TITLE: EVIDENCE OF HORIZONTAL GENE TRANSFER OF THE METHYLTRANSFERASE GENE IN *Burkholderia seminalis* TC3.4.2R3 PRESENT IN THE O-ANTIGEN CLUSTER

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ABSTRACT:

The *Burkholderia seminalis* strain TC3.4.2R3 was isolated from sugarcane roots and showed the ability to inhibit some phytopathogenic fungi, such as *Fusarium oxysporum*, *Ceratocystis paradoxa* and *Colletotrichum falcatum*. Previous studies showed the association between this inhibition ability to the expression of a methyltransferase gene (MTase) present in the O-antigen cluster. Our study aimed to evaluate, in an evolutionary approach, using *in silico* and molecular analysis, the organization of this cluster and the acquisition of this gene by horizontal Gene Transfer (HGT). Phylogenetic analysis based on MLSA was performed withTC3.4.2R3 and other 16 *Burkholderia* species to find the nearest taxon. A maximum likelihood tree was constructed using MEGA 7.0 with Clustal W for alignment by default settings. Firstly, the presence of the O-antigen cluster in other *Burkholderia* species was perfomed in GenBank through BLASTn tool. The GC content of O-antigen cluster and adjacent genes was analyzed by comparing *B. ambifaria* AMMD and *B. cenocepacia* J2315 genomes. Molecular strategy was used to confirm the lacking of MTase in the 16 *Burkholderia* species. A pair of primers annealing about 900 bp up and downstream of MTase was designed and a cPCR was performed using EasyTaq DNA Polymerase. *In silico* comparisons showed that the entire O-antigen cluster had no matches with any *Burkholderia* sequence in GenBank. Moreover, the GC content diverged not only from the O-antigen cluster (mean value of 61%) and the adjacent genes (68%), but also from another *Burkholderia* genomes (67% of GC content). The cPCR reactions showed no positive amplifications for the 16 *Burkholderia* species analyzed, thus the MTase amplicon appeared to be exclusive for TC3.4.2R3, reinforcing the possible HGT. *Burkholderia* genus is studied aiming at diverse biotechnological applications as producers of major molecules of interest. Previous studies showed a Tn5 insertion in the MTase of TC3.4.2R3 causing the loss of the ability in inhibiting phytopathogenic fungi, which suggests the possible involvement of this gene or its cluster in the antimicrobial production. In this way,a better understanding of the mechanisms involved in the phytopathogens inhibition by *B. seminalis* TC3.4.2R3, as well as the role of the methyltransferase gene on this process are very necessary.

Keywords: antimicrobial production, *Burkholderia* spp., cPCR, phylogenetic analysis, phytopathogenic fungi

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