Title: Biodegradation of 2,4-dichlorophenoxyacetic acid (2,4-D) in soil by lignolytic fungi

Authors: Carvalho, M.[1], Pereira, P.H.F.[1], Direito, I.N.C.[1], Ghizelini, A.M [1], Santoro E.P. [1], Macrae, A.[1].

Institution: [1]Laboratorio de Biotecnologia Sustentável e de Bioinformática Microbiana, Instituto de Microbiologia Paulo de Goes, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro (UFRJ); [2]Univesidade Estadual da Zona Oeste (UEZO)

Herbicides are a necessary part of modern industrialized agricultural production. In this study we report on the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). 2,4-D is an important widely used herbicide globally. In Brazil, it is used to control unwanted broadleaf plants (dicots). During the production of sugarcane, wheat and rice where it can be used both pre and post emergence and with soya, pre-emergence. It is relatively mobile in soils, highly toxic and a good candidate for bioremediation. Here we report on lignolytic fungi that degrade 2,4-D. 216 fungal strains, previously isolated from mangrove sediments, were screened for lignolytic activity. Lignolytic activity was tested for using a selective solid medium using the dye POLY-R 478. POLY-R 478 has an aromatic structure similar to lignin and to 2,4-D. Where a halo was seen, lignolytic activity was assumed and these strains cultured for further study. 75 fungal strains produced halos. These strains were then confronted with 2,4-D in liquid culture in a malt extract nutrient solution. The liquid culture was filtered and analyzed by HPLC for the presence of 2,4-D. All of the 75 strains that were lignolytic, were tested to see if they would transform 2,4-D. Chromatograms revealed that the 2,4-D had been transformed by just one strain. Analysis of the chromatogram profile showed that the profile was different to that described for *Ralstonia*. This difference suggests a degradation pathway for 2,4D that has not been fully described previously. The strain was identified as belonging to the species group *Aspergillus awamori.* The *A. awamori* strain and a *Phanerochaete chrysosporium* strain, already described as a 2,4-D degrader, were then cultivated to produce fungal spores. Spore suspensions were made after 14 days and counted using a Neubauer chamber. Counts of 10^9 spores per gram of substrate were recorded. Spore viability and shelf life were tested every 20 days. Spore preparations proved viable for at least 160 days. Further tests are now underway to evaluate the potential of these spores as ingredients for biodegradation formulations. The results are discussed from a biotechnology bio-prospecting view point.

Keywords: Bioremediation, 2,4-D,Lignolytic Fungi, Biodegradation

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