

Bacterial Reduction of Fensulfothion and Its Hydrolysis Product 4-Methylsulfinyl Phenol

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Oxygen-limited cultures of *Klebsiella pneumoniae* reduced 4-methylsulfinyl phenol to 4-methylthiophenol. A study of the effect of 4-methylthiophenol on the growth of *K. pneumoniae* revealed that the specific growth rate was retarded by 40% in the presence of 200 µg of the phenol per ml. A soil bacterium, *Hafnia* sp., was isolated that could reduce the organophosphorus insecticide fensulfothion to fensulfothion sulfide.

The reduction of fensulfothion (*O,O*-diethyl *O*-[4-(methylsulfinyl)phenyl]phosphorothioate) to fensulfothion sulfide has been demonstrated for several bacterial and fungal species (5, 6). A soil isolate, *Pseudomonas alcaligenes* C₁, was found to hydrolyze fensulfothion to 4-methylsulfinyl phenol and diethyl phosphorothioic acid, and the bacterium used the phosphoric ester as a source of carbon and energy (4). However, the 4-methylsulfinyl phenol apparently could not be utilized by *P. alcaligenes* as it accumulated in the growth medium. Hydrolysis of a number of organophosphorus pesticides results in the formation of various substituted phenolic compounds (2, 7), and as these phenolic compounds are toxic and also may form bound residues, knowledge on their biotransformation is important. Although there is little information on the degradation of phenols of this type, reports are available on the oxidation of 4-methylsulfinyl phenol and 4-methylthiophenol (1) and 3-methyl-4-(methylthio) phenol (3) by a *Nocardia* sp. This report describes the isolation and identification of a soil bacterium capable of reducing fensulfothion and the reduction of fensulfothion and 4-methylsulfinyl phenol by *Klebsiella pneumoniae*.

The bacteria used in this study were *K. pneumoniae* UQM 90 from the University of Queensland Department of Microbiology Culture Collection and a soil bacterium that was isolated for its ability to reduce fensulfothion to fensulfothion sulfide. Both bacteria were grown in sucrose mineral salts broth (8) without added Tween 80.

Experiments to determine the effect of 4-methylthiophenol on the growth of *K. pneumoniae* were conducted with liquid sucrose mineral salts broth with or without the phenol, dispensed in 40-ml amounts in 250-ml Erlenmeyer side-arm flasks. After inoculation, the flasks were incubated in a water bath shaker at 36°C, and growth was followed by determining absorbance at 540 nm. The reduction of fensulfothion by *K. pneumoniae* and the soil isolate, and 4-methylsulfinyl phenol by *K. pneumoniae*, was carried out with oxygen-limited cultures of the two bacteria in sucrose mineral salts broth that had been amended with either 50 µg of fensulfothion per ml or 100 to 125 µg of 4-methylsulfinyl phenol per ml. Oxygen limitation was achieved by preincubation of the bacteria in sucrose mineral salts broth for 18 h at 36°C, after which the pesticide or phenol was added and the cultures were reincubated at 36°C. Samples were withdrawn periodically and extracted with high-purity hexane (1:1; vol/vol) containing diazinon (1.8 µg/ml) as the internal

standard and analyzed for fensulfothion and fensulfothion sulfide by gas chromatography. In the case of 4-methylsulfinyl phenol, samples were extracted with ethyl acetate and analyzed by gas chromatography and thin-layer chromatography. Analyses for fensulfothion and fensulfothion sulfide were performed with a dual-column Shimadzu model GC-4A instrument with dual-flame thermionic detectors. Two stainless steel columns (length, 0.4 and 2 m; internal diameter, 4 mm) packed with 10% (wt/wt) 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, and the column oven, detector, and injection port temperatures were 208, 225, and 230°C, respectively. Analyses for 4-methylthiophenol were performed with a Shimadzu GCR1A equipped with dual-flame ionization detectors. Two glass columns (length, 2.1 m; internal diameter, 2.6 mm) packed with 1% SP-1240 DA on 100/120 mesh Supelcoport were used. Column oven and detector temperatures were 170 and 200°C, respectively. Analysis of culture extracts by thin-layer chromatography for fensulfothion, fensulfothion sulfide, 4-methylthiophenol, and 4-methylsulfinyl phenol was done with Merck aluminium sheets precoated with Silica Gel 60 F₂₅₄. The chromatograms were developed by using the solvent, dichloromethane-ethanol (97:3), and the spots were visualized under short-wave UV light. Analytical-grade fensulfothion, fensulfothion sulfide, and 4-methylsulfinyl phenol were provided as gifts by Farbenfabriken Bayer AG, Leverkusen, Federal Republic of Germany. The 4-methylthiophenol was obtained from Sigma Chemical Co., St. Louis, Mo. The soil isolate was able to reduce fensulfothion to fensulfothion sulfide under oxygen-limited conditions similar to those used for *K. pneumoniae* (5). Approximately 50% of the 50 µg of the fensulfothion per ml added to the culture was converted to the sulfide in a 24-h incubation period. The isolate had the following characteristics: gram-negative, rod-shaped cells (0.5 by 1.5 µm); motile with peritrichous flagella; produced acid and gas from glucose, maltose, rhamnose, sucrose, xylose, salicin, mannitol, and sorbitol but had no reaction with arabinose, lactose, raffinose, adonitol, or inositol; utilizes citrate; methyl red negative, Voges-Proskauer positive; positive for catalase, arginine dihydrolase, arginine decarboxylase, nitrate reduction to nitrite, and malonate utilization; and negative for H₂S and indole production. On the basis of these characteristics, the isolate was classified as *Hafnia* sp.

The results (Fig. 1) demonstrate that oxygen-limited cells of *K. pneumoniae* are able to reduce 4-methylsulfinyl phenol to 4-methylthiophenol. The reduction of the phenol followed

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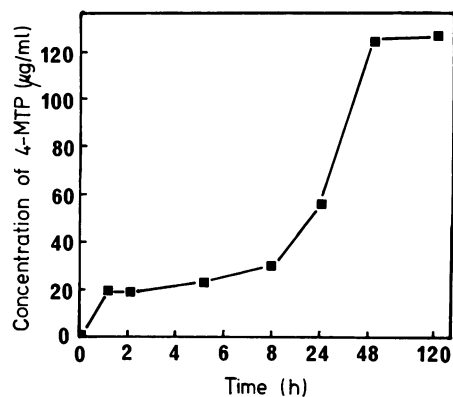


Fig. 1. Formation of 4-methylthiophenol from 4-methylsulfinyl phenol by an oxygen-limited culture of *K. pneumoniae*.

a course similar to the reduction of fensulfothion by *K. pneumoniae* (5). No evidence for the degradation of 4-methylthiophenol was obtained, as ca. 125 µg of the phenol accumulated per ml and persisted in the oxygen-limited cultures. The accumulation of the phenol may have been due in part to its toxicity, as the results obtained in experiments on the effect of 4-methylthiophenol on the growth of the bacterium showed that the specific growth rate was retarded by 40% in the presence of 200 µg of the phenol per ml. The specific growth rates were, respectively, 0.73, 0.63, 0.62, 0.62, and 0.45 h⁻¹ in the presence of 0, 25, 50, 100, and 200 µg of the phenol per ml. Concentrations of more than 400 µg/ml have been found to be toxic to *Nocardia* sp. (1).

Results also were obtained which indicated that 4-methylthiophenol was formed in oxygen-limited cultures of *K. pneumoniae* to which fensulfothion was added. Thin-layer and gas chromatographic analyses showed that a component in culture extracts which had an *R_f* value of 0.7 and a retention time of 7.75 min matched exactly these values for an authentic sample of 4-methylthiophenol. The phenol was probably formed by the hydrolysis of fensulfothion sulfide which is formed rapidly by *K. pneumoniae* at lowered

oxygen levels. The results of these pure culture studies indicate that biotransformation of fensulfothion in soil under oxygen-limited conditions, such as waterlogging or flooding, may lead to the formation and possible accumulation of reduction products, such as fensulfothion sulfide and 4-methylthiophenol. Also, the retardation in the growth rate of *K. pneumoniae* found in the present study indicates that the toxicity of 4-methylthiophenol should be considered when evaluating the overall biological activity of this organophosphorus insecticide.

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