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Short Communication: Methylparathion degradation by *Pseudomonas* sp. A3 immobilized in sodium alginate beads

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An organophosphate-degrading soil isolate of *Pseudomonas* sp. A3, immobilized at 5% (wet wt/v) cell mass in 3% (w/v) sodium alginate beads, detoxified 99% of 1 mM methylparathion in 48 h. The beads were re-usable for five batches, the sixth batch only giving 73% methylparathion removal.

Key words: Cell immobilization, methylparathion, *Pseudomonas* sp.

Methylparathion(*O,O*-dimethyl-*O*-*p*-nitrophenylphosphorothionate; MP) is a non-systemic insecticide used extensively in rice fields for the control of a wide range of insects. It is a potent inhibitor of acetylcholine esterase. We have isolated a soil bacterium, *Pseudomonas* sp. A3, which can utilize organophosphates such as MP, diazinon, monocrotophos and hinosan as sole carbon source. The potential use of immobilized cells of this soil isolate in detoxifying MP and the optimal conditions needed are the subjects of the present study.

Materials and Methods

The cells were grown in mineral salts medium (Saunders *et al.* 1984) with 0.5% (w/v) glucose for 48 h on a rotary shaker and then harvested by centrifugation. The cells were washed twice with sterile saline and the wet pellet was weighed and used to prepare sodium alginate beads. These were activated in mineral salts medium with 0.5% (w/v) glucose for 5 h and then washed again with distilled water. The activated beads (100 g) were packed in a glass column and filled with 200 ml mineral salts medium amended with MP which served as the sole carbon source. The residual MP was estimated (Siddaramappa *et al.* 1973) at intervals in the spent medium. Unless otherwise stated, all the parameters were tested in a column containing 1 mM MP and MP degradation is expressed as the percentage of the MP added initially.

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Results and Discussion

A uniform amount of cells (5%, wet wt/v) was immobilized in beads with alginate ranging from 2% to 5% (w/v). Although 98% degradation of MP occurred within 36 h when 2% alginate was used, considerable splitting of the beads and cell leakage were noted. Beads with 3% alginate offered better stability and their use resulted in 99% degradation in 48 h, compared with 76% and 66% degradation over the same period with the cells entrapped with 4% and 5% alginate gel, respectively. Therefore, the optimal concentration of alginate was 3%; beads prepared from 3% alginate and 5% cell mass offered the highest degradation (Figure 1).

Assessment of cell leakage from the beads indicated that the optimum concentration of $CaCl_2$ needed for immobilization was 3%, although $CaCl_2$ concentration had little affect on the subsequent degradation rate. To assess the impact of pesticide concentration on the degradative potential of the encapsulated cells, varying concentrations of MP (1 to

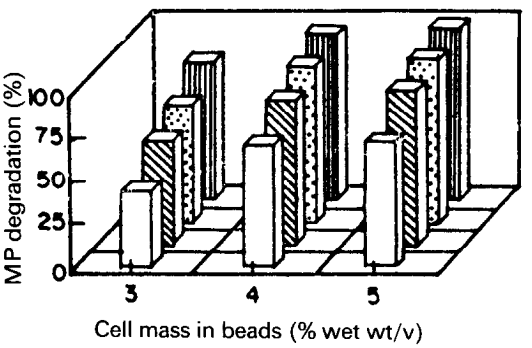

Figure 1. Degradation of methylparathion (MP), initially at 1 mM, after 12 (□), 24 (▨), 36 (▩) and 48 (▥) incubation with cells of *Pseudomonas* sp. A3 immobilized in sodium alginate beads. Cells weighing 1 g wet weighed 0.18 g when dry.

4 mM) were tested in different columns, each with 5% cell mass entrapped in 3% alginate gel and each run for 48 h. Although MP completely disappeared from the column initially containing 1 mM, only 95%, 81% and 63% of the initial MP was degraded when 2, 3 and 4 mM MP was provided, respectively.

Repeated batch experiments were carried out to evaluate the efficacy of immobilized cells under repeated use. The beads containing 5% cell mass were re-used repeatedly for five batches and each batch was run for 48 h. After each run, the spent medium was drained and the beads were carefully washed with distilled water and packed again in the same column. Fresh sterile medium with MP was added to the column and residual pesticide was estimated at the end of each run. The immobilized cells degraded 99% of 1 mM MP in 48 h in the first batch and were stable for four more runs without any significant reduction in the rate of degradation. Only 73% degradation of MP occurred in the sixth run and there was a concurrent increase in cell leakage and bead fragility and deformation. Although *p*-nitrophenol is an intermediate of MP breakdown (Caldwell & Raushel 1991), it did not accumulate in these experiments.

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