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# Reduction of Fensulfothion and Accumulation of the Product, Fensulfothion Sulfide, by Selected Microbes

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Fensulfothion (0,0-diethyl 0-[4-(methylsulfinyl)phenyl] phosphorothioate), marketed under the trademark names of Dasanit and Terracur-P, is an organophosphorus pesticide with activity against both insects and nematodes. The metabolism of this pesticide by plants and animals has been studied in detail, but relatively little is known about its interactions with bacterial and fungal species.

WOOD & MACRAE (1977) first demonstrated the reduction of fensulfothion to fensulfothion sulfide by washed-cell suspensions of the bacterium, Klebsiella pneumoniae. Their work was later extended by TIMMS & MACRAE (1982) who studied in more detail the conversion of fensulfothion by K. pneumoniae and showed that the fensulfothion sulfide formed was rapidly and firmly bound by the bacterial cells. Even heat-killed cells accumulated the sulfide from solution. ROSENBERG & ALEXANDER (1979) isolated 2 pseudomonads able to utilize fensulfothion as a sole phosphorus source. They proposed that hydrolytic attack by a phosphatase or phosphotriesterase was the major pathway for degradation of fensulfothion by these bacteria. If other soil microbes are able to degrade fensulfothion and bioconcentrate fensulfothion sulfide, the activity of topically applied pesticide could be significantly affected.

This study was undertaken to examine several bacterial and fungal species for the ability to reduce fensulfothion and to bioconcentrate the product, fensulfothion sulfide.

## MATERIALS AND METHODS

Microorganisms and growth conditions. The following microorganisms were used: Klebsiella sp., Escherichia coli, Pseudomonas fluorescens, Lactobacillus plantarum, Leuconostoc mesenteroides, Nocardia opaca, Penicillium citrinum and Trichoderma viride.

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All microorganisms, except L. plantarum and L. mesenteroides, were grown in sucrose-mineral salts broth (YOCH & PENGRA 1966) without added Tween 80. The medium was modified for these 2 organisms by adding 0.1% (w/v) yeast extract. Washed-cell suspensions (WCS) were prepared by harvesting logarithmic phase cells, washing twice (4870 g x 30 min), and finally resuspending in 0.25M phosphate buffer (pH 7.2) to a cell dry weight of 0.8-1.0 mg/mL.

Chemicals. Analytical grade fensulfothion and fensulfothion sulfide (0,0-diethyl 0-[4-(methylthio)phenyl] phosphorothioate) were provided as gifts by Farbenfabriken Bayer A.G., Leverkusen, Germany.

Analysis by Gas Chromatography. Analyses were performed with a dual-column Shimadzu model GC-4A instrument having dual flame thermionic detectors. Two stainless steel columns (0.4 and 2 m long, 4 mm i.d.) packed with 10% (w/w) DC 200 silicone oil on 80/100 mesh Gas-Chrom Q were used. The flow rate of oxygen-free nitrogen (carrier gas) was 40 mL/min while the temperatures of the column oven, detectors, and injection ports were 208, 225, and 230°C, respectively. Cell suspensions were extracted with high-purity hexane (1:1 v/v) containing diazinon (1.8 µg/mL) as internal standard. After phase separation, the hexane layer was dehydrated over anhydrous $MgSO_4$ and analyzed by gas chromatography.

Pesticide reduction and bioconcentration assays. Replicate assay flasks were charged with 90 mL phosphate buffer containing approximately 50 µg/mL fensulfothion and 10 mL of WCS. The flasks were incubated without shaking at 36°C. Samples taken at 0, 3, and 24 h were analyzed by gas chromatography to quantify conversion of fensulfothion to fensulfothion sulfide.

Bioconcentration of fensulfothion sulfide by bacterial cells was studied by adding 5 mL lots of WCS to 45 mL phosphate buffer containing approximately 40 µg/mL fensulfothion sulfide. Control flasks contained an extra 5 mL phosphate buffer instead of WCS. All flasks were incubated without shaking at 36°C for 1 h after which 5 mL samples were taken and centrifuged at 1800 g for 15 min. The supernatant and cell pellet (resuspended in 5 mL phosphate buffer) fractions were extracted with n-hexane and the extracts were analyzed for fensulfothion sulfide by gas chromatography. Bioconcentration factors were determined by the method of JOHNSON & KENNEDY (1973).

## RESULTS AND DISCUSSION

Six of the 8 organisms tested reduced fensulfothion to fensulfothion sulfide (Table 1). Only the 2 bacteria requiring yeast extract for growth, L. plantarum and L. mesenteroides, were unable to bring about the conversion. Notably the highest reduction rates were by the 2 enterobacteria tested, Klebsiella sp. and E. coli. Despite prolonged incubation (48 h) no evidence was obtained that would indicate that the microbes could degrade fensulfothion beyond the sulfide.

Table 1. Reduction of fensulfothion (F) to fensulfothion sulfide (FS) by bacteria and fungi

| Organism | 0 h | | 3 h | | 24 h | |
|---|---|---|---|---|---|---|
| | F | FS | F | FS | F | FS |
| | Concn (μg/mL) | | | | | |
| Klebsiella sp. | 45.0 | - | 1.1 | ++++ | ND[a] | ND |
| E. coli | 48.9 | - | 0 | ++++ | ND | ND |
| P. fluorescens | 50.1 | - | 38.8 | ++ | 27.8 | +++ |
| N. opaca | 52.7 | - | 46.6 | ++ | 27.4 | +++ |
| L. plantarum | 56.1 | - | 52.0 | - | 54.1 | - |
| L. mesenteroides | 45.4 | - | 46.0 | - | 47.3 | - |
| P. citrinum | 48.4 | - | 46.1 | + | 32.7 | ++ |
| T. viride | 50.2 | - | 45.3 | + | 34.6 | ++ |

[a]ND, not determined
-, no FS production

In contrast to the variation in fensulfothion reducing-capacity, all organisms tested, concentrated the sulfide within their cells (Table 2). Bioconcentration factors ranged from 7.2 for L. plantarum to 28.8 for Nocardia opaca. Bioconcentration was apparently not related to reducing ability. As previously suggested (TIMMS & MACRAE 1982) the bioconcentration of fensulfothion sulfide probably does not involve biological energy but is simply the result of a high affinity of the sulfide for bacterial cell membrane components.

Table 2. Accumulation of fensulfothion sulfide (FS) by bacterial cells after incubation for 1 h at 36°C

| Organism | FS (μg/mL) | | BCF[a] |
|---|---|---|---|
| | supernatant | cell pellet | |
| Control[b] | 37.4 | 0 | - |
| Klebsiella sp. | 2.9 | 22.8 | 9.8 |
| E. coli | 2.7 | 30.4 | 14.1 |
| P. fluorescens | 3.4 | 33.7 | 12.4 |
| N. opaca | 1.5 | 34.5 | 28.8 |
| L. plantarum | 2.5 | 14.1 | 7.2 |
| L. mesenteroides | 3.2 | 28.1 | 11.0 |

[a]Bioconcentration factor (BCF) = total pesticide residue in bacteria (ng/mg)/pesticide water conc (ng/g)

[b]Control flask did not contain cells.

The widespread ability of microbes to reduce fensulfothion and accumulate fensulfothion sulfide within their cells may well be of ecological significance. Conversion of fensulfothion by soil microflora to the less toxic fensulfothion sulfide ($I_{50}M$ values of $7.3 \times 10^{-6}$ and $9.2 \times 10^{-6}$, respectively (BENJAMIN et al. 1959), may significantly reduce the effectiveness of topically applied pesticide. It nevertheless represents the first step in a possible detoxification pathway, and it is ecologically important that applied pesticide be degraded rapidly before it can be transported from the site of application and affect nontarget organisms.

The use of pure cultures and controlled experimental conditions allowed us to study more precisely the reduction of fensulfothion and bioconcentration of the sulfide. While rates of activity in the more complex soil environment might be variable, the results we obtained in this work should be generally applicable to the field situation.

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