A Versatile Metagenome Purification Method to Identify Uncultivable Bacteria by Denaturing Gradient Gel Electrophoresis (DGGE) from Sediments and Soils

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**Abstract**

Here, we report a versatile metagenome purification method to identify uncultivable species from sediments and soils by nested-PCR-DGGE according to 16S rDNA. This combination of methods uses enzymatic lysis *in situ*, polyvinylpyrrolidone (PVP), Chelex 100, glass bead-silica gel and chaotropic salts, with the advantage that it can be applied to different soils.

**Keywords:** Metagenome purification; Agriculture; Costal lagoon; Hypersaline soils; DGGE; rDNA 16S

Metagenomics is the study of the genomic repertoire of all the organisms living in a particular environment and their activities as a collective [1]. Metagenomes have been purified from soils and other niches, with an estimated $10^3$-$10^7$ species/g [2,3]. To determine the diversity of a microbial community, a common gene present in all species, such as the 16S ribosomal gene (16S rDNA), is amplified by PCR and their amplicons are separated by DGGE, or can be used to generate libraries [4,5]. Since, each amplicon is derived from one genome; their sequences provide taxonomic information and the physiological connections of every species within the community. Therefore, the sequences of the primers, metagenome purity and integrity are essential factors for biodiversity determinations.

A common protocol or commercial kit for metagenome purification from soils or sediments has not been published yet. However, all of them aim to 1) obtain the majority of genomes to represent actual diversity, 2) keep the integrity of each genome, and 3) eliminate humic substances which can inhibit PCR [3,4].

Several protocols were combined to obtain a general method for metagenome purification from sediments of coast lagoons with different salinity concentrations, forests with petrified waterfalls or geysers and soils employed as garbage collector from agricultural and livestock sector (papaya harvest, henequen production and cattle farm). Such method was used to determine the presence of *Lactobacillales* and *Firmicutes* strains with nested-PCR-DGGE.

To prevent cellular lysis by osmotic change and to eliminate debris and humic acids in hypersaline samples, 0.5 g of sediments were homogenized/washed in 5 ml TEN Buffer (100 mM Tris-HCl, pH 7.5; 10 mM EDTA, pH 8; 200 mM NaCl) and centrifuged at 4,000 g for 5 min. All other sediments were washed 4 times in TE Buffer (without NaCl). Cell lysis was carried out *in situ*, the sample was resuspended in 1 ml of TET buffer (100 mM Tris-HCl, pH 8; 10 mM EDTA, pH 8; 1% Triton X-100 (W/V) with 500 μg/ml hen egg white lysozyme (USP)) and was heated in a micro-wave oven [7] at 300 Watts for 5 seconds (applying >400 Watts may result in soil blow ups), and incubated twice for 5 min at 55°C. Proteinase K (500 μg/ml; Invitrogen) and 0.05 ml of SDS at 10% (W/V) were added and incubated as before.

**Figure 1:** Bacterial communities were determined by nested PCR-DGGE. Soil samples were from hypersaline sediment (lane 1), lagoons (2-4), garbage collector from agricultural sectors (5-6), forest with petrified waterfalls and geiser (7-8) and cattle farm (9). Geographic localizations are described in Table 1. The numbers indicate the sequenced amplicons. The amplicons reported in the Gen-Bank appear in italics and underlined. The arrow shows the direction of the urea gradient.

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The differences with other protocols or commercial kits account for the use of this method for diverse soils. The microwave heat-enzymatic-Chlex 100 treatments released complete genomes eliminating the need of phenol-chloroform extractions. Furthermore, PVPP and glass silica beads removed most contaminants that inhibit PCR-DGGE. Therefore, with this strategy bacterial diversity can be determined fast and without the requirement of any special equipment.

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