Genetic and metabolic analysis of the carbofuran degradation pathway in *Sphingomonas* sp. KN65.2

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Introduction

Carbofuran is a carbamate insecticide that has relatively high toxicity in rats as a cholinesterase inhibitor, an endocrine disruptor, an oxidative stress inducer and a nephrotoxin. The compound has been banned in many countries but is still in use in developing countries. Moreover, often several carbofuran metabolites accumulate during the biodegradation of carbofuran and were shown to cause serious effects on the reproductive system in female rats. Many bacterial isolates that degrade carbofuran have been reported, however, only members of the genus *Sphingomonas* seem to mineralize and use the compound as a carbon source. Until now, the genetics and metabolic pathway of carbofuran degradation have been largely unknown. This study aims to unravel the pathway for carbofuran degradation in *Sphingomonas* sp. KN65.2 that was isolated from a carbofuran treated vegetable field in the Mekong delta of Vietnam and that uses carbofuran as the only source of carbon and energy.

Material and methods

*Sphingomonas* sp. KN65.2 was isolated from a vegetable field in SocTrang province, Vietnam. Mutants affected in carbofuran degradation were obtained by screening a plasposon mutant library for growth on carbofuran. Carbofuran degradation and mineralization of mutants were assessed by ultra-fast liquid chromatography and liquid scintillation analysis, respectively. The affected genes were identified by rescue and sequencing of the plasposon flanking genes. Carbofuran metabolites were identified by high-pressure liquid chromatography coupled with a linear ion trap-orbitrap mass spectrometer. The draft genome sequence of strain KN65.2 was done by Illumina sequencing and annotated by RAST. Geneious 6.1.4 software was used to localize the plasposon insertion sites into the genome of strain KN65.2.

Results

Thirty eight mutants that performed slow/retarded carbofuran degradation and/or abolished/slow/retarded growth on carbofuran were identified by screening a KN65.2 plasposon mutant library consisting of 2628 transconjugants. The affected gene products were predicted to function in uptake, methylamine oxidation, hydroxylation and cleavage of aromatic compounds, and alkanoic acid degradation. The genes could be mainly assigned to five gene clusters in the draft KN65.2 genome sequence.

Eight carbofuran metabolites were identified. One persistent metabolite was detected during carbofuran degradation by the wild strain while the remaining seven metabolites were produced by selected mutants impaired in carbofuran degradation. These metabolites enabled us to propose a tentative pathway for carbofuran degradation, consisting of (1) carabamate hydrolysis, (2) monoxygenation of dimethyl-dihydrobenzofuranol, (3) reduction of the ortho-quinone to the corresponding catechol, (4) meta-cleavage of the catechol, (5) hydrolysis of the meta-cleavage product, (6) and (7) nonspecific hydroxylations (Figure 1).
Figure 1. Tentative pathway of carbofuran degradation in *Sphingomonas* sp. KN65.2. Metabolites with CAR labels were identified by MS analysis.

**Conclusion**

This study is the first report about the genetics and metabolism of the carbofuran degradation pathway in *Sphingomonas*.

**Acknowledgement**

This research was funded by VLIR-UOS Belgium (BBTP2007-0012-1087), IFS/OPCW Sweden (C/4563-1) and the EU project BIOTREAT (EU grant n° 266039). We thank K. Simoens for technical support.

**References**

