**Short Communication**

**Formation of a Methylthiolated Metabolite from the Fungicide Chlorothalonil by Soil Bacteria**

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The fungicide chlorothalonil (TPN, Daconil®, 2,4,5,6-tetrachloroisophthalonitrile) is widely used in upland fields in Japan. Chlorothalonil was reportedly degraded without the lag period even in virgin soil after its application,\(^1\) because of the high population level of chlorothalonil-degrading bacteria.\(^2\) There has been no example of an abundance of these bacteria degrading chlorinated aromatic compounds in virgin soils. Therefore, it is of importance to elucidate the metabolic pathway of chlorothalonil and the responsible enzymes in the bacteria. Studies on the metabolic pathway have been carried out only in isolated eucaryotic microorganisms\(^3\),\(^4\) and in soils.\(^5\),\(^6\) This paper reports the finding that the methylthiolated metabolite was produced from chlorothalonil by various soil bacteria, which is a novel pathway in the bacterial degradation of a chlorinated aromatic compound.

The chlorothalonil-degrading soil bacterium A40-2, tentatively identified as *Azomonas* sp.,\(^2\) was used for the identification of the metabolite. The bacterium was incubated in a one-tenth diluted nutrient broth (Eiken Chemical, Tokyo) with (for the sample) or without (as a blank) 0.5 mg/liter of chlorothalonil for 30 hr at 28°C. After the incubation, the culture was mixed with acetone, acidified with 1 N HCl and extracted with hexane. The extract was dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The concentrate was dried under a nitrogen gas stream,

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![Graph A](image1.png)

**Fig. 1.** Mass Spectra of 2,5,6-Trichloro-4-methylthiosalophthalonitrile (A) and a Metabolite of Chlorothalonil in the Culture of *Azomonas* sp. A40-2 (B).

*Abbreviations:* MT, 2,5,6-trichloro-4-methylthiosalophthalonitrile; GC/MS, capillary gas chromatography/mass spectrometry.
dissolved with toluene, and subjected to analysis by capillary gas chromatography/mass spectrometry (GC/MS). The equipment for GC/MS was a Hewlett Packard model 5890/JEOL DX705L equipped with a DB-1 column (15 m length, 0.25 mm inner diameter; J&W, CA, U.S.A.). The mass spectra were recorded after using EI ionization at 70 eV.

The soil used was Anjo upland soil (Typic Dystrochrept), the population of chlorothalonil-degrading bacteria being enumerated as reported previously. The production of the methylthiolated metabolite was examined in cultures of chlorothalonil-degrading bacteria, and the population of methylthiolating bacteria was estimated. For the analysis of chlorothalonil and its methylthiolated metabolite, a Hewlett Packard model 5890 series II gas chromatograph equipped with nitrogen-phosphorus detector and a DB-1701 column (15 m length and 0.53 mm inner diameter; J&W, CA, U.S.A.) was used.

A distinctive peak of a metabolite was observed on the gas chromatogram of the sample culture compared with that of the blank culture. The retention time relative to chlorothalonil was identical with the standard of 2,5,6-trichloro-4-methylthiosophthalonitrile (MT; difference < 0.1%). The metabolite was also found to show the same molecular ion and fragmentation pattern in the mass spectrum as those of standard MT (Fig. 1). This is the first evidence of the bacterial formation of a methylthiolated metabolite from a chlorinated aromatic compound. In the past, the formation of a methylthiolated metabolite from chlorothalonil has been reported in soil, and in cultures of such eucaryotic microorganisms as Saccharomyces pastorianus and Neurospora crassa. The methylthiolation process has also been observed in the degradation of pentachloronitrobenzene and fthalide. However, there has been no previous report that bacteria could transform chlorinated aromatic compounds to methylthiolated metabolites. It is well known in mammals and other animals that methylthiolated metabolites are produced by conjugation with endogenous sulfur-containing compounds in the detoxification process of xenobiotics. The bacterial methylthiolation of chlorothalonil indicates that the bacterium metabolized chlorothalonil through conjugation with an endogenous sulfur-containing compound.

It should be noted that the methylthiolated metabolite-producing bacteria were present at a high population level in the soil. Thirty six percent of the cultures (13/36 cultures) of chlorothalonil-degrading bacteria isolated from Anjo soil produced the methylthiolated metabolite. The population of methylthiolating bacteria corresponds to 6.1 × 10⁶ cfu/g of soil or to 5% of total aerobic bacteria. Gram-positive cocci and Gram-negative rods were observed in the methylthiolating bacteria. These results indicate that methylthiolation is one of the important pathways in various soil bacteria to degrade chlorinated aromatic compounds.

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