TITLE: DGGE (DENATURING GRADIENT GEL ELECTROPHORESIS) MARKER DEVELOPMENT FOR BACTERIAL DIVERSITY STUDIES

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ABSTRACT

The PCR-DGGE (Polymerase Chain Reaction - Denaturing Gradient Gel Electrophoresis) technique has been widely used to compare diversity of complex microbial communities and to monitor dynamics of populations in a variety of ecosystems. This culture independent method is based on partial separation of double strands of DNA, according to nucleotide sequences, during electrophoresis on a denaturing gradient polyacrylamide gel. To allow comparative analysis of DGGE patterns, a marker containing products amplified with different nucleotide sequences and GC contents needs to be used. Therefore, the objective of this study was to develop, in the laboratory, a marker to be applied in the DGGE technique for analysis of bacterial diversity. Bacteria species were selected according to genomic GC-content using the NCBI (The National Center for Biotechnology Information) database. After DNA extraction from bacteria, the amplification of the 16S rDNA gene was performed by PCR. The products were separated by electrophoresis in the gel using denaturing gradient from 45 to 65%. After *in silico* analysis, five bacterial species that possess 27 to 66.8% GC in their genomes were selected, being *Clostridium botulinum* – 27.0%; *Proteus mirabilis* - 38.8%; *Escherichia coli* - 50.5%; *Enterobacter cloacae* - 57.6% and *Pseudomonas aeruginosa* - 66.8%. The 16S rDNA gene amplification from the bacterial isolates generated products with about 198 bp, as expected. Sequences of the PCR products were compared with sequences deposited on NCBI's Genbank, and the identity of the selected bacteria could be confirmed. The GC contents of these sequences varied from 51.02 to 57.31%: *Pseudomonas aeruginosa* – 51.02%; *Proteus mirabilis* – 54.08%; *Escherichia coli* – 54.59%; *Enterobacter cloacae* – 54.59%; *Clostridium botulinum* – 57.31%. After electrophoresis denaturing gradient, the differential migration of the PCR products of the different bacteria was confirmed, allowing them to be used as DGGE markers for the evaluation of bacterial diversity.

Keywords: GC-content, culture independent technique, microbial diversity, NCBI (*The National Center for Biotechnology Information*), rDNA gene.

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