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Biodegradation of Pesticides by Immobilized Recombinant *Escherichia coli*

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The bioremediation of pesticide contamination in the environment using recombinant bacteria is both an increasingly important scientific challenge and a growing business. Many chemicals are released into surface water either as a means of disposal or as a consequence of the methods used in their application. These include pesticides, many of which are toxic or contain toxic contaminants. Pesticides are essential to the high yields of modern agriculture (Kuritz and Wolk 1995) and their use is expected to increase at rates of 6-8% per year over the next few years (Sreenivasulu 2001). In the past forty years, excessive pesticide use has resulted in global problems of pest resistance, resurgence and pesticide residues in crops and soil.

The ability of esterases to degrade organophosphate (OPs) and carbonate (CB) insecticides raises the possibility of using them in bioremediation programs. Bioremediation of pesticides using enzymes as catalytic bioremediants has previously been suggested (Russel et al.1998). OPs can be detoxified by hydrolysis of their phosphoester bonds. Pyrethroids and some OPs like malathion can be similarly detoxified by hydrolysis of their carboxylester bonds, while a diverse range of carbamate insecticides, herbicides and fungicides can be detoxified by hydrolysis of amide or other similar bonds. Most of the candidate bioremediation enzymes identified to date are hydrolases (Russel et al.1998) and a number of enzymes with bioremediation potential have been isolated from pesticide resistant mutants of target (eukaryotic) pest organisms (Beard 1993; Newcomb et al. 1997).

Currently, there has been some limited use of genetically engineered organisms in waste treatment. In order to explore the possibility of using immobilized recombinant *Escherichia coli* (*E. coli*) to detoxify pesticide wastes in polluted water, we focused our work on the transfer of a detoxifying esterase gene from a mosquito into *E. coli*, the ultimate goal being to extend the range of pesticide wastes that can be biodegraded. In this paper we describe the biodegradation of

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acetofenate and chlorpyrifos by immobilized, recombinant *Escherichia coli*. Our results provide a basis for further research on the use of detoxifying enzymes in bioremediation.

MATERIALS AND METHODS

Acetofenate (7504) and chlorpyrifos were obtained from the Qingdao Pesticide Factory, China. Aqueous solutions were prepared by dissolving these substances in sterilized distilled water. An esterase gene was cloned and inserted into *E. coli* of the strain pRL-439, the recombinant strain was designated pRL-B1 (Yan et al. 2000). pRL-439 (supplied by Dr. Wolk, Michigan University) was used as a control.

Degradation of the insecticides using the immobilized recombinant cells was performed using the methods described in Yan et al. 2000. The recombinant cell suspension was prepared by growing the culture to high cellular densities at 37℃ according to Qiao (Qiao and Yan 2000). Cells were harvested by centrifuging the cell suspension at 6000*g* for 20 min at 4℃. 5g cells were washed twice in sterilized distilled water then suspended in 50 ml sterilized distilled water. 6 % (w/v) sodium alginate was then added and the suspension gently stirred for 1 hour (Yan et al. 2000). The ability of the immobilized cells to degrade insecticides was tested with respect to the organochlorine insecticide acetofenate (7504) and the OP insecticide chlorpyrifos.

The suspension of immobilized cells was placed in a 100 ml flask to which the following was added: 20 ml distilled water, 1 ml 2% Tritonx-100, and a suitable concentration of either 7504 or chlorpyrifos. The flask was incubated at 37℃ and placed on a mechanical shaker. 1ml of the solution was removed at regular intervals from which degradation products were extracted with redistilled hexane and analyzed by a Hewlett-Packard 5890 gas chromatograph (GC).

The gas chromatograph setup for analysis of insecticide 7504 was a Ni63 electron capture detector on a fused silica capillary column (length 25m, 0.32 mm id, Supelco Corp., USA). Nitrogen was used as the carrier gas at 1 ml/min. The injector, column, and detector temperatures were set at 320℃, 250℃and 290℃ respectively. The minimum threshold of detection for 7504 was 0.1 ng/μL. An identical setup was used for the analysis of chlorpyrifos except that the injector, column and detector temperatures were set at 250 ℃, 230 ℃ and 300 ℃ respectively. The minimum threshold of detection for chlorpyrifos was 0.001 ng/μL.

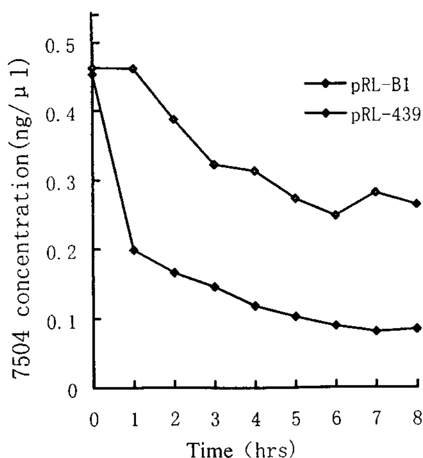
RESULTS AND DISCUSSION

The immobilized cells were used to degrade the organochlorine insecticide Acetofenate (7504) and the OP insecticide chlorpyrifos. When 7504 was incubated with immobilized cells at 37℃, 56 % of the compound was degraded after 1 hr and 78% after 8 hr (Fig. 1). When chlorpyrifos was incubated with immobilized cells at 37℃, 21% of the compound was degraded after 1 hr and 45% after 8hr (Fig. 2). It showed that immobilized transgenic *E. coli* cells could degrade insecticides that contain ester bonds. The different degradation rates of the two insecticides probably reflects the fact that different ester bonds have different degradation rates (Huang et al. 2001). Consequently, insecticides with carboxylester bonds are more rapidly degraded than those with other ester bonds (Huang et al. 2001). Preliminary experiments on the ability of these immobilized recombinant cells to hydrolyze other pesticides indicate that they can also hydrolyze methyl parathion (data not shown). Additional experiments are under way to determine the range of ester compounds that can be degraded and whether steric hindrance at the active site or other some factor is responsible for any inability to hydrolyze substrates.

Leng and Qiao (Leng et al. 1986) found that *in vivo* rat liver cells could effect a 93.9% degradation of 7504 within 40 min of incubation and an 88.7% degradation of deltamethrin within 90 min of incubation. These insecticides were degraded by the hydrolysis of their ester bonds. However, because rat liver cells contain many different enzymes they can only be used as a model in the degradation of some chemicals.

Carboxylesterase is a collective term for the enzymes which hydrolyze carboxylic esters. The majority of OPs are esters of phosphoric acid and can therefore be hydrolyzed by carboxylesterases. Most OPs are administered in the biologically inactive phosphorothionate form which is converted into the active organophosphate form by monooxygenases within the insect (Hemingway and Karunaratne 1998). The rate of interaction of carboxylesterases with organophosphates is very fast. For example, the bimolecular rate constant for the reaction between Est B1 and parathion-oxon is $1.5 \times 10^8\ M^{-1}min^{-1}$(Karunaratne et al. 1993). However, because the deacylation rate is very slow, it may take about 32 hr for one molecule of enzyme to completely hydrolyze a paraoxon molecule (Karunaratne et al. 1993). Deacylation rates are significantly higher for the aphid paraoxon inhibited esterase E4, where one molecule is completely hydrolyzed in 3hr (Devonshire and Moores 1982).

In order to more easily obtain a high, stable concentration of immobilized cells, it is very important to select a suitable alginate concentration. We found that 6%



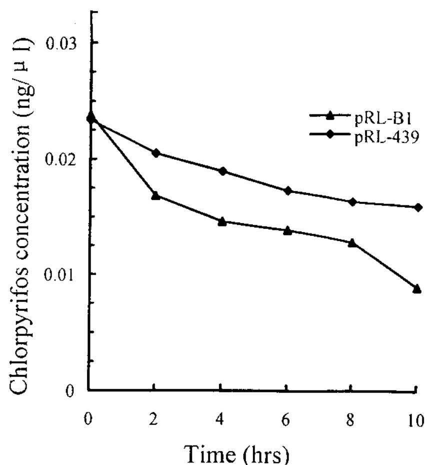


Figure1. Degradation of 7504 by immobilized *E. coli* cells

Figure 2. Degradation of chlorpyrifos by immobilized *E. coli* cells

alginate is suitable for the immobilization of *E. coli*. We also found that after immobilized cells have been cultured for two weeks, then the immobilized cells were washed and activated at 37℃ overnight in LB medium, they can be reused once more (data not shown). Such reimmobilized cells were capable of degrading α-naphthylacetate and β-naphthylacetate. Recombinant cells have proved useful in the bioremediation of surface water contaminated by pesticides and other organochlorides (Yan et al. 2000, unpublished data). Assays showed that immobilized recombinant cells can degrade 61% of deltamethrin within one hour. Experiments indicate that the detoxifying enzyme in expression strains of *E. coli* can detoxify organophosphate insecticides, and was effective in detoxifying hens poisoned by organophosphate insecticides (Qiao and Yan, 2000).

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