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Dehydrochlorination of δ-Isomer of Hexachlorocyclohexane by a Soil Bacterium, *Pseudomonas* sp.

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Commercial formulations of an insecticide, hexachlorocyclohexane (HCH), currently used on a large scale in India, contain α-, β-, γ-, δ- and other isomers. According to earlier reports (Raghu and MacRae, 1966; Castro and Yoshida, 1971; Sethunathan, 1973), these HCH isomers persist in aerobic soil and water systems, but disappear rapidly from predominantly anaerobic ecosystems such as flooded soils and lake sediments. Bacterial strains (facultative or strict anaerobes), isolated from these anaerobic systems rapidly degraded α- and/or γ-isomer, but not β- and δ-isomers of HCH under anaerobic conditions (MacRae et al., 1969; Sethunathan et al., 1983). Recent reports show that bacteria mainly Pseudomonads, isolated from aerobic soils could degrade not only γ-isomer (Senoo and Wada, 1989; Sahu et al., 1990), but also α- and β-isomers of HCH (Sahu et al., 1990) under aerobic conditions. There is no report of the degradation of δ-HCH in pure culture by either aerobic or anaerobic bacteria although it is a common constituent in widely used commercial formulations of HCH. We report the rapid degradation of δ-HCH by a Pseudomonas sp. under aerobic conditions.

MATERIALS AND METHODS

The Pseudomonas sp., used in this study, was the same as isolated earlier from the rhizosphere of HCH-treated sugarcane plants and shown to degrade α-, γ- and β-isomers of HCH (Sahu et al., 1990). A mineral salts medium (Sahu et al., 1990) supplemented with technical grade δ-HCH (purity 99.1 %, Lachat Chemicals, Wisconsin, U.S.A.) was sterilized by filtration through a 0.45 μm Millipore filter. Ten milliliter portions of this medium contained in 100 ml Erlenmeyer flasks were inoculated with 0.1 ml of a suspension of Pseudomonas sp. in sterile distilled water containing 7×10^4 cells and incubated in a shaker at 28±2°C. Uninoculated flasks served as control. At periodic intervals, residues in 1 to 2 ml of the media from duplicate flasks were extracted by shaking with 1 to 5 ml of hexane and 50 mg of sodium sulfate for 2 min and analysed in a Varian 3600 gas chromatograph equipped with an electrolytic conductivity detector (Tracor model 1000 HALL detector) operated in the halogen mode. The gas chromatograph was fitted with a glass column (2 mm O.D.; 2 m length) packed with 10% OV 101 on Chromosorb WHP 80/100 mesh. Operating

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conditions for the gas chromatograph were : column, 190°C; injector 220°C; detector 240°C; carrier gas, helium, 31 ml/min; hydrogen, 73 ml/min; operating conditions for the HALL detector were reactor temperature, 820°C and solvent (n-propanol) flow, 36 ml/min vent, 1.5 min and detector attenuation x5. Under these conditions, the retention time of δ-HCH was 6.4 min. The sensitivity of the HALL detector was 2×10^{-12} g of δ-HCH.

RESULTS AND DISCUSSION

The concentration of δ-HCH in the inoculated medium decreased rapidly and reached undetectable levels within 8 days after inoculation with Pseudomonas sp. under aerobic conditions (Table 1); during the corresponding period, decrease in δ-HCH level in uninoculated medium was negligible. Bacterial degradation of δ-HCH led to the accumulation of a transitory metabolite, possibly δ-pentachlorocyclohexene (δ-PCH) formed by dehydrochlorination.. This metabolite and authentic δ-PCH (obtained from Dr. N. Kurihara, Kyoto, Japan) showed the same retention time of 7.1 min.

Table 1. Degradation of δ-HCH in a mineral salts medium inoculated with Pseudomonas sp. under aerobic conditions

Incubation (days)	Compound recovered (μM)[1]			
	Uninoculated		Inoculated	
	δ-HCH	δ-PCH	δ-HCH	δ-PCH
0	36.6±0.5	0	36.1±1.0	0
2	35.3±0.9	0	16.2±1.0	10.8±1.0
4	35.8±1.4	0	13.8±1.7	11.0±0.8
6	35.6±0.5	0	9.1±1.2	8.8±0.2
8	33.5±0.9	0	0	0

[1] Mean of duplicate estimation ± standard deviations.

The release of chloride from δ-HCH by Pseudomonas sp. was examined as described earlier (Sahu et al., 1990). Deionised water supplemented with δ-HCH was inoculated with Pseudomonas sp. In inoculated samples incubated under aerobic conditions, the concentration of δ-HCH decreased to undetectable levels in three days with concomitant accumulation of chloride almost in stoichiometric amounts (Table 2). In uninoculated controls, no chloride was detected.

The data presented in this study provide first evidence for dehydrochlorination of δ-HCH by a bacterium, (Pseudomonas sp.) under aerobic conditions with concomitant formation of δ-PCH and chloride. This versatile bacterium with exceptional capacity to degrade not only α- and γ-, but also thermodynamically more stable β- and δ-isomers of HCH (this report; Sahu et al., 1990) may be useful in developing a technology for decontamination of HCH-polluted environments.

Table 2. Chloride released from δ-hexachlorocyclohexane added to deionised water as sole source of carbon by Pseudomonas sp. under aerobic conditions

Incubation (days)	Compound recovered (μM)[1]			
	Uninoculated		Inoculated	
	Parent[2]	Chloride	Parent[2]	Chloride
0	3.54±0.1	0	3.85±0.1	0
1	3.85±0.58	0	3.03±2.75	0.89±0.28
3	3.78±0.34	0	0	2.7 ±0.84

[1]Mean of duplicate estimations ± standard deviations

[2]Parent δ-HCH recovered

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