New insights into the biodegradation of β-triketone herbicides:

Role of nitroreductase enzymes in mesotrione transformation

Louis Carles1,2, Pascale Besse-Hoggan3,4, Muriel Joly1,2 and Isabelle Batisson1,2

1Clermont Université, Université Blaise Pascal, LMGE, F-63000 Clermont-Ferrand, France

2CNRS, UMR 6023, Laboratoire Microorganismes: Génome et Environnement, F-63177 Aubière, France

louis.carles@univ-bpclermont.fr, [muriel.mourguy@univ-bpclermont.fr](file:///C%3A%5CUsers%5Cpabesse%5CAppData%5CLocal%5CTemp%5Cmuriel.mourguy%40univ-bpclermont.fr), isabelle.batisson@univ-bpclermont.fr

3Clermont Université, Université Blaise Pascal, ICCF, F-63000 Clermont Ferrand, France
4CNRS, UMR 6296, Institut de Chimie de Clermont-Ferrand, BP 80026, F-63171 Aubière Cedex, France

pascale.besse@univ-bpclermont.fr

Mesotrione is a selective herbicide belonging to the triketone family commonly used on corn since 2003. In order to predict its potential toxicological impacts, its environmental fate has to be identified. A mesotrione-degrading strain, *Bacillus megaterium* Mes11, isolated from an agricultural soil was used as a reference model to study the mesotrione biodegradation pathway (Batisson et al., 2009). Three metabolites were identified using complementary analytical tools (NMR and LC-MS/MS), from reduction of the nitro group of mesotrione into hydroxylamine derivatives, and then from hydrolysis leading to the final accumulation of AMBA (2-amino-4-methylsulfonylbenzoic acid). The aim of this research work was to identify the enzymes involved in mesotrione degradation.

Proteomic studies on Mes11 revealed a network of differential expressed proteins allowing us to emphasize a direct link with nitroreductase (NR) enzymes (Bardot et al., 2015). These results are consistent with the chemical structures of metabolites produced (Batisson et al., 2009) and strongly suggest the involvement of a NR in the reduction of mesotrione NO2 group into NH2 during the transformation into AMBA. To test the Mes11 NR activity on mesotrione, the 9 genes coding for *B. megaterium* NR, showing 11% to 64% similarity in their amino acid sequences, were amplified from Mes11 strain DNA, and expressed in *E. coli*. The resulting His-tag enzymes were purified on Ni-NTA column. Their nitroreductase activities were tested against mesotrione and monitored both by NAD(P)H cofactor oxidation dosages at 340 nm and by HPLC analysis.

Only two Mes11 isoenzymes (named NfrA1 and NfrA2) were capable of reducing the nitro group of mesotrione. They belong to oxygen-insensitive type I nitroreductases and to the super-family of Nitro FMN reductases, including *E. coli* NfsA and *B. subtilis* NfrA1 and YcnD nitroreductases, already described as having activity on different nitroaromatic compounds. Both Mes11 NR show optima pH and temperature of 5.5 and 25°C. Mes11 NfrA1 and NfrA2 use NADPH and NADPH/NADH as cofactor, have a KM(Mesotrione) of 42 and 61.7 µM, a KM(NADPH) of 46.2 and a KM(NADH) of 178.6 µM, and a Vmax of 13.6 and 30.5 µM.min-1, respectively. Kinetics assays show a ping-pong Bi-Bi mechanism for both enzymes.

This study constitutes the first identification of genes and enzymes involved in mesotrione biodegradation, allowing a better knowledge of mesotrione biodegradation pathways. These enzymes (or the corresponding genes) may be used as biomarkers to predict the capacity of ecosystems to degrade mesotrione and to assess their contamination both by the parent molecule and/or by the metabolites. These enzyme biomarkers could thus provide a powerful tool to determine the environmental risk assessment of such an herbicide.

Batisson I., Crouzet O., Besse-Hoggan P., Sancelme M., Mangot J-F., Mallet C., Bohatier J. 2009. Environ. Pollut. 157: 1195-1201.

Bardot C., Besse-Hoggan P., Carles L., Le Gall M., Clary G., Chafey P., Federici C., Broussard C., Batisson I. (2015). Environ. Pollut. 199: 198-208.