Biochemical assessment of chlordecone transformation by the use of fungal biocatalysts

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Context: chlordecone biotransformation

The persistence of chlordecone, a highly chlorinated cyclodiene insecticide, is explained by its high affinity for soil organic matter, but also by its specific chemical structure recalcitrant to biodegradation. A few publications deal with its biodegradation. They remain poorly convincing, but suggest the presence of neighbouring molecules, monohydro- and dihydro-chlordecone, formed from a biological transformation process. Accordingly, our actual knowledge does not demonstrate any biological transformation of chlordecone in soils. Since the eighties, numerous bibliographic syntheses (for example the most recent Bath *et al.*, 2007; Rubilar *et al.*, 2008) reported the microbial and fungal mechanisms involved in the dehalogenation and transformation of chlorinated chemicals, but without taking chlordecone into account. A few biological processes are actually known to be involved in chlordecone dissipation. *Pseudomonas* species have been shown to transform chlordecone in liquid cultures to compounds co-eluting with hydrochlordecone or dihydrochlordecone (George and Claxton, 1988). In humans and several vertebrates, chlordecone is reduced to chlordecone alcohol (Fariss *et al*., 1980; Soine *et al*., 1983; van Velde P.A. *et al.*, 1984). Functionalised metabolites can then be subjected to conjugation with glucidic compounds. If anaerobic conditions seem favourable to dehalogenase activity (Wohlfarth and Diekert 1997), numerous studies also suggest the interest of oxidative metabolism, the main process occurring in soils.

It is thus important to develop new studies of biochemistry, which complete molecular (soil metagenomics…) or biotechnological (DNA shuffling of microbial sequences of dehalogenases) approaches. In addition, biochemical approaches could be developed according to the findings of genomic studies. Several ways should be investigated concerning chlordecone biological transformation, which represents currently a main environmental and societal stake. Fungal biocatalysts exhibit properties offering advantages in that context (Mougin *et al.*, 2009).

Proposal for future ways of research for chlordecone transformation

1-Screening for filamentous fungi producing ligninolytic enzymes involved in xenobiotic transformation

One first step should consist in screening a fungal collection (formed from strains available in and out of INRA) in order to evidence new potentialities of chlordecone transformation among ligninolytic fungi. A study performed in liquid cultures (Kennedy *et al*., 1990) has shown a low mineralization of Mirex (an insecticide of the chlordecone family) in the presence of the basidiomycete *Phanerochaete chrysosporium*. A polar metabolite was also detected in liquid cultures and soils, although in very low amounts. New developments should based approaches on the oxidative dechlorination, possibly mediated by oxidative enzymes involved in the breakdown of ligninolytic materials, namely peroxidases, laccases… That approach should integrate the evolution of our knowledge acquired during the last 20 years in the scope of mechanisms of action of these ligninolytic enzymes (Mougin *et al.*, 2003). It is important to consider laccases, potent oxidases exhibiting a high potential in terms of catalytic and biotechnological developments. Understanding the catalytic mechanisms involved in chlordecone transformation could allow the development of

strategies considering enzyme optimisation and evolution, a multidisciplinary approach intending to merge several partners of distinct skills.

In addition to well-used ligninolytic enzymes (Peroxidases, laccases), one interest should be paid to cellobiose dehydrogenases, able to produce reactive radicals contributing to the transformation of pollutants (Cameron and Aust, 1999).

As a general way, filamentous fungi are easy to produce. We master also several protocols for their inoculation into soils. One potent way implies the use of inocula comprising lignocellulosic pellets coated with fungi (Rama *et al.*, 2001).

2-Screening for fungi producing enzymes induced by chlordecone exposure

In that second approach, fungal strains could be screened without *a priori* to evidence proteins/enzymatic systems specifically secreted/induced during fungal growth in the presence of chlordecone. These strains could be either isolated from chlordecone-polluted soils, or obtained from collections and exposed to chlordecone. It is likely that chlordecone transformation in soils, because of its poor bioavailability, involves exocellular enzymes. Extracellular fluid obtained from cultures will be subjected to protein analysis. That strategy should allow identification of actually unknown proteins, possibly capable of transforming the insecticide. That approach should benefit from actual research in fungal secretomics (2D electrophoresis) and proteomics (mass spectrometry analysis). One hypothesis consists in the fact that these proteins could be enzymes able to transform chlordecone.

3-Screening for other biocatalysts involved in chemical dechlorination

Mild catalytic conditions could allow the dechlorination and the further breakdown of chlordecone. The use of vitamins as biocatalysts (hemoproteins, porphyrins and corrins) offers a high potential. That approach has been successfully used in the past for the degradation of the organochlorine insecticide lindane (Marks *et al.*, 1989; Mougin *et al.*, 1996). Schrauzer and Katz showed in 1978 the interest of vit B12 as a catalyst for the dechlorination of Mirex and chlordecone. The reaction leads to the fragmentation of chlordecone and the formation of an organocobalamin. That approach, never studied in more details to date, is worth to be completed and adapted to environmental conditions related to these present in soils. In a first time, commercially available biocatalysts will be used. In a second time, the presence of relevant biocatalyst will be then assessed in fungal strains.
Vit B12 has been also involved in the CO dehydrogenase enzyme complex of the methanogenic archaebacteria *Methanosarcina thermophila,* described to decompose chlordecone (Jablonski *et al.*, 1996).

Concerning ligninolytic enzymes and other proteins, it will necessary to develop processes to make their use easy, and ensure their stability. Encapsulation (alginate beads…) or immobilisation (grafted membranes…) are two possible way of development (Jolivalt *et al.*, 2000).

4-Identification of transformation products and assessment of their ecotoxicity

Previous experiments are performed in liquid conditions allowing the collect, purification and identification of transformation products. That identification must require the use of chromatographic methods associated with mass spectrometry.

Transformation products will be purified and concentrated (if possible synthesised by organic chemists) and used to spike Terrestrial Model Ecosystems (TMEs). In these experimental devices,

ecotoxicity is easy to be assessed on earthworms or global soil enzymatic activities as described (Igel-Egalon *et al*., in press).

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