

Degradation of Dieldrin to Carbon Dioxide by a Soil Fungus *Trichoderma Koningi*

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The breakdown of dieldrin, one of the most persistent of the chlorinated hydrocarbon insecticides, by a number of different microorganisms has been reported in recent years. Bacteria known to degrade dieldrin to several metabolites are *Pseudomonas melophthora*, a symbiote of the apple maggot (2), other *Pseudomonas* sp., and several *Bacillus* sp., isolated from dieldrin contaminated soils as well as the fungus, *Trichoderma viride* (4). *Mucor alternans*, another soil fungus, showed twenty-six percent breakdown of the compound in two days, but no metabolites were identified (1).

Attempts have been made to understand the possible detoxification of dieldrin through the identification of some of these metabolites. Four strains of *Trichoderma viride*, closely related taxonomically to *Trichoderma koningi*, were found to hydrolyze this insecticide to 6,7-trans-dihydroxydihydroaldrin (5). This degradation product was also produced from dieldrin by the bacterium, *Aerobacter aerogenes* (9). Several metabolites were identified from a soil *Pseudomonas* sp. (6). Further studies with this bacterium demonstrated no evolution of radioactivity from the dieldrin isotope labeled on the chlorinated carbons. This *Pseudomonas* sp. apparently degraded the non-chlorinated segment of the dieldrin molecule and not the chlorinated carbon ring.

Experimental

Trichoderma koningi was selected from thirty other strains of bacteria and fungi through enrichment culture. These thirty microbes were originally isolated from contaminated sources such as dieldrin treated soil and agar petri plates coated with dieldrin as well as a chemostat containing dieldrin. Of these thirty cultures twenty-one showed considerable growth on the mineral salts broth (8) and 0.5 percent dieldrin with no growth in the controls. *Trichoderma koningi*, originally isolated from a cranberry mold, grew most abundantly on this medium with dieldrin as a sole carbon source.

This fungus was inoculated into a culture tube of 50 ml of yeast extract and mannitol broth (3). Gas containing 80:20 nitrogen to oxygen ratio was bubbled continually through the culture tube at flow rate of ca. 35 ml/min. The carbon dioxide

present in the affluent air was removed by passage through a tube containing 5 g of soda lime and a flask containing 200 ml of saturated barium hydroxide solution. The air was then bubbled through ethyl alcohol and filtered through glass wool before going through the medium. The gas evolved from the mycelium was filtered with cotton and carried through the carbon dioxide trap (7) of the ethanolamine solution (1:2 monoethanolamine to 2-methoxyethanol).

^{14}C -Dieldrin, labeled at all chlorine attached carbons, 33,000 counts per min., and $1.5 \times 10^{-5}\text{g}$ dieldrin (99.0%), both in acetone, were dried on sterile glass wool and aseptically inoculated into the fungal culture.

Sampling of the ^{14}C -carbon dioxide was made periodically from the ethanolamine solution and detection was by the liquid scintillation counting technique on the Packard Tri-carb 314 E (Packard Instrument Company, Inc., Downers Grove, Illinois).

The ^{14}C -UL-glucose, specific activity of 2.8 mCi per mM, was added at 1,300 counts per min. per culture.

Results and Discussion

The ^{14}C -carbon dioxide evolution is presented in percent radioactivity in the following table and demonstrates the complete degradation of a segment of the carbon structure of both the ^{14}C -UL-glucose and the ^{14}C -dieldrin by the fungus, Trichoderma koningi. The average radioactivity given off from the ^{14}C -dieldrin substrate is 3.1 percent, and the average radioactivity evolved from the ^{14}C -UL-glucose is 48.0 percent. No ^{14}C -carbon dioxide evolved from either of the control tubes containing the ^{14}C -dieldrin and the medium but no fungus.

Table 1.

Isotope	T. koningi replicate	Radioactivity (Counts per Min.)			Total CPM	Percent Isotope Evolved
		3 da.	10 da.	17 da.		
^{14}C -Dieldrin	1	0	711	351	1,062	3.2
	2	0	638	340	979	2.9
^{14}C -UL-Glucose	1	59	370	269	698	53.1
	2	114	388	106	608	46.8
	3	135	322	118	575	44.2
	control	0	0	0		

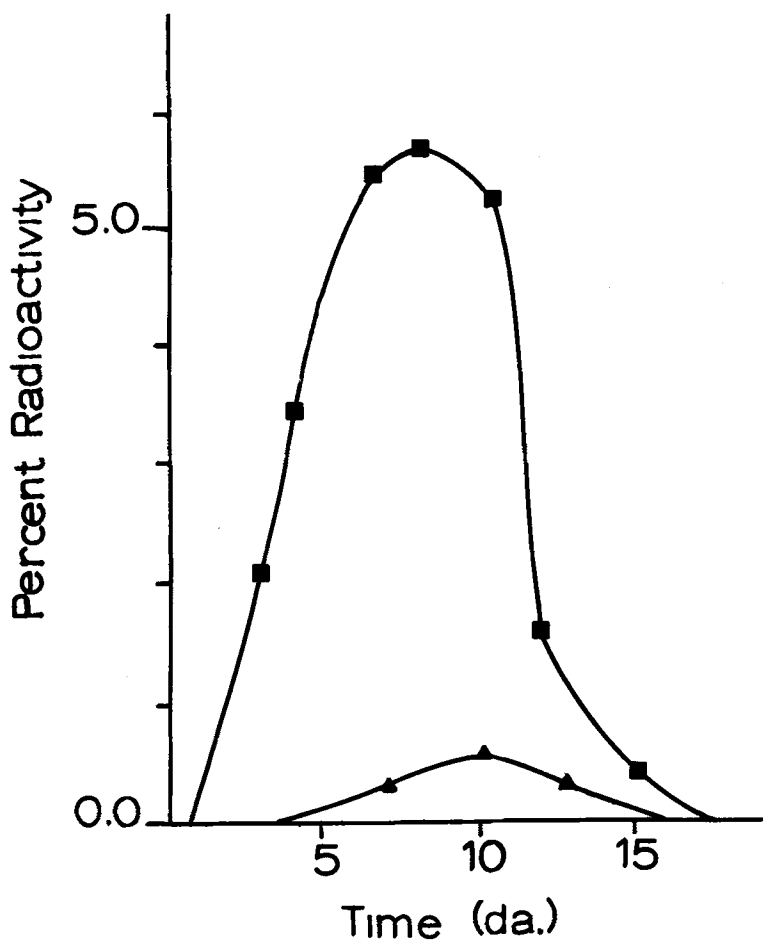


Figure 1. The distribution of the percent radioactivity evolved as ^{14}C -carbon dioxide from a culture of the fungus, *Trichoderma koningi*, labeled with the isotopes, ^{14}C -UL-glucose and ^{14}C -dieldrin, over an incubation period of 17 days. ^{14}C -UL-Glucose, —■—■—■—, ^{14}C -Dieldrin —▲—▲—▲—.

It is concluded that *T. koningi* is capable of degrading one or more of the carbons on the chlorinated ring of the dieldrin molecule.

The level of the remaining dieldrin in the spent medium of the culture containing ^{14}C -UL-glucose was negligible. The amount of radioactivity remaining in the spent medium with the ^{14}C -dieldrin isotope was approximately 15 percent. It is probable that the radioactivity unaccounted for is contained within the mycelium.

No degradation products other than ^{14}C -carbon dioxide have been identified.

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