

ENHANCEMENT OF SOIL DNA EXTRACTION BY THE USE OF A HAND HELD MIXER

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SHORT COMMUNICATION

ABSTRACT

The efficiency of soil DNA extraction using a bead beater homogenizer (Biospec Products - Germany) was compared to that obtained with a hand held mixer (Moulinex - Brazil). The hand held mixer costs 100 times less and extracted seven times more crude DNA than the bead beater.

Key words: molecular tools, DNA extraction.

Several procedures have been useful in the evaluation of the microbial ecology in a specific environment. However, some of the methods present limitations regarding sensibility. The traditional technique of plating on agar media fail to recover many populations, since more than 90% of the microorganisms that inhabit the soil can not be cultivated (1). A new methodological approach of molecular genetics to monitor the environment has been developed. It requires the soil DNA extraction. Once DNA is extracted, other methodologies based on the PCR amplification of a conserved region of genes in different groups of microorganisms (3,6) have been used to study soil diversity. For this reason, techniques for direct extraction of soil DNA have been tested and enhanced (2,5). In this study, the efficiency of DNA extraction in four soil samples using two different protocols was evaluated. In one, a mini bead beater, developed by Biospec Products (Germany) as a cell homogenizer was used. In the other, a common household kitchen mixer, made by Moulinex (Brazil), was tested.

Soil samples from fields facing different levels of degradation were collected in a core of 10 cm depth and immediately frozen at -70°C. Genomic DNA was then extracted from soil samples following a modification of the method of Smalla *et al.* (5), using the mini-bead beater as well as the hand held mixer to induce cells lyses. Four grams from each soil sample were suspended

in 4 ml of 0.12 mol⁻¹ phosphate buffer (pH 8.0). The resulting suspensions were shaken for 5 min at room temperature and incubated at 4°C for 30 min. After incubation, 480 mL of 20% duodecil sodium sulphate (SDS) was added and suspensions were kept on ice. From this point on, the buffer used in the mini bead beater was amended with 0.8 g of glass beads (0.1 mm diameter), and the buffer used for the hand held mixer was supplemented with lysozyme (Sigma, Co.) at a final concentration of 5 mg mL⁻¹. In both methods the mixtures were shaken three times for 90 s. at the highest speed and then were centrifuged at 14,000 g at room temperature. The resulting suspensions were submitted to the same extraction procedures described by Smalla *et al.* (5). The crude DNA preparations were then subjected to two sequential cleaning steps: cesium chloride (CsCl) and potassium acetate (Kac) (4). A final wizard spin column (Wizard DNA clean-Up System, Promega, Madison, WI, USA) purification was performed according to the manufacturer's instructions. The DNA obtained by using both methods was quantified in a fluorometer and also estimated on 1.5% agarose gel, stained with ethidium bromide and photographed with an Eagle Eye System (Stratagene). The Gibco-BRL 100 bp ladder was used to determine the sizes of the fragments. The amount of DNA recovered in the crude preparations by cells lyses induced in the mini bead beater resulted in 0.5 to 1.0 mg of DNA

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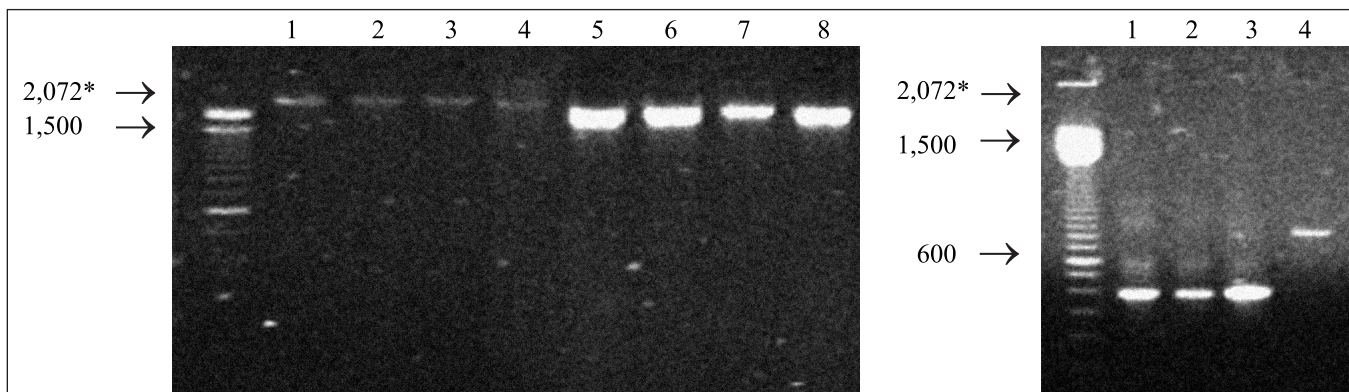


Figure 1. A: DNA (10 mL sample per well) obtained from 4 soil samples by using two procedures. Lanes 1 – 4: DNA extracted with a mini bead beater; Lanes 5 – 8: DNA extracted with a hand held mixer; **B:** Polimerase reaction (PCR) products of amplified soil DNA extracted with a hand held mixer. Lanes 1-2-3: amplification of the nuclear 28S subunit rDNA gene; Lane 4: amplification of the nuclear 18S subunit rDNA gene. * Fragments size (100 bp, Gibco-BRL, Gaithersburg, MD, USA).

per gram of soil. The cells lyses induced by the hand held mixer resulted in a considerable improvement of DNA recovery, i.e. from 5.0 to 7.5 mg of DNA per gram of soil (Figs. 1 and 2). It was also tested if the DNA yielded by the hand held mixer was clean enough to be amplifiable. For this reason the DNA extraction products obtained by using the hand held mixer were submitted to a PCR amplification of the nuclear 18 S and 28 S small-subunit rDNA according to standard protocols (3,6). The products were successfully amplified generating around 400 bp for 28 S region and 950 bp for 18 S region (Fig. 1). This procedure is a reliable, simple and affordable for enhancement of crude soil DNA extraction. The DNA extracted by using the hand held mixer yielded higher amounts of pure DNA and it accounts for a 100 times less the cost of the mini bead beater.

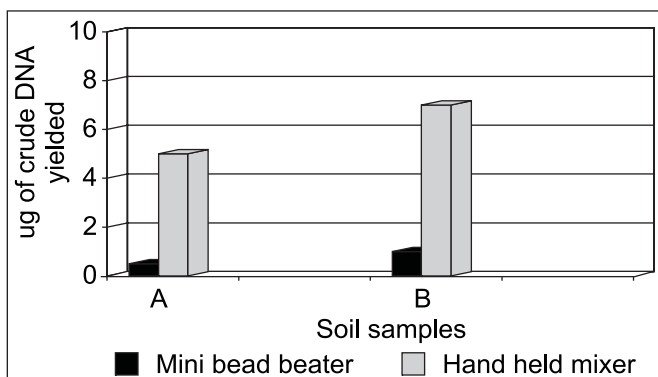


Figure 2. Amount of DNA yielded by the two extraction methods: a hand held mixer and the mini bead beater.

RESUMO

Otimização da extração de DNA do solo utilizando um homogeneizador manual

A eficiência da extração de DNA do solo utilizando um homogeneizador *bead beater* (Biospec Products – Alemanha) foi comparada àquela obtida utilizando um homogeneizador manual (Moulinex - Brasil). O homogeneizador manual tem custo 100 vezes menor e revelou-se mais eficiente que o *bead beater* extraíndo sete vezes mais DNA.

Palavras-chave: ferramentas moleculares, extrato de DNA

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