

Microbial dehalogenation of polychlorinated biphenyls in aerobic conditions

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SUMMARY

From soils contaminated with polychlorinated biphenyls (PCBs) a strain of *Alcaligenes* sp. able to grow in a mineral medium with a commercial mixture of PCBs as carbon source was isolated. This strain consumed up to 200 ppm in seven days in laboratory conditions. After 24 h of incubation, some new congeners of PCBs could be recognized by mass spectrometry. Through the identification of these compounds it was possible to postulate examples of possible transformations by dechlorinations of penta- and tetra-chlorinated congeners into tri-chlorinated ones. The properties of the isolated strain are appropriate for bioremediation of contaminated areas and also for using in bioreactors in order to remove the xenobiotic chemical.

Key words: PCBs biodegradation, PCBs dechlorination, *Alcaligenes* sp.

RESUMEN

Dehalogenación microbiana de bifenilos policlorados en condiciones aeróbicas. A partir de suelos contaminados con bifenilos policlorados (PCBs) se aisló una cepa de *Alcaligenes* sp. capaz de crecer en medio mineral con una mezcla comercial de PCBs como fuente de carbono. Esta cepa consumió hasta 200 ppm de PCBs en siete días de incubación en condiciones de laboratorio. En 24 horas de incubación se han podido detectar nuevos congéneres de PCBs mediante espectrometría de masa. La identificación de estos compuestos ha permitido postular transformaciones de congéneres penta- y tetra-clorados que originarían derivados triclorados. Las propiedades de la cepa aislada son apropiadas para biorremediación y para su uso en biorreactores para eliminar estos compuestos xenobióticos.

Palabras clave: biodegradación de PCBs, dehalogenación de PCBs, *Alcaligenes* sp.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of very stable synthetic compounds composed of a biphenyl nucleus with 1-10 chlorine substitutes. Of the 209 different congeners theoretically possible, only about half are actually produced by synthesis due to steric hindrance. The commercial mixtures were formerly used widely as heat transfer agents in electrical transformers and capacitors, and hydraulic fluids (1). PCBs are widely distributed and they are environmentally persistent, specially those with higher degree of chlorination. These products are important environmental contaminants and they were found in many natural samples and accumulate in higher trophic levels (13, 18). They were associated with toxic effects in wildlife such as deformities and lowered reproductive success (12), and with hepatic tumors in rats (15).

Bioremediation is being considered as a cheaper, less disruptive and more publicly acceptable alternative to current remediation technologies, such as incineration. Besides, bioreactors are an excellent way to eliminate these compounds in a closed system, avoiding the possibility of environmental pollution. (2, 10).

Both aerobic and facultative anaerobic bacteria using PCBs have been isolated from the environment (7). Screening methods to isolate and characterise polychlorinated biphenyls degrading microorganisms have been developed (3, 19). Highly chlorinated congeners are slowly dechlorinated, and these transformations generally reduce the chlorine content of PCBs mixtures. The major products are mono- and dichlorobiphenyls (17).

Generally the isolated PCBs degrading microorganisms are aerobic gram-negative soil bacteria, which includes species of *Pseudomonas*, *Acinetobacter* and *Achromobacter* (1), *Alcaligenes* (4) *Klebsiella* (6) and *Moraxella* (7). Gram-positive organisms such as *Arthrobacter* and *Corynebacterium* species were also isolated (1). Many of these strains commonly use biphenyls as a sole source of carbon and energy, and they cometabolize PCBs through a principal oxidative route. The biodegradability and catabolic fate of PCBs by bacteria are greatly influenced by chlorine substitution on the biphenyl molecule. Analysis of metabolic intermediates showed that a number of PCBs congeners were converted to the corresponding chlorobenzoic

acids via an oxidative route (11).

PCBs can be also degraded *Aspergillus niger* (9) and by white rot fungi, such as *Phanerochaete chrysosporium* (5) and *Trametes trogii* (14).

In this paper, some features observed with a novel strain of *Alcaligenes* sp isolated from PCBs contaminated soil are described.

MATERIALS AND METHODS

Alcaligenes sp. strain K was enriched from contaminated soils by the procedure of Chou et al (8). The methods for isolation and culture in mineral medium with PCBs as sole carbon source, and also to obtain bacterial mass and the estimation of xenobiotic compound consumption were already described (6, 20). The identification of the isolated strain was performed by API test from bioMérieux. A commercial mixture of PCBs, type Aroclor 1150, sterilized by Tyndall effect, was used as substrate. The sterilized xenobiotic compound was sonified in the mineral culture medium in order to allow a correct dispersion. At different times the reaction was stopped with hydrochloric acid, final concentration 1%, and the remain fraction of PCBs congeners was extracted with two volumes of *n*-hexane. The hexane extracts were twice washed with OHNa 5%, and finally, with distilled water. Biphenyl (0.2-mg l⁻¹) has been used as an inner standard. Heated cells (80° C during 30 min) were incubated for 7 days as control. The washed hexane extracts were analysed by using a gas-liquid chromatograph coupled with a mass spectrometer Trio 2-VG using a 25 m length methylsilicon column, ID 0.25 mm and pressure of 70 Kpa. The mass spectra obtained were compared with database of MassLinx v. 3.0. Identifications were performed with probabilities over 90%.

RESULTS

Alcaligenes sp. (K strain) exhibited a duplicating time of 20 min in Veal Broth medium and 100 min in the mineral medium.

Figure 1 shows the chromatograms obtained with the extracts in *n*-hexane at zero time and after 24 h of incubation. This figure shows a series of peaks that disappear, for example *Tr* 14.60, 15.05, 15.37, 16.15, 17.10, 18.03, 18.25, 19.58 and 19.70 min of extracts from non incubated mixtures (Day0). Some other peaks change its area significantly: 15.55, 16.55 and 17.57 min. Finally there are some others not present in the original mixture, such as *Tr* 16.37, 18.02 and 18.59 min obtained after 24 h of incubation (Day1). At the first 24 h of incubation, 50% of the PCBs were consumed. Table 1 is a summary of the most outstanding facts, indicating the peaks that change significantly after the first 24 h of incubation and their identification. In Figure 2 the mass spectra of two congeners are shown. Figure 2A shows the spectra of the peak *Tr*19.70 min that disappear during the first 24 h of incubation. Figure 2B shows the spectra of a new congener, peak *Tr* 18.59 min.

Seven days incubations produce the total consumption of PCBs mixture. Heated cells did not affect the original mixture of PCBs, even to seven day of incubation.

DISCUSSION

Alcaligenes sp. strain K consumes 50% of PCBs (200 ppm initial concentration) in 24 h and 100% of them in seven days of incubation. This special feature suggests its possible use in bioremediation and/or in bioreactors to eliminate these compounds.

It was possible to detect the ions corresponding to the derivatives with different numbers of chlorine atoms. Most of the mono-, bi- and trichlorinated congeners were fast consumed in the reaction mixture, like some tetra- and penta-chlorinated ones. The appearance of some trichlorinated compounds was also detected.

All these results suggest that the most halogenated compounds are transformed into derivatives with a less chlorination degree without involving oxygenating enzymes. This process yields derivatives with free positions 2 and 3 on the biphenyl molecule. Reductive dehalogenation activity in anaerobic microorganisms, is most commonly observed at the *meta* and *para* positions, but recent reports indicate that dehalogenation in *ortho* chlorines is also possible (17). This fact seems to be similar to that observed with the congeners formed in 24 h of incubation with *Alcaligenes* sp strain K. Other reductive dehalogenation activities have been reported, such as the biodegradation of the pentachlorophenol (PCP). It is important to note that this degradation occurs by an aerobic pathway (16). Figure 3 shows several examples that illustrate the obtained results. Those

compounds with *Tr* 19.13, 19.58, 19.70 and 20.17 min lost one of its chlorine atoms and after 24 h of incubation *Tr*. 18.59 min compound appears as a result of this process. It is important to note that *Tr*18.59 min congener does not exist in the original mixture. Positions 2 and 3 on one benzene residue of the produced congeners are free and able to react with the dioxygenase enzyme, and therefore to be consumed in a similar way to those consumed in 24 h incubation.

Figure 3 also tries to explain this fact by the difference of speed between dehalogenation and the later catabolism of the compound.

As a conclusion, it has been possible to isolate a strain capable of consuming all the congeners of a commercial mixture of PCBs as a unique carbon and energy source. This strain promises to have a very useful role in PCBs bioremediation or bioreactor biodegradation.

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Table 1. Peaks in Figure 1 that change significantly its area in 24 h incubation

Day 0		Day 1	
<i>Tr</i> (min)	Identification	<i>Tr</i> (min)	Identification
14.60	2,2'-dichloro-1-1'-biphenyl		
15.05	3,5-dichloro-1-1'-biphenyl		

15.37	4,4'-dicloro-1-1'-biphenyl		
15.55	3,3'-dichloro-1-1'-biphenyl	15.60	3,3'-dichloro-1-1'-biphenyl
16.15	2,3,6-trichloro-1-1'-biphenyl		
16.55	2,4',5-trichloro-1-1'-biphenyl	16.56	2,4',5-trichloro-1-1'-biphenyl
17.02	2,3', 5-trichloro-1-1'-biphenyl		
17.10	2,3,4'-trichloro-1-1'-biphenyl		
17.28	3,4,4'-trichloro-1-1'-biphenyl		
17.57	2,4,5-trichloro-1-1'-biphenyl	17.57	2,4,5-trichloro-1-1'-biphenyl
17.83	2,3,4-trichloro-1, 1'-biphenyl		
		18.02	2,4,6-trichloro-1-1'-biphenyl
18.25	2,2',5,5'-tetrachloro-1-1'-biphenyl		
18.38	2,2',3,5-tetrachloro-1-1'-biphenyl		
18.43	2,2',5,6-tetrachloro-1-1'-biphenyl		
		18.59	2,4,4'-trichloro-1-1'-biphenyl
18.83	2,2',3,5-tetrachloro-1-1'-biphenyl		
19.13	2,3',4,6-tetrachloro-1-1'-biphenyl		
		19.25	2,2',4,5'-tetrachloro-1-1'-biphenyl
19.58	2,3',4',5-tetrachloro-1-1'-biphenyl		
19.70	2,3',4,4'-tetrachloro-1-1'-biphenyl		
		19.83	2,2',4,6-tetrachloro-1-1'-biphenyl
20.17	2,2',4,4'-tetrachloro-1-1'-biphenyl		

Figure 3. Proposed dehalogenation reactions for the higher chlorinated congeners of PCBs by *Alcaligenes* sp. strain K.

Figure 1. Chromatograms at zero time and 24 h of incubation.

Figure 2. (A) Mass spectra of a tetrachlorinated congener (*Tr* 19.70 min.) that disappears in 24 h of incubation. (B) Mass spectra of a trichlorinated congener (*Tr* 18.59 min.) formed in 24 h of incubation.

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