Microbial Cometabolism of 2,4,5-Trichlorophenoxyacetic Acid

by R. S. HORVATH[1]
Department of Agronomy, Cornell University
Ithaca, N. Y.

The term cooxidation was first used by Foster (1) to describe the phenomenon by which a microorganism oxidizes a compound without being able to utilize the energy derived from the oxidation to sustain growth. Jensen (2) later suggested the more general term cometabolism to describe this phenomenon, because, for example, bacteria unable to utilize chlorine-substituted pesticides as carbon sources for growth, occasionally do dehalogenate the pesticide. Cometabolism may account for the degradation of many pesticides in nature and thus may be of considerable ecological significance.

The occurrence of microbial cometabolism of chlorinated aromatic compounds is well documented. An Arthrobacter was isolated by the author and was shown to cometabolize m-chlorobenzoate to 4-chlorocatechol (3). A Brevibacterium was subsequently isolated which cometabolized the herbicide 2,3,6-trichlorobenzoate to 3,5-dichlorocatechol (4). An Achromobacter was shown to be capable of cometabolizing 3-methylcatechol as well as the 4-chloro- and 3,5-dichlorocatechols produced in the previous work (5,6).

Alexander and Aleem (7) reported that no compound having a chlorine in the meta-position on its aromatic nucleus was transformed to an appreciable extent. Their conclusion was based on studies of chloro-aromatics, including 3,4-dichloro- and 2,4,5-trichlorophenoxyalkyl carboxylic acids (7). However, previous results obtained by the author (3,4,5,6) indicated that a cometabolic degradation of chloro-aromatic compounds was not inhibited by a chlorine substituent in the meta-position. For this reason, a study of the cometabolism of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was begun.

---

[1]Present address: Department of Biology
Bowling Green State University
Bowling Green, Ohio 43403

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 6, 1971, published by Springer-Verlag New York Inc.

Although 2,4,5-T is generally regarded as a recalcitrant molecule, an organism was isolated which was capable of effecting a significant degradation of this herbicide by a cometabolic process. This paper reports on the organism employed and the results obtained which clearly show microbial degradation of 2,4,5-T.

Materials and Methods

The organism employed in this study was isolated by enrichment culture technique and was maintained in a benzoate-salts medium as previously described (3). Manometric studies employed a resting cell suspension of benzoate-grown cells in phosphate buffer (3). The isolate was characterized according to A Guide to the Identification of the Genera of Bacteria (8).

Oxygen uptake by the isolate on 2,4,5-T was determined by standard manometric techniques (9). Inorganic chloride was determined according to the procedure of Bergmann and Sanik (10). The procedure of Arnow (11) was employed to detect the presence of catechols.

Thin layer chromatography employed Eastman Chromagram silica gel plastic sheets with fluorescent indicator. Chloroform, butanol-benzene-water (1:9:10) and butanol-acetic acid-water (4:1:5) served as the solvent systems. Chromatograms were developed to a height of 100 mm and spots were detected under ultraviolet light.

Results and Discussion

The organism employed in this study was identified as a Brevibacterium sp. It was a nonphotosynthetic, nonsporing, gram positive rod which was motile via peritrichous flagella. It produced acid but no gas from glucose, liquefied gelatin, reduced and peptonized litmus milk, did not produce indole and exhibited a white effuse growth on nutrient agar slants.

The Brevibacterium cometabolized 2,4,5-T with the comsumption of 1 μmole of oxygen and the release of 1 μmole of chlorine as inorganic chloride per 1 μmole of herbicide oxidized. These results indicated the possible formation of a dichlorocatechol. As seen in Figure 1, the oxygen uptake occurred in a bimodal fashion with each rise corresponding to about 0.5 μmole of oxygen per μmole of 2,4,5-T cometabolized.

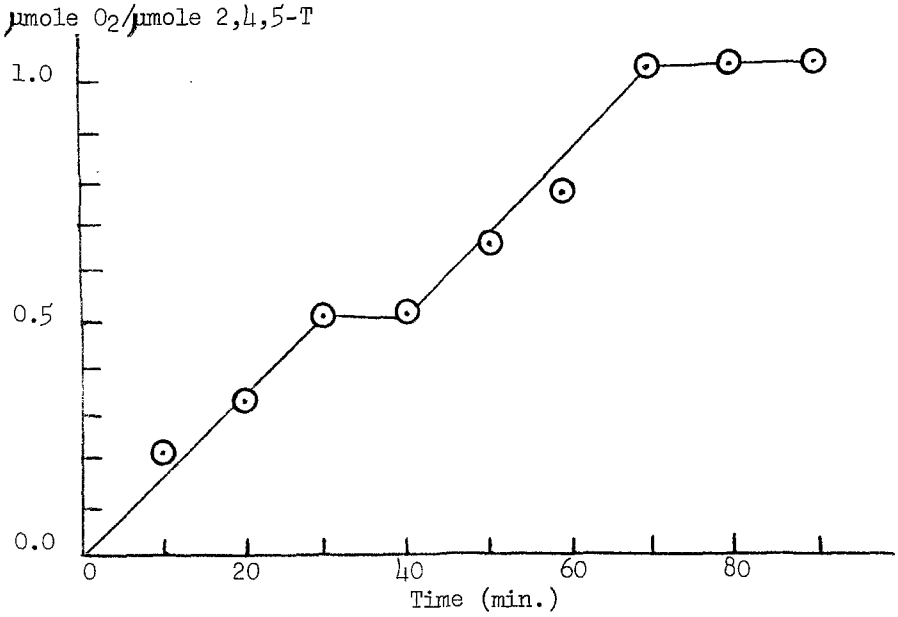

Figure 1. Oxidation of 2,4,5-T by a Brevibacterium sp.

Oxidation of 2,4,5-T by resting cell suspensions of benzoate-grown cells occurred without an initial adaptive lag period. This indicated that either the enzyme which catalyzed the initial oxidation was constitutive or that this enzyme was induced by growth on benzoic acid. The adaptive lag period which occurred between the first and second rise in oxygen uptake was reproducible in four experiments. It appeared that the enzyme involved in the second oxidation differed from that which catalyzed the first oxidation.

When oxidation of the 2,4,5-T was complete, as determined by the return of the respiration rate to that of endogenous, an Arnow positive material could be detected in the culture supernatant. This material gave R_f values on thin layer chromatograms identical to those of authentic 3,5-dichlorocatechol. The R_f values obtained in three solvent systems were as follows: chloroform, R_f = 0.17; butanol-benzene-water (1:9:10), R_f = 0.40; butanol-acetic acid-water (4:1:5), R_f = 0.94. On this basis the product of cometabolism of 2,4,5-T by the Brevibacterium sp. was tentatively identified as 3,5-dichlorocatechol.

The results of this investigation are important from the points of view of microbial biochemistry, microbial ecology and environmental pollution. Prior to this work, no organism had been found which could effect a significant change of the 2,4,5-T molecule (12). The results presented in this paper demonstrate the microbial degradation of this herbicide. The degradation of both 2,3,6-trichlorobenzoate (4) and 2,4,5-trichlorophenoxyacetate by microbial cometabolism indicates the environmental importance of cometabolism in the degradation of some supposedly recalcitrant molecules. The end product of 2,4,5-T degradation, 3,5-dichlorocatechol, has also been found to be a product of the microbial degradation of 2,3,6-TBA (4) and 2,4-D (13). This catechol was shown to be completely metabolized by the Arthrobacter responsible for 2,4-D degradation (13). In addition, the author demonstrated the cometabolism of this compound by an Achromobacter sp. (5,6). Thus, it would appear that both 2,3,6-TBA and 2,4,5-T can be completely degraded by a series of cometabolic reactions or by the action of two or more microbial species. In light of these findings, the concept of molecular recalcitrance (14) should be reexamined. Inability of microorganisms to grow at the expense of an organic compound can no longer be interpreted to mean that the compound is recalcitrant, or the microorganisms fallible. It would appear that under the correct environmental conditions, most, if not all, organic compounds can be degraded either by complete mineralization of the molecule or by the phenomenon of cometabolism.

References

1. J. W. FOSTER, The Oxygenases (O. Hayaishi, ed.), p. 241 (1962), Academic Press, New York.

2. H. L. JENSEN, Acta. Agr. Scand. 13, 404 (1963).

3. R. S. HORVATH and M. ALEXANDER, Appl. Microbiol. 20, in press (1970).

4. R. S. HORVATH, J. Agr. Food Chem., in press (1970).

5. R. S. HORVATH, J. M. DUXBURY, and M. ALEXANDER, Bact. Proc., p. 9 (1970).

6. R. S. HORVATH, Biochem J., in press (1970).

7. M. ALEXANDER and M. I. H. ALEEM, J. Agr. Food Chem. 9,44 (1961).

8. V. B. D. SKERMAN, A Guide to the Identification of the Genera of Bacteria, 2nd. ed. (1967), Williams & Wilkins, Baltimore.

9. W. W. UMBREIT, R. H. BURRIS, and J. F. STAUFFER, Manometric Techniques, 4th. ed. (1964), Burgess Publishing Co., Minneapolis.

10. J. G. BERGMANN and J. SANIK, Anal. Chem. 29, 241 (1957).

11. L. E. ARNOW, J. Biol. Chem. 118, 531 (1937).

12. M. ALEXANDER, Principles and Applications in Aquatic Microbiology (H. Heukelekian and N. C. Dondero, eds.), p. 15, (1964), John Wiley & Sons, Inc., New York.

13. M. A. LOOS, R. N. ROBERTS, and M. ALEXANDER, Can. J. Microbiol. 13, 679 (1967).

14. M. ALEXANDER, Adv. Appl. Microbiol. 7, 35 (1965).

15. This investigation was supported by Public Health Service Training Grant number ES-00098 from Division of Environmental Health Sciences.