Abstract

Synthetic carbamates constitute a significant pesticide group with oxamyl being a leading compound in the insecticide/nematicide market. Microbial degradation constitutes one of the main processes controlling the environmental dissipation of oxamyl. Paradoxically, microbial degradation, which was initially viewed as a desirable process for reducing environmental hazards, has turned into a double-edged sword with the development of the phenomenon of enhanced microbial degradation, which under conducive condition can lead to loss of the biological efficacy of pesticides. Considering the importance of biodegradation in the environmental fate and efficacy of soil-applied carbamate insecticides we aimed (1) to isolate and identify bacteria degrading the carbamate oxamyl and to characterize the genes involved in its transformation, (2) to explore the ecology, distribution and function of carbamate hydrolase genes in soils and (3) to get insights into their origin and evolution

As a source for the isolation of oxamyl-degrading bacteria we used a soil from a commercial banana plantation located in the area of Sitia, northeast Crete, in Greece with history of previous treatment with oxamyl. A rapid microbially driven hydrolysis of oxamyl to oxamyl oxime was observed in the studied soil which is in line with the reduced biological efficacy of oxamyl in the given field. Subsequent enrichment cultures inoculated with the studied soil resulted in the isolation of four oxamyl-degrading bacterial strains which identified, based on multilocus sequence analysis (MLSA), as *Pseudomonas*. The isolates were able to metabolize oxamyl to oxamyl oxime which was not further transformed by our strains in contrast to its gradual dissipation in soil. Soil sterilization resulted in a complete halting of the degradation of oxamyl oxime suggesting that its transformation was biologically driven. However, our repeated attempts to isolate oxamyl oxime degraders following the same enrichment cultures method failed, suggesting that its transformation in soil is probably a co-metabolic process performed by non-specialized soil bacteria or fungi. All the isolated strains carried the carbamate-hydrolase gene *cehA* which was shown, via transcription analysis, to be responsible for the hydrolysis of oxamyl. Our isolates were able to utilize the methyl carbamate moiety (released during hydrolysis of oxamyl) as a C and N source, in agreement with the high mineralization levels of the ^{14}C-carbomoyl-labelled

oxamyl by all isolates, and their capacity to grow on methylamine, that is released from the decomposition of the unstable methyl-carbamate moiety.

We extended our investigations from the *in vitro* bacterial hydrolysis of oxamyl to the role of the soil microbiota in the *in situ* biodegradation of carbamates in soils. We studied the degradation of oxamyl and we determined the abundance of the three most studied carbamate hydrolase genes *cehA*, *mcd* and *cahA* in 16 soils from a potato monoculture area in Greece where oxamyl is regularly used. Oxamyl showed low persistence (DT_{50} = 2.4-26.7 days) and qPCR detected the *cehA* and *mcd* genes in 10 and three of the studied soils, respectively. The abundance of the *cehA* gene was positively correlated with pH, while both *cehA* abundance and pH were negatively correlated with oxamyl DT_{50}. In light of the detection of *mcd* in the studied soils, despite the absence of carbofuran, its main substrate which has been banned and has not been used in the studied region for over 10 years, we tested the hypothesis that other carbamates used in the region might serve as substrates for *mcd*. None of the alternative carbamates tested increased the abundance of *cehA* and *mcd* apart from (i) oxamyl which stimulated the abundance and expression only of the *cehA* gene and (ii) carbofuran that stimulated the abundance and expression of both genes suggesting an interesting catabolic functional redundancy first time reported for carbamates. The *cehA* gene was also detected in pristine soils upon repeated treatments with oxamyl and carbofuran and only in soils with pH $\geq$ 7.2, where the most rapid degradation of oxamyl was observed. The occurrence of *cehA* gene in agricultural and pristine soils suggested its widespread distribution that could be a result of a parallel evolutionary mechanism from a common ancestor, probably involved in the detoxification of natural carbamate soil compounds produced by soil microorganisms and plant roots.

Overall, we studied the degradation of oxamyl by oxamyl-degrading bacteria and its biodegradation in soils. We reported the isolation and identification of four oxamyl-degrading *Pseudomonas* strains and we determined the microbial transformation pathway of oxamyl. All isolates carried the *cehA* gene which was shown to be responsible for the hydrolysis of oxamyl. Soil microcosm studies further reinforced the role of *cehA* gene in the biodegradation of oxamyl, whereas both the *cehA* and *mcd* genes are involved in the biodegradation of carbofuran. The detection of the *cehA* gene in agricultural and pristine soils, after oxamyl and carbofuran application

suggests its widespread occurrence and stresses the significant role of pH as a driver of the distribution of the *cehA* in soils. These results have major implications regarding the maintenance of carbamate hydrolase genes in soils, have practical applications regarding the agricultural use of carbamates and provide insights into the evolution of *cehA* gene.