Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Microbiological Methods 60 (2005) 83–92
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Capillary electrophoresis single-strand conformation polymorphism analysis for monitoring soil bacteria

Stephanie King[a], Bruce R. McCord[a], R. Guy Riefler[b,*]

[a]Department of Chemistry and Biochemistry, 171 Clippinger Laboratories, Ohio University, Athens, OH 45701, United States
[b]Department of Civil Engineering, 139 Stocker Engineering Center, Ohio University, Athens, OH 45701, United States

Received 21 July 2004; received in revised form 24 August 2004; accepted 24 August 2004
Available online 27 September 2004

Abstract

The ability to effectively monitor a microbial community is necessary to design and implement remediation strategies for contaminated soil. Single-strand conformation polymorphism (SSCP), a technique which separates DNA fragments based on their sequence, was used to analyze amplified 16S rRNA gene fragments of 12 common soil bacteria. Separation was performed using capillary electrophoresis (CE), as opposed to other common gel techniques, to eliminate the need for band analysis on gel matrices. Four different universal bacterial primer sets were used for DNA amplification: 341–534, P11–P13, Er10–Er11, and Er14–Er15 corresponding to the V3, V8, V2, and V4 regions, respectively. The forward strand of each primer was labeled with 6-carboxy fluorescein fluorescent dye. Analyses were performed on the Applied Biosystems 310 genetic analyzer using GeneScan Analysis Software version 3.5. The best results were obtained using primer 341–534, in which 6 of the 12 bacteria could be distinguished. By combining primer sets 341–534 and Er10–Er11, all 12 of the bacteria could be separated, indicating various degrees of polymorphism within the selected primer regions. When performing simultaneous amplification and analysis of all 12 species some preferential amplification occurred, as not all peaks could be observed. However, SSCP profiles obtained for pure bacterial cultures show the potential of CE-SSCP for bacterial community analysis.

Keywords: Capillary electrophoresis; Single-strand conformation polymorphism; PCR; Soil bacteria

1. Introduction

The use of molecular biological techniques for the analysis of bacterial communities has become standard practice. One of the most important of these techniques is the polymerase chain reaction (PCR). This process has enabled scientists to target specific regions of DNA for species identification, and is independent of culture techniques which can show biases associated with selective media (Duthoit et al., 2003; Kirk et al., 2004). Recent advances in DNA extraction and purification permit an improved representation of the total bacterial population directly from environmental samples. Through these DNA-based

* Corresponding author. Tel.: +1 740 593 1471; fax: +1 740 593 0625.
E-mail address: riefler@ohio.edu (R.G. Riefler).

0167-7012/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.mimet.2004.08.014

molecular methods, the role of bacteria in the treatment of contaminated soil can be examined more thoroughly, resulting in improved remediation strategies. Phytoremediation is an emerging technique in which the removal, containment, or neutralization of environmental contaminants is carried out using plants and their associated bacteria (Susarla et al., 2002). Phytoremediation can be enhanced through bioaugmentation of beneficial bacterial species (Siciliano and Greer, 2000; Van Aken et al., 2004). The ability to track the introduced bacteria as well as the native population is essential for determining the effectiveness of the phytoremediation strategies (Siciliano et al., 2003).

There are several published electrophoretic methods for monitoring microbial communities in soil without cultivation, including denaturing gradient gel electrophoresis (DGGE) (Muyzer and Smalla, 1998; Gelsomino et al., 1999; Kozdrój and van Elsas, 2000; Niemi et al., 2001; Watanabe et al., 2001), temperature gradient gel electrophoresis (TGGE) (Muyzer and Smalla, 1998; Eichner et al., 1999; Miethling et al., 2000), and single-strand conformation polymorphism (SSCP) analysis (Lee et al., 1996; Schmalenberger et al., 2001; Schmalenberger and Tebbe, 2003; Junca and Pieper, 2004). The use of capillary electrophoresis (CE) as opposed to the gel slab techniques has become a promising alternative to these methods (Ren, 2000; Turenne et al., 2000). CE systems have higher analysis speed, lower sample and reagent requirements, and excellent temperature control, a necessary feature for high resolution and reproducibility. When using laser-induced fluorescence detection, CE systems have significantly greater sensitivity than traditional electrophoretic separations. No further purification of the amplified product, such as removal of phosphorylated strands or excess primers, is necessary, and unlike gel electrophoresis techniques, different conditions are not necessary for separation of sequences with varying GC content (Duthoit et al., 2003). With the development of multi-capillary systems, CE separations have comparable throughput to that of gel slabs (Hayashi, 1999).

While originally utilized for DNA sequencing and fragment size analysis, SSCP has been recently applied in the detection of human genetic mutations (Orita et al., 1989a,b; Anderson et al., 2003). SSCP is a powerful structural analysis technique in which DNA fragments of the same length can be separated based on their sequence (Tebbe et al., 2001). In this technique, PCR-amplified DNA is added to formamide and heat-denatured to create single-stranded fragments. Electrophoretic separation is then performed under non-denaturing conditions, allowing the fragments to partially renature and form folded conformations due to the different intramolecular interactions between the bases. These secondary structures result in different electrophoretic mobilities, and hence separation of DNA strands differing by as little as a single base pair is possible. SSCP does not require complex apparatus for the preparation of gradient gels or the addition of GC clamps to the primers (Tebbe et al., 2001). However, it can be limited by the formation of single- and double-stranded fragments, and multiple conformations for the same fragment. SSCP analysis is optimal for DNA fragments ranging from 175 to 400 base pairs in length, as sensitivity of mutation detection decreases with increasing fragment length (Kourkine et al., 2002).

The objective of this study was to develop a CE-SSCP technique for the analysis of 12 common soil bacteria. Four universal bacterial primer sets were used to amplify various regions of the 16S rRNA gene. The CE-SSCP mobility values obtained for individual pure cultures are presented, along with results from simultaneous amplification and identification of all species.

2. Materials and methods

2.1. Bacterial strains

Purified, genomic DNA of the microbial strain *Pseudomonas putida* ATCC 47054D was purchased from the American Type Culture Collection (ATCC; Manassas, VA). Two microbial strains obtained from ATCC included *P. putida* ATCC 700478, and *Rhizobium leguminosarum* biovar *trifolii* ATCC 14479. Additional type cultures analyzed were *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Bacillus megaterium*, *Clostridium sporogenes*, *Clostridium perfringens*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. All bacteria except the anaerobic *Clostridium* species were stored