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Simple DNA extraction protocol for a 16S rDNA study of bacterial diversity in tropical landfarm soil used for bioremediation of oil waste

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ABSTRACT. Landfarm soil is used to bioremediate oil wastes from petrochemical industries. We developed a simplified protocol for microbial DNA extraction of tropical landfarm soil using only direct lysis of macerated material. Two samples of tropical landfarm soil from a Brazilian refinery were analyzed by this protocol (one consisted of crude oil-contaminated soil; the other was continuously enriched for nine months with petroleum). The soil samples were lysed by maceration with liquid nitrogen, eliminating the need for detergents, organic solvents and enzymatic cell lysis. Then, the DNA from the lysed soil sample was extracted using phenol-chloroform-isoamyl alcohol or guanidium isothiocyanate, giving high DNA yields (more than 1 μg DNA/g soil) from both soil types. This protocol compared favorably with an established method of DNA template preparation that included mechanical, chemical and enzymatic treatment for cell lysis. The efficiency of this extraction protocol was confirmed by polymerase chain reaction amplification of the 16S rRNA gene, denaturing gradient gel electrophoresis and cloning assays. Fifty-one different clones were obtained; their sequences were classified into at least seven different phyla of the Eubacteria group (Proteobacteria - alpha, gamma and delta, Chloroflexi, Actinobacteria, Acidobac-

teria, Planctomycetes, Bacteroidetes, and Firmicutes). Forty percent of the sequences could not be classified into these phyla, demonstrating the genetic diversity of this microbial community. Only eight isolates had sequences similar to known sequences of 16S rRNA of cultivable organisms or of known environmental isolates and therefore could be identified to the genus level. This method of DNA extraction is a useful tool for analysis of the bacteria responsible for petroleum degradation in contaminated environments.

Key words: Microbial DNA; Bacterial diversity; Direct lysis; Petroleum degradation; Cloning assay; DGGE

