TITLE: GENETIC CHARACTERIZATION OF ACTINOBACTERIA ISOLATED FROM DIFFERENT ENVIRONMENTS

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ABSTRACT:
The Actinobacteria phylum is an important group of Gram-positive bacteria, widely distributed in terrestrial and aquatic environments. The genus *Streptomyces* stands out as the largest producer of antibiotics. Besides antibiotics, these microorganisms also produce several other secondary metabolites with biological activities. Some of these metabolites are antagonistic agents, pharmacological agents that act as antitumor, immunomodulators, neurological agents and enzyme inhibitors or agrobiological agents such insecticides, pesticides, and herbicides. Identifying these organisms is still a challenge due to the diversity and the large number of species. Sequencing the 16S rDNA region has been used to identify members of the genus *Streptomyces*, but these sequences are not very effective in discriminating related species. In this report, the diversity of 86 actinobacterial isolates was investigated using BOX-PCR and URP-PCR primers. The fragment patterns generated from each PCR technique were clustered in dendrograms and the Pearson correlation coefficient was chosen to measure similarity. Simple matching analysis generated the data matrix and dendrograms were constructed using UPGMA. Cluster analysis of the BOX-PCR profiles resulted in 12 groups with a cut-off value of 0.6 for the coefficient of similarity. The amplification profiles of the isolates using URP-PCR primers revealed a high diversity with the nine primers used. One representative of each cluster formed with BOX-PCR and URP-PCR amplification products was analyzed by sequencing the 16S rDNA (n=44). URP and BOX1AR primers produced different amplification patterns with the isolates tested. Phylogenetic analyses of the 16S rDNA sequences showed that most of the isolates clustered in the *Streptomyces* genus. However, analysis of the 16S rDNA did not result in the same diversity observed with BOX-PCR and URP-PCR. Genomic DNA fingerprinting based on BOX-PCR is a valid tool for the detection of highly related genomes since it can identify small genomic variations, whereas 16S rDNA sequencing is not able to unravel the high genetic diversity existing in this group of bacteria. URP-PCR primers were used for the first time with actinobacterial isolates in this work. With the results it was possible to observe intraspecific diversity among isolates and reveal the potential of these two fingerprinting techniques for further studies of actinobacterial diversity.

Keywords: *Streptomyces*; URP-PCR; BOX-PCR; diversity

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