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MOLECULAR IDENTIFICATION AND PHYLOGENY OF TWO ENDOPHYTIC PLANT GROWTH-PROMOTING SPORE-FORMING BACTERIA ISOLATED FROM YERBA MATE (ILEX PARAGUARIENSIS ST. HIL.)

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RESUMO

Introduction: *Ilex paraguariensis* St. Hil., also called yerba mate, is one of the most economically important crops in Argentina. It is widely marketed in South America; but also, it is consumed worldwide. It is emphasized that despite this overall consumption, yerba mate can only grow in certain regions of Argentina, Paraguay, and Brazil due to unique soil characteristics, such as lateritic soils, and warm and moist weather. Currently, there are plantations of very good performance in the region; however, there are concerns about the increase of yerba mate degraded crops, as a result of the monoculture system, erosion and compaction of soil, and nutrient loss combined with little or no soil fertilization. In our group, this motivated the research and development of a biofertilizer from native bacteria to recover crop performance. For this reason, two endophytic bacteria coded as 19RS3 and T5S-T4 were isolated from *I. paraguariensis* St. Hil. roots and selected for their plant growth-promoting (PGP) properties showed *in vitro*. The strains were morphologically identified as *Bacillus*. However, bacteria closely related cannot be distinguished from other species simply by *16S* rRNA gene sequence. Objective: The aim of the present work was to molecularly identify two endophytic plant growth-promoting spore-forming bacteria isolated from yerba mate by the analysis of the *16S* rRNA, *23S* rRNA, and *gyrB* concatenated gene sequences. Methods: *Bacillus* sp. 19RS3 and T5S-T4 genomes were sequenced. The reported genome of *B. altitudinis* strain SGAir0031 (GenBank accession number: CP022319) was used as a reference for the detection of *16S* rRNA, *23S* rRNA, and *gyrB* genes. Gene search and analysis were performed with Geneious 11.0.1 software. The sequences obtained were compared with the nucleotide and protein databases of the National Center for Biotechnology Information (NCBI), using the BLASTn and BLASTx platforms, respectively. Sequences of the *16S* rRNA, *23S* rRNA, and *gyrB* genes from different species belonging to the genus *Bacillus* were selected and manually concatenated using the Mega 6.06 software. The sequences were analyzed by the Neighbor-Joining, Maximum Likelihood, and Maximum Parsimony methods using the Bootstrap test with 1000 replicates. Results: Neighbor-Joining, Maximum Likelihood and Maximum Parsimony trees with high bootstrap values were constructed. Monophyletic clades were generated for each *Bacillus* species. *Bacillus* sp. 19RS3 and T5S-T4 were grouped in a monophyletic clade supported by a bootstrap of 100% and were identified as *B.*

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altitudinis. Conclusions: The use of *16S* rRNA, *23S* rRNA, and *gyrB* concatenated genes sequences provides an accurate identification for bacterial isolates that involve a complex and extensive biochemical identification procedure. It is an efficient method for the identification and taxonomic analysis of closely related isolates such as those of *Bacillus* genus that are involved in plant growth promotion and have a potential application as biofertilizer.

PALAVRAS-CHAVE: Bacillus altitudinis, Bacterial identification, Bioinformatics, Concatenated gene sequences.

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