Microbial Degradation of DDT Metabolites to Carbon Dioxide, Water, and Chloride

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The current awareness of environmental problems has prompted innumerable studies on the fate of DDT in soil and water ecosystems. Until several years ago, DDT was considered a "recalcitrant molecule" in lieu of its persistence in soil (1). Wedemeyer (2, 3) demonstrated the removal of the trichloromethyl moeity from DDT in a series of several reactions brought about by A. aerogenes. One of the degradation products formed from DDT was p,p'-dichlorodiphenylmethane (DDM), which was shown in a later study by Focht and Alexander (4) to be converted to p-chlorophenylacetic acid; this compound was then cometabolized through the fission of the benzene ring. Though cleavage of both benzene rings of DDM by Hydrogenomonas was thus shown, the bacterium was unable to dehalogenate both ring-fission products. It was postulated that the chlorinated fragment formed from DDM degradation was probably a 3-chloro-substituted acid.

The report which follows describes the growth and metabolism of a fungus that is capable of converting these chlorinated bacterial degradation products to H_2O, CO_2, and HCl.

MATERIALS AND METHODS

The bacterial isolate employed in this study was isolated and cultured as previously described (4). The fungal isolate was obtained from sewage effluent by elective culture with 3-chloro-cis-crotonic acid (3-CCC) as the sole carbon source in a mineral salts medium. The isolate was streaked onto agar plates, obtained in pure culture form, and maintained in liquid media and on agar slants containing 3-CCC (1000 ppm).

Growth of both fungus and bacterium was determined turbidimetrically and by viable plate counts. The latter method was used for distinguishing between the two mixed populations: bacteria were counted on nutrient agar, and fungi were selected on potato-dextrose agar, pH 4.0.

Chloride was measured turbidimetrically with acidified $AgNO_3$. Degradation products formed from DDM and p-chlorophenylacetic acid by resting cell suspensions of Hydrogenomonas were extracted as previously described (4).

Substrates tested for their ability to support growth (Table 1) were used in 1000 ppm concentrations.

RESULTS AND DISCUSSION

The isolate was a hyaline Moniliaceae fungus producing fusiform phialogenous conidia in slimy clusters or in long chains. It was able to utilize all of the substances tested for growth except diphenylmethane as shown in Table 1. Surprisingly, Hydrogenomonas was capable of utilizing several chlorinated acids for growth, which certainly indicates that it does possess dehalogenase activity. Growth was particularly rapid and luxurious

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with chlorosuccinate. The bacterium was not able to grow on nor

TABLE 1
Utilization of substrates as sole carbon sources for growth of Hydrogenomonas and Fusarium

Substrate	Hydrogenomonas	Fusarium
3-chloropropionic acid	-	+
3-chloropropanediol	+	+
3-chlorobutyric acid	+	+
chlorosuccinic acid	+	+
3-chloro-cis-crotonic acid	-	+
3-chloro-trans-crotonic acid	-	+
cis-crotonic acid	+	+
trans-crotonic acid	+	+
phenylacetic acid	+	+
diphenylmethane	+	-

cometabolize the chlorocrotonic acids, though it did grow poorly on the nonchlorinated crotonic acids. The enzyme(s) necessary for dehalogenating the chlorocrotonic acids and the degradation products of DDM and p-chlorophenylacetic acid may possess high substrate specificity in accordance with the hypothesis advanced by Horvath and Alexander (5) to explain the nature of microbial cometabolism.

Extracted products from bacterial cell suspensions incubated with DDM and p-chlorophenylacetic acid were each re-extracted into basal salts media, purged with N_2 gas and filter sterilized. The fungal isolate was inoculated from a slant culture into each of the respective media. Growth was evident within five days, and both fungal supernatant liquids were positive for chloride. Uninoculated controls were negative. The fact that the fungus was able to grow on both ring fission products clearly established the breakdown of DDM--a DDT metabolite--to CO_2, H_2O, and HCl.

Mixed resting cell suspensions incubated with DDM rarely yielded more than 15% of the substrate as chloride with results from p-chlorophenylacetic acid only slightly better. Growing mixed culture systems yielded more chloride although in all experiments, a large portion of the substrate was unchanged.

Increased substrate concentrations of p-chlorophenylacetic acid increased the total growth. No correlations existed between cell density and the amount of p-chlorophenylacetic acid converted to chloride. The results shown in Table 2 only measure total growth and do not distinguish between the two species. Many other combinations were employed but revealed nothing of significance. The results here are complicated by the ability of both organisms to utilize each substrate for growth, although phenylacetic acid was a better substrate for growth of the bacterium, while crotonic acid was a better growth substrate for the fungus.

TABLE 2

Relationship of crotonic and phenylacetic acid concentrations to maximal growth and chloride production from p-chlorophenylacetic acid (100 ppm)

Crotonic acid (ppm)	Phenylacetic acid (ppm)	Maximal turbidity	Chloride %
50	50	.09	56
0	25	.04	37
50	25	.07	34
100	100	.14	29
100	0	.07	22
0	100	.13	22
25	50	.09	20
25	25	.05	17
0	0	.01	trace

The relationships of maximal cell numbers to diphenylmethane concentration and degradation of DDM are shown in Table 3.

Bacterial growth at zero substrate concentration is probably due to exogenous diphenylmethane carried over from the inoculum. Nevertheless the corresponding optical density was nil for bacterial cell concentrations of this magnitude. DDM definitely appears to have an inhibitory effect on bacterial growth as noted by the ten-fold or greater reduction in numbers for the 50 and 100 ppm levels of diphenylmethane. The numbers of fungi

TABLE 3

The influence of diphenylmethane (DPM) concentration upon microbial growth and DDM degradation

DPM (ppm)	With DDM (100 ppm)	Without DDM
200	7.0×10^8 (a)	1.4×10^9
	<10(b)	<10
	--(c)	--
100	4.6×10^7	5.4×10^8
	4.9×10^3	5.3×10^2
	12%	--
50	6.6×10^6	2.7×10^8
	7.7×10^3	1.2×10^3
	23%	--
0	9.9×10^5	2.3×10^6
	97	10
	trace	--

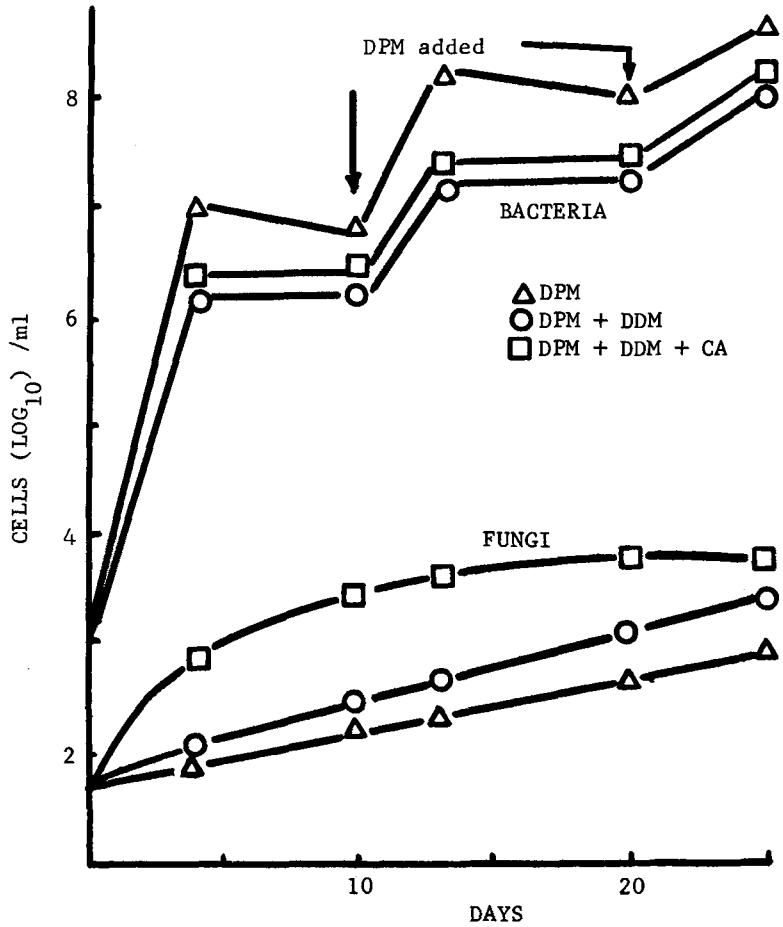

Figure 1. Microbial growth responses in mixed culture to diphenylmethane (DPM) at 50 ppm initial and supplemental concentrations, p,p'-dichlorodiphenylmethane (DDM) at 100 ppm, and crotonic acid (CA) at 1000 ppm.

(a) bacteria/ml
(b) fungi/ml
(c) % Cl^-

were higher with DDM than without, probably due to utilization of the bacterial degradation products for growth. The higher diphenylmethane concentration (100 ppm) results in a smaller total of fungal cells and less production of chloride than in the lower concentration of 50 ppm. This probably due to the more rapid growth of the bacterium, which in some way inhibits fungal growth, because when concentrations of 200 ppm diphenylmethane or higher were used, fungi could not be recovered.

Fungal growth was enhanced further by the addition of crotonic acid and by adding diphenylmethane over periodic intervals so that bacterial growth and cometabolism were sustained at levels that did not inhibit fungal growth. The results (Fig. 1) show the staggered pattern of bacterial growth as dependent on diphenylmethane, while fungal growth is not greatly affected by periodic supplements of diphenylmethane. Again, bacterial growth is lower where DDM is present, while the opposite is true of fungal growth. The production of chloride, where DDM was added, coincided with fungal growth curves. Only about 25% of total chloride present in DDM was produced from both treatments with and without crotonic acid. The differences between the two treatments did not appear significant despite the difference in numbers of fungi.

In conclusion, further work is needed to establish the intricate and subtle control mechanisms which influence the growth, regulation, and interactions of two quite different microorganisms in proximity of one another. The absence of complete degradation of DDM to chloride is certainly influenced by the extremely limited solubility of the compound and its toxicity to the bacterium. Fungal growth in such a system is totally dependent on degradation products excreted by the bacterium. In order to maintain the level of these products and the maintenance of the Hydrogenomonas, an exogenous energy source is needed because no energy can be supplied by the cometabolism of DDM. Yet, if too much of an energy source is used, bacterial growth "swamps out" fungal growth. This study demonstrates that when conditions approaching optimal are met, the degradation of DDM can proceed to CO_2, H_2O, and HCl. Future studies involving the use of controlled mixed culture systems with relation to metabolism of recalcitrant molecules should be seriously considered over the pure culture tecynique since the situation which occurs in nature is far removed from the metabolism of a single substrate by a single microorganism.

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