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Metabolic Breakdown of Kaneclors (Polychlorobiphenyls) and Their Products by *Acinetobacter* sp.

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Biodegradability of commercial polychlorobiphenyl mixtures (Kaneclors, KC 200 to KC 500) and their metabolic products by *Acinetobacter* sp. strain P6 were studied by gas chromatography-mass spectrometry analysis. KC 200 (primarily dichlorobiphenyls) rapidly degraded after 4 h of incubation with the P6 resting cells, showing predominant accumulation of monochlorobenzoic acids. KC 300 (primarily trichlorobiphenyls) were also degraded after 4 h of incubation, producing various metabolic intermediates such as mono- and dichlorobenzoic acids, dihydroxy biphenyl compounds with two and three chlorines, and the ring meta-cleavage compounds with two and three chlorines. KC 400 (primarily tetrachlorobiphenyls) were also susceptible to biodegradation by the same organism. Chlorobenzoic acids (chlorine number 1 to 3), dihydroxy compounds (chlorine number 2 to 4), and the ring meta-cleavage compounds (chlorine number 2 to 3) were observed as the products from KC 400. In addition to such products, a large amount of unknown compounds with two chlorines in the molecule, which can be derived from 2,3,2',3'- or 2,3,2',5'-tetrachlorobiphenyls or both, accumulated. In contrast to KC 200, KC 300, and KC 400, KC 500 (primarily pentachlorobiphenyls) were resistant to degradation and hardly metabolized. Only dihydroxy compounds of certain pentachlorobiphenyls were detected.

Since Ahmed and Focht (1) first reported that two species of *Achromobacter* were capable of degrading mono- and dichlorobiphenyls, a number of studies have indicated that pure cultures of microorganisms and naturally occurring microbial populations are capable of degrading isomeric polychlorobiphenyl (PCB) components and commercial PCB mixtures (2–5, 7, 10, 11, 16–20). Degradation of highly chlorinated biphenyls by bacteria can be considered a cometabolic mechanism (14). Baxter et al. (3) observed that degradation of PCBs by a *Nocardia* sp. and a *Pseudomonas* sp. increased upon addition of biphenyl. Clark et al. (7) demonstrated that the gas chromatography (GC) profile of Aroclor 1242 was greatly altered after 5 days of incubation with mixed cultures including predominantly *Alcaligenes* sp. Tucker et al. (18) showed that commercial PCB mixtures containing predominantly mono- and dichlorobiphenyls readily undergo primary biodegradation by activated sludge microorganisms, and as the level of tri-, tetra-, and pentachlorobiphenyls increase, the degradation rates decrease accordingly. Liu (16) observed similar results for various Aroclors by *Pseudomonas* sp. However, there is relatively little information available on how microorganisms attack commercial PCB mixtures and what kinds of products are formed and accumulated. Commercial PCBs are mixtures of many different isomers with various chlorine contents. In previous studies (12, 13), we have studied the biodegradability and metabolic fate of 36 pure isomers of PCBs by two single bacterial strains of *Alcaligenes* sp. and *Acinetobacter* sp. Based on the results obtained for various isomeric PCBs, we have investigated how commercial PCB mixtures are catabolized by bacteria.

MATERIALS AND METHODS

Microorganism and cultivation. *Acinetobacter* sp. strain P6 was used throughout the experiment. The properties and characteristics of this strain have been described previously (11–13). The cells were grown in the following mineral medium supplemented with 4-chlorobiphenyl (1 mg/ml) as a sole source of carbon: $(NH_4)_2SO_4$, 1 g; KH_2PO_4, 0.2 g; NaCl, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; deionized water, 1 liter (pH 7.5).

Incubation and extraction. The organism was grown for 3 to 4 days, and the culture fluid was filtered once to remove remaining solid 4-chlorobiphenyl. The harvested cells were washed twice in 0.05 M phosphate buffer (pH 7.5). The washed cells were resuspended in 10 ml of phosphate buffer to which 500 μg of Kaneclors (KC 200 to KC 500) was added. The final absorbance of the cells was adjusted to 1.0 at 660 nm

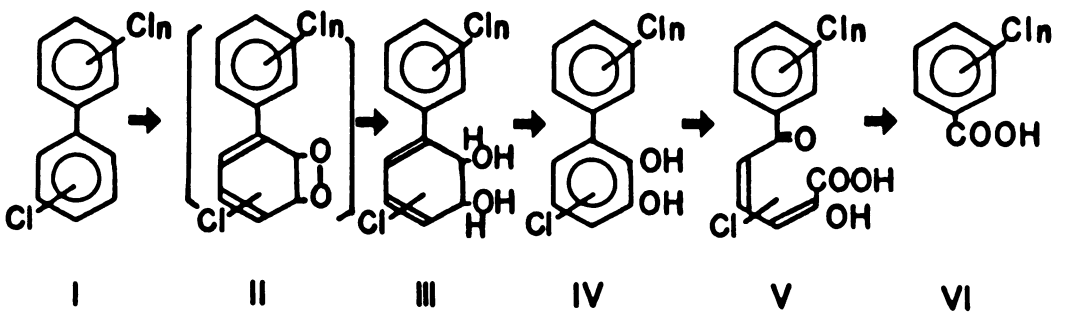

FIG. 1. Major metabolic sequence of PCBs by *Acinetobacter* sp. strain P6.

(A_{660}; 4.4 × 10^8 cells per ml). Incubation was carried out at 30°C with shaking for 1 h to 5 days, depending on the ease of degradability of Kaneclors. The entire incubation mixtures were then extracted with 10 ml of ethylacetate after acidification to pH 1 with concentrated HCl.

GC-mass spectrometry. The ethylacetate layer was removed and evaporated under a gentle stream of nitrogen gas and was dissolved in a small amount of ethylacetate. Bistrimethylsilyl acetamide was added to obtain trimethylsilyl derivatives of PCB metabolites. The samples were analyzed with a combined gas chromatograph-mass spectrometer (JEOL Ltd. model JMS D-300) with a coiled glass column (1 m by 4 mm [inner diameter]) packed with silicon OV 1 at 2% on 80- to 100-mesh Chromosorb G. Helium was used as a carrier gas at a flow rate of 20 ml/min. The column temperature on GC was increased from 140 to 250°C at a rate of 8°C/min. The electron impact masses were measured at a 20-eV ionization potential, 300-μA trap current, and 200°C ion source temperature. In this study, mass data of PCB metabolites were obtained as trimethylsilyl derivatives at electron impact mode. After measurements, electron mass (*m/z*) of base peak

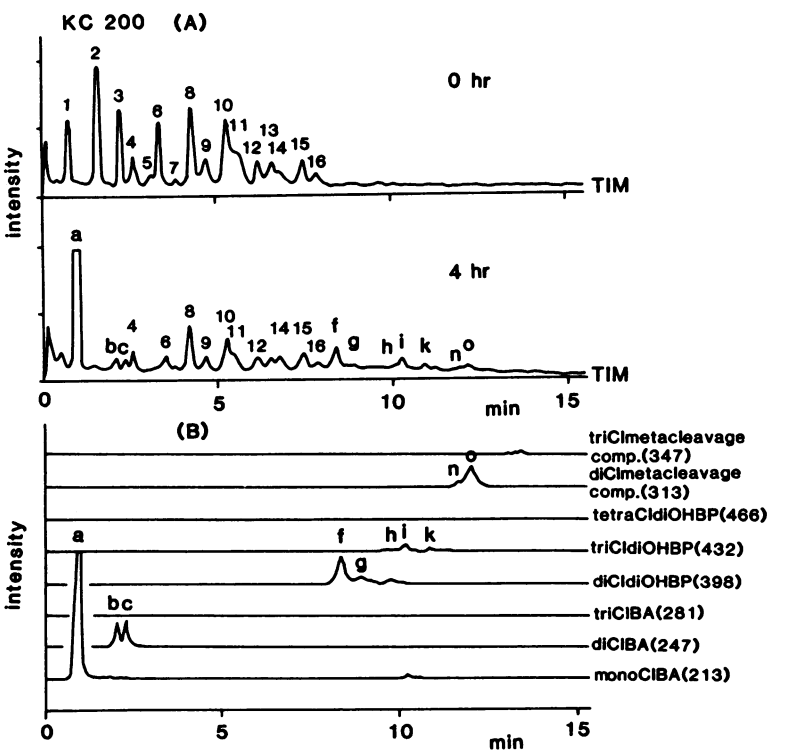

FIG. 2. Biodegradation of KC 200 by *Acinetobacter* sp. strain P6 and their metabolic products. (A) TIM on GC-mass spectrometry of KC 200 at time zero and after 4 h of incubation with resting cells. (B) Mass chromatogram of metabolites. The electron mass (*m/z*) of the base peak for each metabolite was monitored. BA, Benzoic acid; BP, biphenyl.

for each metabolite was monitored to obtain the mass chromatography.

Chemicals. Kaneclor (KC) 200, 300, 400, and 500 were purchased from Gasukuro Kogyo Inc. (Tokyo).

RESULTS

In previous papers (12, 13) we have demonstrated the metabolic fates of various isomeric PCBs by *Acinetobacter* sp. strain P6. Many PCB components were converted to the corresponding chlorobenzoic acids through an oxidative route (Fig. 1). However, dihydroxy compounds (III in Fig. 1), meta-cleavage compounds (IV), or certain unknown products specifically accumulated from several PCB components, depending on the chlorine substitution. Based on these findings, metabolites from various KCs were searched and identified by GC-mass spectrometry analysis.

KC 200 is a PCB mixture containing primarily dichlorobiphenyls. The entire mixture was extracted with ethylacetate immediately after addition of the cells (time zero), so that approximately 16 peaks of PCBs which are numbered were detected by total ion monitor (TIM in Fig. 2A). After 4 h of incubation, major PCB peaks (peaks 1 to 3) disappeared and other PCB peaks were greatly decreased. Various PCB metabolites (designated by letters in Fig. 2) appeared at both sides of PCB components. As shown in the mass chromatogram (Fig. 2B), monochlorobenzoic acids (peak a) accumulated in the reaction mixture because *Acinetobacter* sp. strain P6 cannot attack chlorobenzoic acids, as described previously (12).

KC 300 (primarily trichlorobiphenyls) were easily susceptible to microbial attack (Fig. 3). After 4 h of incubation, a variety of PCB metabolites were detected, including monochloro- (peak a) and dichloro- (peaks h, i, j, and k) dihydroxybiphenyls and the ring meta-cleavage compounds with two chlorines (peaks n and o) and three chlorines (peaks p, q, and r) in the molecules.

KC 400 (primarily tetrachlorobiphenyls) were incubated with the organisms for 24 h. The TIM profile was greatly changed by decrease of PCB components and concomitant formation of PCB metabolites (Fig. 4). The metabolites of KC 400 were assigned as follows: monochlorobenzoic

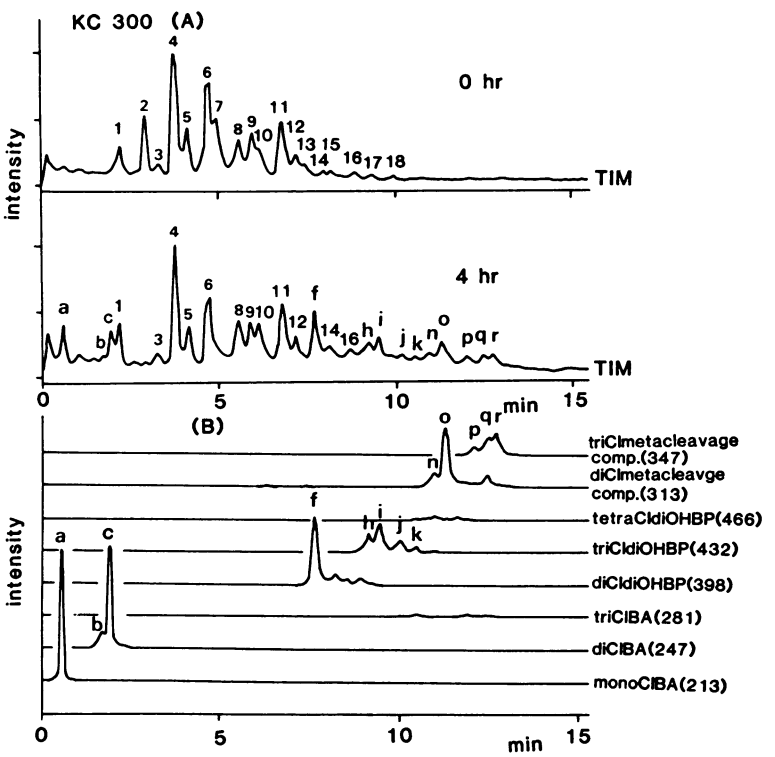

FIG. 3. Biodegradation of KC 300 by *Acinetobacter* sp. strain P6 and their metabolic products. (A) TIM on GC-mass spectrometry of KC 300 at time zero and after 4 h of incubation with resting cells. (B) Mass chromatogram of metabolites. The electron mass (*m/z*) of the base peak for each metabolite was monitored. BA, Benzoic acid; BP, biphenyl.

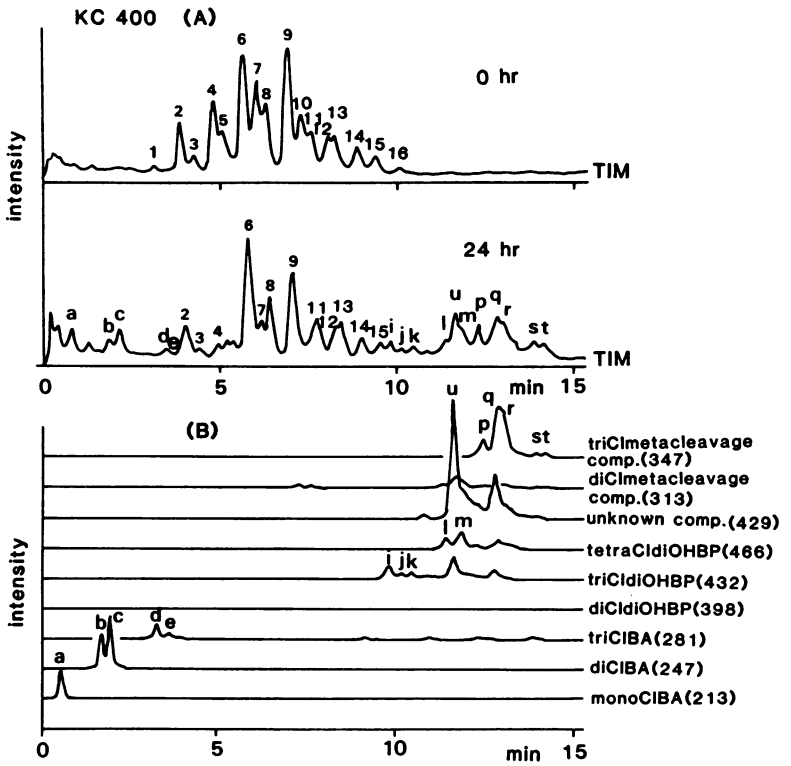

FIG. 4. Biodegradation of KC 400 by *Acinetobacter* sp. strain P6 and their metabolic products. (A) TIM on GC-mass spectrometry of KC 400 at time zero and after 24 h of incubation with resting cells. (B) Mass chromatogram of metabolites. The electron mass (*m/z*) of the base peak for each metabolite was monitored. BA, Benzoic acid; BP, biphenyl.

acids (peak A), dichlorobenzoic acids (peaks b and c), trichlorobenzoic acids (peaks d and e), trichlorodihydroxybiphenyls (peaks i, j, and k), tetrachlorodihydroxy biphenyls (peaks l and m), and the ring meta-cleavage compounds with three chlorines (peaks p, q, r, s, and t). In addition to these metabolites, a significant amount of the unknown compound (peak u) was detected. The mass spectrum of the unknown compound (Fig. 5) indicates that this compound has two chlorines in the molecule (M^+, *m/z* 444 as trimethylsilyl derivative; M^+-CH_3, *m/z* 429 as the base peak). In previous studies (12), we showed that this kind of product always accumulated in the metabolism of 2,3,2′,3′- and 2,3,2′,5′-tetrachlorobiphenyls. This is evidence that KC 400 contains tetrachloro-isomers which commonly contain two chlorines at the 2,3-position in one of the biphenyl rings. The metabolic profile of KC 400 was basically identical even after 5 days of incubation.

KC 500 (primarily pentachlorobiphenyls) were quite resistant to microbial attack. The TIM profile of PCB components was not significantly altered after 5 days of incubation (data not shown). Only metabolites that could be detected were dihydroxy compounds of pentachlorobiphenyls.

DISCUSSION

The results obtained in the present study with commercial PCB mixtures are in good agreement with the results obtained previously (12, 13) from 36 individual isomeric PCBs in terms of biodegradability and metabolic behaviors. Degradation rate markedly decreases as chlorine substitution increases with KCs, as demonstrated with various Aroclors (16, 18). Liu (16) showed that Aroclor 1221 (primarily dichlorobiphenyls) was degraded much faster (980 μg/h per mg of cell (dry weight) than Aroclor 1254 (primarily pentachlorobiphenyls, 43 μg/h per mg of cell [dry weight]) by the resting cells of *Pseudomonas* sp. KC 500, which corresponds to Aroclor 1254, was also quite resistant to microbial attack by *Acinetobacter* sp. However, many PCB components can be converted to the corresponding chlorobenzoic acids through the oxidative route (Fig. 1) by the same organism, as

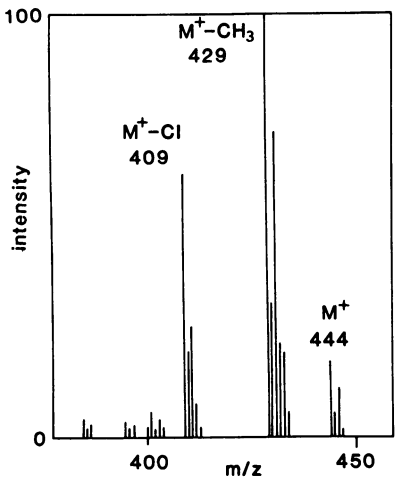

FIG. 5. Mass spectrum of the unknown compound derived from 2,3,2',3'- or 2,3,2',5'-tetrachlorobiphenyl or both in KC 400.

demonstrated with KC 200, KC 300, and KC 400. This chlorobenzoate pathway thus seems to be a major metabolic process in the environmental degradation of PCBs by bacteria, since members of other bacterial genera such as *Alcaligenes* (10), *Arthrobacter* (9), *Achromobacter* (1), and *Pseudomonas* (5) also convert chlorinated biphenyls to chlorobenzoic acids. In the natural environment, a mixed culture might be expected to give more complete degradation than do single organisms. Chlorobenzoic acids formed from many PCB components by bacteria can be decomposed by other bacteria. Horvath and Alexander (15) demonstrated that 20 isolates representing nine bacterial genera co-metabolized 1 or more of 22 substituted benzoates. DiGeronimo et al. (8) reported that sewage microorganisms decomposed *o*-, *m*-, and *p*-chlorobenzoic acids and 3,4-dichlorobenzoic acid. Furukawa and Chakrabarty (9) demonstrated total degradation of mono- and dichlorobiphenyls by the mixed culture of *Acinetobacter* sp. strain P6 or *Arthrobacter* sp. strain M5 harboring pKF1 plasmid specifying degradation of chlorobiphenyls to chlorobenzoates and genetically constructed *Pseudomonas putida* (6) harboring pAC27 or pAC31 specifying utilization of mono- or dichlorobenzoates.

It should be noted that some alternative metabolites other than chlorobenzoates also accumulated from commercial PCBs. Dihydroxy compounds (IV in Fig. 1) of various PCB components and the ring meta-cleavage compounds (V) with various chlorine contents accumulated in the catabolism of various KCs. The unknown compounds also can be produced from 2,3,2',3'- or 2,3,2',5'-tetrachlorobiphenyls or both in KC 400. Biological degradability might lead to metabolites of increased toxicity (21). Since PCBs are still ubiquitous environmental pollutants and the metabolic breakdown by microorganisms is considered to be one of the environmental degradation processes for these widespread materials, it should be important to investigate the effects of these metabolic products upon living organisms in the natural ecosystem.

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