Metabolism of DDT by Fresh Water Diatoms

by S. MIYAZAKI and A. J. THORSTEINSON
Department of Entomology, The University of Manitoba
Winnipeg, Canada R3T 2N2

Introduction

Reductive dechlorination of DDT to TDE (DDD) under anaerobic conditions has been demonstrated in baker's yeast (1), a fungus (2), several actinomycetes (3), and bacteria (4,5,6, 7,8,9,10). DDE, the dehydrochlorination product of DDT, was also found to occur with several bacteria (5,10) and a marine diatom (11). Keil and Priester (11) suggested a possible role of diatoms in detoxifying DDT in aquatic environments. Our paper reports on metabolism of DDT by fresh water diatoms collected from a mosquito breeding site near Winnipeg, Manitoba, Canada.

Materials and Methods

Ten diatom cultures were isolated from a sample of ditch water by adding 5 ml of sample to Werner's agar (12) in sterile petri dishes. The plates were incubated at 20°C for 2 weeks with a photoperiod of 16 hours-light and 8 hours-dark, at about 3200 lumens per square meter, using cool white fluorescent tubes. Individual clones were selected and inoculated in Werner's liquid media (12). After incubation, these cultures were transferred to fresh Werner's agar to insure culture purity. Each of these pure cultures was transferred to a 125 ml Erlenmeyer flask containing 50 ml of Werner's medium and incubated at 20 - 22°C in a gyratory water-bath shaker for 2 weeks at 120 cycles per minute after addition of 10^{-8}M (0.71 ppm) of uniformly ring-labeled ^{14}C-DDT (1,1, 1-trichloro-2,2-bis(p-chlorophenyl) ethane), specific activity 5.48 mc/mmole. DDT was introduced to the medium in 10 µl of benzene. At the end of the incubation period 0.5 ml of glacial acetic acid was added. The mixture was then immediately extracted 3 times, each time with 50 ml of n-hexane-ether (1:1). The medium was then filtered and the cells were extracted 3 times with 10 ml of acetone. The acetone extract was combined with the n-hexane-ether extracts. The solvent phase was then dried over anhydrous sodium sulfate, and further concentrated by a gentle air stream to 0.1 ml. Thin-layer chromatography was used to separate DDT from metabolites. A 10 µl portion of the concentrated material was spotted on a No. 6060 Eastman chromatographic sheet 2 cm from the edge. The sheet was then developed in a solvent mixture of n-hexane, ether and glacial acetic acid (100:1:1) (13) until the solvent front was 15 cm from the origin. The resulting chromatogram was exposed to Ilford Ilflex^{R} X-ray film for 4 weeks.

Cultures of Nitzschia species and an unidentified diatom species (designated hereinafter as Culture A) were selected for further studies based on the results of the preceding test. The procedure outlined above was repeated in 4 replicates of each diatom culture and the control consisting of the media without diatom inoculation. Another 10 µl portion of the same concentrated material

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was spotted on a second sheet and chromatogrammed using a mixture of cyclohexane and glacial acetic acid (24:1) to separate polar metabolites. Standard authentic samples of DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene), DDMU (1-chloro-2,2-bis(p-chlorophenyl)ethylene), DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane), TDE (DDD) (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane), DBP (4,4'-dichloro-benzophenone), dicofol (4,4'-dichloro- -(trichloromethyl)benzhydrol, DDA (2,2-bis(p-chlorophenyl) acetic acid), and DDOH (2,2-bis(p-chlorophenyl) ethanol) were spotted as references at the edge of each sheet. The portions of the silica gel adsorbent that corresponded to the radioactive spots detected by the X-ray film were cut out of the sheets, and the radioactivity of each spot was determined in a liquid scintillation counter (Nuclear Chicago 725).

Results and Discussion

Table 1 shows that the total radioactivity recovered from the control media was greater than in the diatom culture media and the difference was significant for Culture A. DDE, identified by thin-layer chromatography, was the only metabolite of DDT produced by either culture. The radioactive spots found on the chromatograms other than those of DDT and DDE were disregarded as impurities, because the radioactivity of these spots did not vary significantly from the corresponding spots of the control. Culture A degraded more DDT to DDE than the Nitzschia species but most of the DDT added to the media remained unchanged in both cultures. The fact that the total radioactivity recovered from the diatom culture media was less than from the control suggests that some of the DDT or its metabolite(s) were bound intracellularly, or were otherwise not extractable by the solvent system used. Evaporation of DDT from the chromatogram may also account for some loss of radioactivity. Our results suggest that some species of fresh water diatoms may be significant in the degradation of DDT to the non-insecticidal metabolite, DDE in nature.

TABLE 1

Metabolism of DDT by fresh water diatoms after 2 weeks incubation

Diatom	No. Repl.	Radioactivity Recovered[1]			
		DDT	DDE	Water Phase[2]	Total
Nitzschia sp.	4	58.6±6.7	0.4±0.0**	2.3±0.2*	63.3±6.5
Culture A	4	55.3±3.7**	0.9 ±0.1***	3.1±0.1**	60.4±6.5**
Control[3]	4	71.1±1.3	0.2 ±0.0	1.5±0.2	75.7±1.3

[1]Data are expressed in percentages of applied DDT (Mean±S.E.); * = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$.

[2]Media left after solvent extraction.

[3]DDT added to media and incubated without diatoms.

Summary

Laboratory cultures of fresh water diatoms (a Nitzschia species and an unidentified species) metabolized DDT to DDE only but the amount of conversion was relatively slight.

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