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 activities 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 (Buffer TEN without NaCl) and centrifuged. Cell lysis was carried out in situ [6], 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 (USB)) 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.

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RNASpA (50 mg/ml; USB) was mixed and incubated for 5 min at 55°C. Samples were then centrifuged at 10,000 g for 10 min. To precipitate the SDS, the solution was chilled on ice for 10 min and centrifuged at 11,000 g for 5 min at 25°C. Metagenome was precipitated with 0.3 vol. of phenol-chloroform extractions. Furthermore, PVPP and glass silica beads removed most contaminants that inhibit PCR-DGGE. Therefore, Chelex 100 treatments released complete genomes eliminating the need of this method for diverse soils. The microwave heat-enzymatic process released DNA from diverse soils for studies in microbial ecology. Curr Issues Mol Biol 5: 1-8.

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The average metagenome concentration in hypersaline and coast sediments was 60 μg/ml, whereas for other soils ranged from 2 to 3 times more (Table 1). To determine the metagenome performance, we monitored Lactobacillales and Firmicutes strains diversity by means of nested PCR-DGGE with the regions V1-V2 from rDNA 16S genes. Monitoring with Ribosomal Data Base Project, indicated that 3 of them (from in extremis soils and in cattle farms) were ~42% homologous to Firmicutes; 4 were 41-56% homologous to Proteobacteria; and the homology of 5 amplicons was lower than 17%. The sequence from the other 13 amplicons was not determined (Table 1).

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