Title: Analysis of the degradation of the herbicide 2,4-dichlorophenoxyacetic acid by bacteria of the genus *Ochrobactrum.*

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Abstract:

Brazil is the world's leading consumer of pesticides and their management and fate is important to avoid contamination of soil and water resources. Within the pesticides, the use of herbicides to control weeds in crops is wide spread in Brazil. After glyphosphate, 2,4-dichlorophenoxyacetic acid (2,4-D) is the second most commonly used pesticide in Brazil. It is used on a massive scale in particular with sugar cane production. 2,4-D is highly toxic to humans (Level II), it is mobilized by water and although biodegradable it often persists in soils and water. An integrated approach to manage its fate requires a reduction in its use and improved biodegradation where it is used. A significant study of soils with and without a history of using 2,4-D was published by our group. A part of that study included selecting and identifying 70 strains of bacteria considered 2,4-D degraders. HPLC analyses of 2,4-D transformation by those strains revealed that *Ochrobactrum* strain MF13.2C has potential as biodegradation agent for a future biotechnology. The strain was reactivated on solid Luria-Bertani medium (LB) and then inoculated in liquid LB medium enriched with 300 $mg.L^{-1}$ of 2,4-D. Bacteria were then transferred to test tubes (n=4) containing minimal liquid medium with 500 $mg.L^{-1}$ of 2,4-D as the sole carbon source until they reached an $O.D_{600nm}$ of 0.3. HPLC was used to confirm the transformation of 2,4-D. To confirm strain identity, the DNA of the strain was extracted, and 16S rDNA PCR amplified using the primers 27F and 519R. Strain MF13.2C was putatively identified as an *Ochrobactrum intermedium* strain. Analysis of the control chromatogram revealed a single peak, with retention time of 11.88 minutes, showing the standard chromatographic profile of 2,4-D. In the chromatogram with MF13.2C no peak formation was observed at 11.88 minutes, although other smaller peaks are visible at but are not compatible with the chromatographic profile of 2,4-D. This result strongly suggests that this strain was able to use 2,4-D as a carbon source and has potential as a bioremediation agent of 2,4-D. Experiments are ongoing to verify if this strain can be used for a novel biotechnology product.

Keywords: Biodegradation, 2,4-dichlorophenoxyacetic acid (2,4-D), *Ochrobactrum* spp.

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