TITLE: IDENTIFICATION OF BACTERIAL STRAIN MF13.2C AND CONFIRMATION OF ITS CAPACITY TO DEGRADE THE HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) FOR BIOTECHNOLOGICAL PURPOSES.

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ABSTRACT:

Brazil is an agricultural powerhouse. Not surprisingly large scale agricultural production has its costs. Environmental costs include Brazil being a leading user of herbicides and insecticides, however, these chemicals help guarantee crop yields and global food security. The herbicide known commonly as 2,4-D is extensively used globally and has been for the last fifty years. It is second only to glyphosphate in its importance in Brazil. Consequently 2,4-D reaches and contaminates soils, rivers and aquifers. The purpose of this specific study was to reactivate a bacterial strain that had previously shown great biotechnological potential. The laboratory collects interesting bacteria, yeasts and filamentous fungi for biotechnological solutions. In this study we describe the reactivation and identification of a bacterial strain that has been dormant in glycerol for the last 10 years and how to confirm that it is still a 2,4-D degrader. The strain MF13.2C was revived on Luria Bertani (LB) medium. Molecular identification was first by cell lysis for DNA extraction using heat shock. Genomic DNA was PCR amplified using universal primers for 16S rDNA. The 16S rDNA PCR product was sequenced and sequence alignments made with NCBI-BLAST. After the alignment with the BioEdit program, a phylogenetic tree was inferred using Neighbour-joining method MEGA 7.0. To confirm if the strain still retained the capacity to degrade 2,4-D, it was grown in liquid LB medium and then passed to tubes containing a minimal medium with 500 $mg.L^{-1}$ of 2,4-D as the sole source of carbon. After 30 days HPLC analysis of the growth medium was done. Chromatographic analyzes were performed using a Shimadzu SPD-M10A equipped with a LC-10AD pump and Diode array detector CBM-10. The results of the sequencing confirmed that the strain MF13.2C shared 99% identity with *Ochrobactrum ciceri*, strain DZQ2a. MF13.2C was confirmed as strain belonging to *Ochrobactrum ciceri*. Regarding degradation activity and biotechnology potential,

after 30 days the 2,4-D was not detected in samples. These finding confirm that strain MF13.2C continues to be very bioactive after ten years storage and will now be used to develop novel microbial biotechnology.

Keywords: *Ochrobactrum* sp., biodegradation, herbicide, biotechnology, pesticide, bacteria, 2,4-D

Development Agency: PIBIC-UFRJ, CNPq, CAPES, FAPERJ