TITLE: A DGGE (DENATURING GRADIENT GEL ELECTROPHORESIS) MARKER FOR FUNGAL DIVERSITY STUDIES: *IN SILICO* ANALYSIS

AUTHORS: SANTAREN, K.C.F.; FRANÇA, D.A.; BARONI, F.A.; COELHO, S.M.O.; SOUZA, M.M.S.; COELHO, I.S.

INSTITUTION: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO, SEROPÉDICA, RJ (RODOVIA BR 465, KM 07, S/N, CEP 23890-000, SEROPÉDICA – RJ, BRAZIL)

ABSTRACT:

The DGGE (Denaturing Gradient Gel Electrophoresis) technique has been used to estimate diversity and to compare microbial communities in different ecosystems. This method allows the separation of DNA fragments according to nucleotide sequences, based on the electrophoretic mobility of their double-stranded molecules partially separated in the polyacrylamide gel. DGGE can be used for simultaneous analysis of multiple samples. However, differences in the denaturing gradient between gels that causes gel-to-gel variations in band positions is a major obstacle in experiments where a large number of samples requires simultaneous comparisons across different gels. To overcome this limitation, standard markers should be included in all gels to monitor gel-to-gel variability. From this perspective, the present work aims to propose a marker to be applied in the DGGE technique to evaluate the fungal diversity, with accessible methodology and low cost for reproduction and application in different laboratories. Nucleotide sequences from yeasts *Candida albicans, Geotrichum candidum, Malassezia pachydermatis, Rodotorula glutinis, Saccharomyces cerevisiae and Sporothrix schenckii* were selected using the Primer-Blast tool with the primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and FUNG (5'-ATTCCCCGTTACCCGTTG-3'). These primers amplify fungal rDNA 18S gene generating fragments of approximately 350 bp. The sequences were aligned and edited in MEGA software and GC content of the fragments was determinate at website http://www.endmemo.com/bio/gc.php. In the *in silico* analysis, 18S rDNA gene sequences from yeasts *Saccharomyces cerevisiae, Candida albicans, Geotrichum candidum, Malassezia pachydermatis, Rodotorula glutinis, and Sporothrix schenckii* were obtained having a size of 349, 347, 341, 344, 344 and 346 bp, respectively. The percentage of GC content of yeasts was 36.9, 38.3, 40.8, 41.3, 42.2 and 43.9%, respectively. Based on the information obtained, it is considered that these yeast species are promising to be used as markers for the analysis of fungal diversity using the DGGE technique.

Keywords: Microbial diversity, PCR (Polymerase Chain Reaction), 18S rDNA gene, yeast

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