Transformation of Malathion by a Fungus, *Aspergillus oryzae*, Isolated from a Freshwater Pond

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Although fungi proliferate in aquatic environments under conditions of high nutrient availability, their role in pesticide transformation in aquatic ecosystems has received little attention. This has been partly due to the difficulties involved in quantification of fungal growth and metabolism. The purpose of this work was to investigate the rates and products of malathion [0,0-dimethyl S-(1,2 dicarbethoxy)ethylphosphorodithioate] transformation by a fungus, commonly isolated from aquatic environments, in order to indicate whether or not transformation rates and products are similar to those resulting from bacterial metabolism or chemical reaction.

MATERIALS AND METHODS

Organism and Growth Conditions. The fungal isolate, Aspergillus oryzae, was isolated from a local pond and identified by Gene E. Michaels, Department of Microbiology, University of Georgia, using the scheme of RAPER and FENNEL (1965). The Aspergillus flavus-oryzae group has been isolated from most types of freshwater aquatic habitats (COOKE, 1963) and is expected to be active in an aquatic environment provided sufficient nutrients were available. Plate cultures of the fungus exhibited a tendency to vary from the primary variety to a more flocculent variety of the same species resembling A. oryzae var. effusus. A parasitic species of Penicillium was also noted on some subcultures of the original cultures submitted for identification. However, Penicillium was not directly confirmed in the original cultures and was not noted by direct observation of any cultures used in malathion experiments.

Stock cultures of A. oryzae were maintained on plates containing Saboraud's medium (Difco) diluted 1:10. Inocula for experiments were suspensions prepared by agitating 10 ml of sterile water in a plate

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containing the sporulating fungus. A pH 6.8 basal salts medium (PAYNE and FEISAL, 1963) containing 0.05 g (0.278 mmol) glucose per liter of distilled water was used as the liquid culture medium. This medium yielded 25 ± 4 mg dry weight of fungi per 50 mg glucose. Fungi were separated from liquid cultures for dry weight determinations by filtering, first through tared pre-filters then through tared 0.22 micron Nucleopore filters, and drying to a constant weight at 90°C.

Duplicate 500 ml flasks of the various weights of suspended fungi were prepared by measuring appropriate portions of liquid medium inoculated with a fungal suspension. The flasks were then incubated on a gyrotory shaker at 28°C for three days. After incubation, the cultures were adjusted to a final volume of 200 ml with a basal salts/malathion solution containing a predetermined amount of dissolved malathion (99.9% pure from American Cyanamid). Stock solutions of basal salts/malathion were prepared by stirring malathion overnight in basal salts solution and filter-sterilizing through a 0.22 micron Millipore filter.

Thin-Layer and Gas-Liquid Chromatography. Determinations of malathion concentration were made by gas-liquid chromatography of 2,2,4-trimethylpentane extracts of culture medium and control solutions. Extraction efficiency was 96-97% at a ratio of 1:1 sample:extraction solvent. The instrument used was a Tracor MT-220 gas-liquid chromatograph equipped with a high temperature nickel-63 electron capture detector. A one-meter glass column (4 mm ID) containing 80/100 mesh GasChrom-Q with 3% silicone SE-30 liquid phase was used. Malathion eluted as a single symmetrical peak in 0.85 min at a column temperature of 170°C with a nitrogen carrier flow of 120 ml/min. Detector and inlet temperatures were set at 240°C and 195°C, respectively. A linear response range was observed between 0.05 and 0.5 ng, and malathion quantities were determined by peak height comparison using standards with closely matched peak heights within this range of linearity.

Silica gel plates with hexane:acetic acid:ethyl ether (75:15:10) developing solvent were used for thin-layer chromatography of malathion transformation products. Thin-layer elutions were visualized by spraying developed plates with 2,6-dibromo-N-chloro-p-quinoneimine (MENN et al.,1957). β-Malathion monoacid and malathion dicarboxylic acid standards were synthesized and their molecular structures were established by mass spectrometry, ^{13}C and proton nuclear magnetic resonance analysis, and infrared spectroscopy

(WOLFE et al., 1974). For quantitative determination of β-malathion monoacid by gas-liquid chromatography, samples of liquid cultures were acidified and extracted with ethyl ether and methylated with diazomethane (WEBB et al., 1973).

RESULTS AND DISCUSSION

Malathion concentrations rapidly decreased in A. oryzae liquid cultures and virtually all malathion lost could be accounted for in the form of transformation products. Filtrates of liquid cultures containing malathion showed no change in malathion concentration; therefore, transformation by chemical or exoenzymatic activity was ruled out. The velocity of malathion transformation was proportional to dry weight of fungal biomass, and would be expected to remain proportional to fungal biomass as long as the fungi are well enough suspended and sufficient turbulence is maintained so that transport into fungal conglomerates or mats does not become a limiting factor.

During the course of laboratory experiments with malathion, no growth of fungi could be detected. Fungal biomass was therefore regarded as constant and a constant enzyme concentration was assumed for the duration of the experiments.

Malathion transformation for a given fungal concentration was first order with respect to malathion concentration, [S], from 0.01 to 20.0 mg/l malathion (Figure 1). A maximum velocity, V_{max}, was reached at approximately 40 mg/l malathion and the velocity of transformation was zero order with respect to malathion concentration up to 90 mg/l malathion, the maximum concentration used.

The observed velocities of malathion transformation can be accurately described using the Michaelis-Menten equation (WHITE et al., 1964)

$$V = \frac{V_{max}[S]}{K_m + [S]} \qquad (1)$$

where V_{max} is the maximum velocity of malathion uptake, [S] is malathion concentration, and K_m is the Michaelis-Menten constant.

Using a rearranged form (LINEWEAVER and BURKE, 1934) of the above equation

$$\frac{[S]}{V} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}} \qquad (2)$$

experimental values of [S]/V were plotted as a function of [S]. The slope of the resulting plot, $1/V_{max}$, determined by a computer program employing least squares analysis, yields a value for V_{max} of (7.8 $\pm$ 2.6) x 10^{-3} mg malathion per hr per mg fungus. K_m was determined from the y intercept, K_m/V_{max}, to be 16.3 $\pm$ 6.7 mg/l malathion. At velocities much below V_{max}, malathion transformation may be limited by transport to enzyme sites, while velocities of transformation at or near V_{max} may be limited by enzyme concentration.

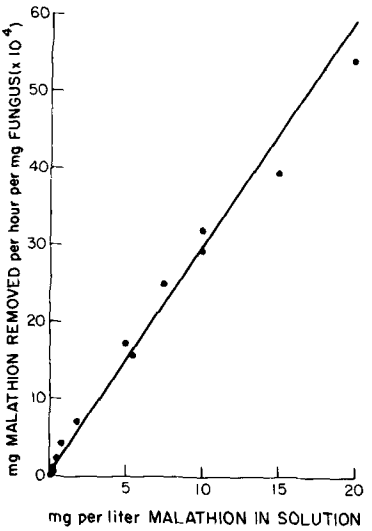

Figure 1. Rate of Malathion Transformation Versus Malathion Concentration.

In studies of bacterial transformation of malathion (PARIS et al., 1974), growth based on viable plate counts was significant at concentrations from approximately 10^5 to 10^9 bacteria per liter. At very high bacterial concentrations, approximately 10^{12} viable bacteria per liter, no growth was observed and transformation was first order with respect to malathion concentration as in fungal experiments. A comparison of velocities of malathion transformation

for the bacteria (under no growth conditions) and fungi used in our laboratory studies indicated that transformation velocities (per unit dry weight of cell material) for bacteria were about 5,000 times greater than those for fungi under similar conditions of malathion concentration ($<<K_m$), temperature, and agitation rate.

Comparison of thin-layer chromatographic R_f values of transformation products with those of known standards showed the presence of β-malathion monoacid and malathion dicarboxylic acid. Quantitative gas-liquid chromatographic analysis of extracts methylated with diazomethane showed that approximately 97% of the malathion removed from solution could be accounted for in the form of β-malathion monoacid. In bacterial cultures discussed earlier, approximately 99% of the malathion in solution was converted to β-malathion monoacid. Both bacterial and fungal cultures were demonstrated to use ethanol, the probable by-product of malathion monoacid formation, as a sole carbon source. Conversion of malathion primarily to the beta isomer of malathion monoacid may therefore be a representative pathway of heterotrophic transformation of malathion. This is different from chemical hydrolysis of malathion, which proceeds primarily to the alpha isomer of malathion monoacid (WOLFE et al., 1974).

In conclusion, our studies with A. oryzae indicate that fungal transformation of malathion yields the same major metabolities as those produced by bacteria; however, the rate of transformation by A. oryzae is much slower. Transformation rates reported for bacterial and fungal cultures in the laboratory are not expected to be the same as those observed for microbial populations in the field, but they do provide some basis for comparison with other degradative processes.

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DISCLAIMER

Mention of commercial products does not necessarily constitute endorsement by the Environmental Protection Agency.

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