 ± 0.01
Orange orchard soil	8.6 ± 0.3	n.a[a]	1.46 ± 0.06	n.a[a]	0.51 ± 0.09	n.a[a]

[a]n.a, not assayed

By using the silica-based method described here, it was possible to extract up to 14.9 µg of DNA per gram of sediments and up to 8.6 µg from orchard soil. The lowest DNA yield was obtained from poultry farm soil where 6.5 µg of DNA per gram of sample was obtained (Table 2). The contamination of samples with polyphenol derivatives was evident in the 230/260 absorbance ratio that was over 0.5 being the soil taken from poultry farm the more contaminated.

Washing of samples was critical and for sediments, at least two washes must be done to eliminate salt, because otherwise the DNA may not bind to the silica. It is also helpful to remove soluble contaminants, such as metal ions and organic acids, which can inhibit PCR. Although some authors recommend the use of PVPP to adsorb humic compounds [14, and references therein], in our trials this did not improve DNA quality and decreased DNA yield (also reported by Zhou et al. [13]), although PVPP may be helpful in clay or sand-rich soils.

The DNA extracted had a high molecular weight, with a size near to 20 kb and showed scarce evidences of degradation (Fig. 2a). Inhibition on Taq polymerase caused by contaminants co-purified along with DNA could be overcome by adding BSA (0.1% or 0.5%, see Fig. 2 legend) to PCR reaction [15]. The magnesium concentration was also carefully standardized as it may fluctuate according to primer combination and sample source. Once the optimal reaction conditions were determined, a unique PCR product of the expected size was obtained for both primers combination tested (Fig. 2b, c). In order to reveal the metagenomic character of the extracted DNA, a semi-nested PCR was performed on amplicons obtained with primers FirF:369/FirR:1244. The nested primer was gcPedio2:644 (forward) and FirR:1244 was used as reverse (Table 1). This primer pair generated a unique band in agarose gel electrophoresis corresponding to a fragment of 600 bp (data not shown), but after a separation by DGGE, up to ten major bands were visible (Fig. 3) demonstrating that the template DNA contains several genomes.

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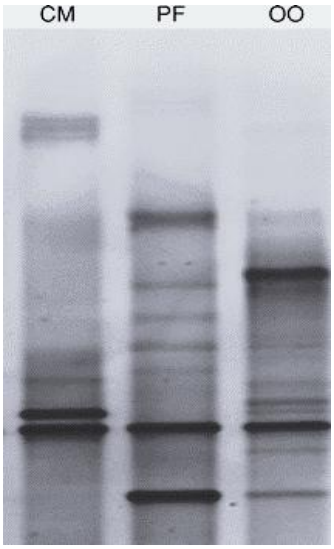

Fig. 3 DGGE patterns of $_{mg}$DNA extracted from sediment and soil samples by the silica method and amplified with primers FirF:669/FirR:1244 and reamplified with gcPedio2:644/FirR:1244. CM, sediments from coastal marsh. PF, soil from poultry farm. OO, soil from orange orchard

Recently, a protocol for purification of DNA from soil was published [16] that included an additional purification step by using Q-Sepharose that reduced the humic acids content by 84%. Unfortunately no evidence is shown neither on the amplificability of the resulting DNA (Table 3) nor on the applicability of this method to different soil samples. In the same way, some features from other protocols described in the literature are summarized in Table 3. Conversely, the protocol described in the present paper rendered a $_{mg}$DNA that can be efficiently amplified and that is applicable to soil samples with different organic matter and salt load.

Table 3 Performance of the silica-based method as compared to other extraction methods described in the literature

Extraction method	Sample	Time h)	Amplificability	Metagenomic character of DNA	References
Tsai and Olson	Soil and sediments	7	Yes[a]	n.t.	[18]
Jacobsen and Ramussen	Soil (sandy loam)	4	n.t.[b]	n.t.	[8]
Zhou et al.	Soil	6	Yes[a]	n.t	[13]
Sharma et al.[c]	Soil (hot springs)	4–5	n.t.	n.t.	[16]
Silica-based	Soil and sediments	4–5	Yes	Yes	This study

[a]An additional column purification step is required
[b]Not tested
[c]Only encompasses an additional purification step to the method of Zhou et al. [13]

In this paper we report a simple and cheap method without the use of column purification, which yields a good quality and high molecular weight $_{mg}$DNA from different types of soils and sediment that can be successfully amplified by PCR for studying microbial diversity and functionality in complex communities. We are currently applying this method to extract

DNA from sediments and soils to study the diversity and distribution of lactic acid bacteria in coastal marshes and associated to stockbreeding. This research is focused on finding new bacterial strains with novel characteristics that possibly will bear new biotechnological applications.

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