

### Short Research Article

# Production and use of mycotoxins uniformly enriched with stable isotopes for their dosage in biological samples: (2) production of mycotoxins and their characterization<sup> $\dagger$ </sup>

## OLIVIER PUEL<sup>1,\*</sup>, SOURAIA TADRIST<sup>1</sup>, NICOLAS LOISEAU<sup>1</sup>, MICHEL PEAN<sup>2</sup>, FRÉDÉRIQUE BRAVIN<sup>3</sup> and MARCEL DELAFORGE<sup>3</sup>

<sup>1</sup> INRA, UR 66 Laboratoire de Pharmacologie-Toxicologie, Toulouse, F-31931, France
<sup>2</sup> CEA Cadarache, DEVM/GRAP, Group de Recherches Appliquées en Phytotechnologie, St Paul les Durance, F-13108, France
<sup>3</sup> CEA Saclay, DSV/DBJC/SBFM and URA CNRS 2096, Gif sur Yvette Cedex 91191, France

Received 3 August 2006; Revised 18 January 2007; Accepted 20 January 2007

 $\textbf{Keywords:} \ \text{mycotoxins;} \ ^{13}\text{C-labelling;} \ \text{MSn;} \ \text{fusariotoxins;} \ \text{mycophenolic acid;} \ \text{fumitremorgin C}$ 

#### Introduction

Mycotoxins are naturally occurring secondary metabolites produced by fungi. They are implicated in several toxic effects in animal and humans, and represent a real health hazard in all countries of the world.<sup>1</sup> Toxinogenic fungi from three genera (*Aspergillus*, *Penicillium*, and *Fusarium*) are widespread in various agricultural products and constitute an economically important worldwide problem. Pharmaco-



Figure 1 Fumonisin B1 specific isotopic pattern. Comparison between measured and expected isotopic ratios.



<sup>\*</sup>Correspondence to: Olivier Puel, Institut National de la Recherche Agronomique, Laboratoire de Pharmacologie-Toxicologie, 180 chemin de Tournefeuille, BP 3, Toulouse 31490, France.

E-mail: Olivier.Puel@toulouse.inra.fr

<sup>&</sup>lt;sup>†</sup>Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

564 O. PUEL ET AL.



Figure 2 Mass spectra of deoxynivalenol <sup>12</sup>C (left) and deoxynivalenol 10% U-<sup>13</sup>C from raw sample (right).



Figure 3 Mass spectra of 10% U-<sup>13</sup>C mycophenolic acid from *Penicillium brevicompactum* culture on enriched wheat.

logical studies of these compounds often encounter problems.

My cotoxin production was done using autoclaved 10%  $^{13}\mathrm{C}$  and  $^{15}\mathrm{N}$  grains and plant dry material as the only nutrient source for the fungi culture. The grains or plant materials were moistened for reach  $a_{\mathrm{W}}$  value of 0.98, before sterilization. Then around 30 g of this plant material were inoculated with a suspension of  $2\times10^5$  conidia.

#### **Results and discussion**

Uniformly enriched <sup>13</sup>C and <sup>15</sup>N fumonisins (B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>) productions were performed from *Fusarium verticilloides* strain culture on corn grains and the isotopic ratios were close to that expected for a 10% <sup>13</sup>C and 10% <sup>15</sup>N content (Figure 1).

Using *F. graminearum* and enriched wheat, we produced <sup>13</sup>C zearalenone and <sup>13</sup>C deoxynivalenol (Figure 2). Other <sup>13</sup>C fungal secondary metabolites having therapeutic interest such as mycophenolic acid (immunosuppressor) (Figure 3) or the breast cancer resistance protein (BCRP) inhibitor, fumitremorgin C or ergot alkaloids can be produced following the same techniques (data not shown).<sup>2,3</sup>

#### REFERENCES

- Riley RT. Mechanistic interactions of mycotoxins: theoretical considerations. In *Mycotoxins in Agriculture and Food Safety*, Sinha KK, Bhatnagar D (eds). Marcel Dekker Inc.: New York, 1998; 227–253.
- Allison AC, Eugui EM. Immunopharmacology 2000; 47: 85–118. DOI:10.1016/S0162-3109(00)00188-0
- 3. Rabindran SK, Ross DD, Doyle LA, Yang W, Greenberger LM. *Cancer Res* 2000; **60**: 47–50.