

TITLE: APPLICATION AND IMPLEMENTATION OF THE MOLECULAR TECHNIQUE OF POLYMERASE CHAIN REACTION (PCR) FOR TAXONOMIC MODERNIZATION IN THE BACTERIA COLLECTION OF THE AMAZON

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ABSTRACT:

Brazil holds 20% of the world's biodiversity and this wide variety of specimens are deposited in biological collections and are responsible for their conservation. The biological collections have become important, both for a basic analysis and for the economic base of a country and are used as strategic resources when they are organized, documented, classified and providing maintenance, storage and donation of biological material. Among the Brazilian collections of microorganisms are the Amazonian Bacteria Collection (CBAM) located at the Institute Leônidas and Maria Deane / FIOCRUZ / AM, composed of 706 strains identified through traditional taxonomy (BBL Crystal Kit). Currently, the molecular technique used to identify species is the Polymerase Chain Reaction that provides faster and more reliable identifications. The objective of this work was to genotypically authenticate bacteria from the CBAM collection. The bacterial DNA was extracted through QUIAGEN Kit. For the confirmation of the species was used to the amplification of the 16S gene, where was used the primers 530 F and 1492R. The PCR amplification products were submitted to 1.5% agarose gel electrophoresis stained with Gel Red and visualized in photodocumentator. The products obtained in the PCR were purified by the Polyethyleneglycol (PEG) precipitation method. The sequencing was performed on Applied Biosystems 3130 sequencer. The sequences were assayed with Genius 9.0 software. For identification of species, the sequences were inserted into the BLAST program. Among the 706 strains, 176 were authenticated by sequencing, of these 36.93% (65), the phenotypic and genotypic tests did not confirm the same species and 63% (111) confirmed the species. The molecular method has revolutionized the identification of microorganisms, due to the fast result and high reliability. However commercial kits are based on biochemical reactions colorimetrically grouped into phenotypic characteristics, which most often occur problems in the interpretation of the readings, causing results with a low level of confidence when compared to the results obtained by molecular biology. As the Biological Collections are important repositories for scientific research, it has been observed the increasing need to to identify quickly and confidently, resulting in taxonomically more reliable microbiological collections.

Keywords: Biological collection, bacteria, molecular technique

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