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Aerobic secondary utilization of a non-growth and inhibitory substrate 2,4,6-trichlorophenol by *Sphingopyxis chilensis* S37 and *Sphingopyxis*-like strain S32

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Abstract

This paper reports 2,4,6-trichlorophenol (246TCP) degradation by *Sphingopyxis chilensis* S37 and *Sphingopyxis chilensis*-like strain S32, which were unable to use 246TCP as the sole carbon and energy source. In R2A broth, the strains degraded 246TCP up to 0.5 mM. Results with mixtures of different 246TCP and glucose concentrations in mineral salt media demonstrated dependence on glucose to allow bacterial growth and degradation of 246TCP. Strain S32 degraded halophenol up to 0.2 mM when 5.33 mM glucose was simultaneously added, while strain S37 degraded the compound up to 0.1 mM when 1.33 mM glucose was added. These 246TCP concentrations were lethal for inocula in absence of glucose. Stoichiometric releases of chloride and analysis by HPLC, GC-ECD and GC-MS indicated 246TCP mineralisation by both strains. To our knowledge, this is the first report of bacteria able to mineralize a chlorophenol as a non-growth and inhibitory substrate. The concept of secondary utilization instead of cometabolism is proposed for this activity.

Abbreviations: 4C3MP – 4-chloro-3-methylphenol; 24D – 2,4-dichlorophenoxyacetic acid; 24DCP – 2,4-dichlorophenol; 246TBP – 2,4,6-tribromophenol; 245TCP – 2,4,5-trichlorophenol; 246TCP – 2,4,6-trichlorophenol

Introduction

Chlorophenols are chloroaromatic xenobiotics widely used as biocides in wood treatment, flame-retardants, solvents and as reagents in synthetic chemistry (Atuanya et al. 2000). These compounds are also secondarily produced in the bleaching process of Kraft cellulose pulp mills (LeueBNBerger et al. 1985). Aerobic biodegradation of chlorophenols has been extensively studied, and several strains of bacteria that completely degrade and use these compounds as primary substrate for growing have been reported (Saber & Crawford 1985; Radehaus & Schmid 1992; Steinle et al. 1998; Lee et al. 1998; Andreoni et al. 1998). Chlorophenols supporting growth metabolism enhanced by other readily metabolizable carbon sources (Topp et al. 1988) and chlorophenol cometabolic transformation using other primary substrates (Wang & Loh 1999) have been also published. The easily metabolizable compounds may better accomplish the energy requirements for biomass maintenance (Rittmann 1992) or may act as co-substrates in xenobiotic metabolism by participating as an electron-donor compounds (Perkins et al. 1994). Although, there are agreement that the presence of easily metabolizable carbon sources could be important for natural bioremediation of a toxic compound, scarce attention has been focused on this process as well as on the xenobiotic degradations that support growth (Loh & Wang 1998).

Previous studies have shown that chlorophenol compounds exert their toxic effect on membrane mainly by acting as uncoupling agents (Beltrame et al. 1988; Dapaah & Hill 1992; Sharma et al. 1997). Hence, bacterial strains that use these compounds as growth substrate should have developed tolerance mechanisms that allow bacteria to regulate the chlorophenols flow across their membranes (Brözel & Cloete 1993) or to obtain high energetic yield from its catabolism to overcome uncoupling or any other toxic effect. On the other hand, preliminary studies have shown that the bacterial abilities to tolerate some chlorophenols and to degrade them are independent process (Martínez et al. 2000). Keeping in mind the available data, it is possible to postulate the arrange of genes involved in chlorophenols metabolism and tolerance that define a bacterial phenotype for mineralisation of these compounds without growth. The aim of this work was to investigate the chlorophenol degradative activity dependence upon easily metabolizable carbon sources in bacterial strains isolated from the environment without a selective pressure.

Materials and methods

Chemicals

2,4,5-Trichlorophenol (245TCP), 2,4,6-trichlorophenol (246TCP), 2,4-dichlorophenol (24DCP), 2,4,6-tribromophenol (246TBP) and glucose were purchased from Sigma Chemical Co. (St. Louis, USA). 4-Chloro-3-methylphenol (4C3MP), 2,4-dichlorophenoxyacetic acid (24D), pyruvate, maltose, sulfuric acid, dichloromethane, and n-hexane for GC-MS, were purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade), ammonium acetate and phenol were purchased from J.T. Baker (Phillipsburg, USA).

Culture media

R2A agar (DIFCO Laboratories, Detroit, USA) was used as solid nutritive medium. R2A broth (DIFCO Laboratories, Detroit, USA) was diluted twofold in phosphate buffer ($Na_2HPO_4 2H_2O$ 1.14 g l^{-1} and KH_2PO_4 0.58 g l^{-1}) at pH 7.0 to prepare a buffered nutritive broth (BNB). The chloride free-mineral salt medium (MSM) contained (g l^{-1}): Na_2HPO_4 $2H_2O$ 0.715, KH_2PO_4 0.365, $(NH_4)_2SO_4$ 0.5 and $MgSO_4$ $7H_2$ 0.1, pH 7.0. After sterilizing by autoclave, MSM was supplemented with 0.5% (vol/vol) trace element solution (also sterilized by autoclave), which contained (mg l^{-1}): $Ca(NO_3)_2$ $4H_2O$ 880, $FeSO_4$ $7H_2O$ 200, $ZnSO_4$ $7H_2O$ 10,H_3BO_3 10, $CoSO_4$ $7H_2O$ 10, $CuSO_4$ $5H_2O$ 10, $MnSO_4$ H_2O 4, $Ni(NO_3)_2$ $6H_2O$ 4, Na_2MoO_4 $2H_2O$ 3, Na_2WO_4 $2H_2O$ 2 and H_2SO_4 0.05 N. When required, Wolfe's vitamins solution was added as described for ATCC 1019 medium (Gherna et al. 1992). All reagents were purchased from Merck (Darmstadt, Germany). Purified water from a Barnstead Easy Pure System was used in MSM, and distilled water was used in BNB. For degradation assays, chlorophenols, 246TBP, 24D and phenol were added from stock solutions at 2.0 g l^{-1} in 0.1 N NaOH. Easily metabolizable substrates and phenolic compounds used in growth assays were added from stock solutions of 1.0 g l^{-1} and 5.0 g l^{-1}, respectively, both prepared in MSM. All these stock solutions were sterilized by filtration using 0.2 μm Millipore membranes.

Bacterial strains

Two strains isolated previously (Godoy et al. 1999) from a polluted river (receiving a non-treated effluent from a bleached Kraft cellulose pull mill) identified as *Sphingopyxis chilensis* S37 (Godoy et al. 2002) and strain S32 were used. These strains were selected because they were able to degrade 246TCP in resting cell conditions, as previously reported for strain S37 (ex *Pseudomonas paucimobilis*) (Aranda et al. 1999). Strain S32 has the same morphological and physiologic properties by API 20 NE System (bioMériux, France) than strain S37 (Godoy et al. 2002). As control, two strains able to use 246TCP as growth substrate were included: *Ralstonia* sp. PZK (Padilla et al. 2000), isolated from the same river, and *Ralstonia eutropha* JMP134, which is also able to grow with 24DCP and besides have genes for 24D degradation in the plasmid pJP4 (Perkins et al. 1990; Padilla et al. 2000).

Halophenols degradation in nutritive broth

Strains were cultured in 5 ml of BNB at 25 °C until late exponential phase. The cultures were 1 : 1000 diluted in 20 ml of BNB in 50 ml Erlenmeyer flask containing 0.1 mM of 246TCP or 245TCP, or containing 0.2 mM of 24DCP, 4C3MP, 24D or phenol, and incubated in an orbital shaker (80 rpm) at 25 °C for three days. 246TCP was also tested at 0.1, 0.2, 0.3, 0.4 or 0.5 mM in BNB cultures for four days. Daily samples of 1 ml were centrifuged at 12,000 *g* for 1 min and stored at −80 °C for the posterior spectral or HPLC analysis

(see below). Culture growths were measured daily by cell density (OD_{630}). Biotic and abiotic controls were included.

Assays of growth with a sole carbon source

Cells of 24 h cultures in BNB were washed three times by centrifugation and resuspension in MSM and were inoculated at cells density of 10^5 CFU ml^{-1} in 20 ml of MSM containing glucose (0.14, 0.28, 0.56, 2.78 or 5.56 mM), maltose (0.07, 0.15, 0.29, 1.46 or 2.92 mM), phenol (0.27, 0.53, 1.06, 5.32 or 10.6 mM), pyruvate (0.29, 0.59. 1.18, 5.88 or 11.8 mM), 246TCP (0.06, 0.13, 0.25, 0.51, 2.53 or 5.07 mM) or 246TBP (0.06, 0.13, 0.25, 0.51, 2.53 or 5.07 mM) as sole carbon source. The cultures were incubated for two weeks as previously described. Samples were obtained at 2 days intervals for viable cell count (Herbert 1990) and cell density measurement (OD_{630}).

246TCP degradation in the presence of glucose

Strains were cultured until late exponential phase in BNB supplemented with 246TCP 0.1 mM. Previously washed cells of these cultures were inoculated at cell densities of 10^5 CFU ml^{-1} in 20 ml of MSM containing both glucose and 246TCP at various concentrations. Each concentration of glucose (0.17, 0.33, 0.66, 1.33, 2.67 or 5.33 mM) was used in combination with each concentration of 246TCP (0.025, 0.05, 0.10, 0.20 or 0.40 mM). Controls without glucose or 246TCP were also included. Cultures were incubated for 9 days as previously described. Samples of 1 ml were centrifuged at 12,000 *g* for 1 min and stored at −80 °C for the posterior spectral and chloride analysis (see below). In other experiment, the strains were cultured at 30 °C for 2 days in 150 ml of MSM with glucose (1.33 mM). These cultures were supplemented with 246TCP 0.1 mM and then incubated for 3 days as previously described. At the beginning and at the end of this incubation, samples of 60 ml were centrifuged at 15,000 *g* for 5 min and stored at −80 °C for the posterior spectral, HPLC, GC-ECD, GC-MS and chloride analysis (see below).

Analytical methods

Degradation of phenolic compounds was initially measured spectrophotometrically (Cecil 3000 UV/Visible scanning spectrophotometer, Cambridge, England) (200 to 350 nm). The maximum wavelength for respective band contribution of each compound was determined by second derivative analysis. A molar absorbance coefficient ($A_{310\,nm}$) of 4.56×10^3 was used to calculate 246TCP concentration. When required, degradation of phenolic compounds was determined with a high performance liquid chromatograph (HPLC) (Agilent Series 1100, California, USA) equipped with a diode array detector, the column was a Zorbex Eclipse XDB-C8 (4.6 mm × 15 cm). For this purpose, 20 μl of samples, previously filtered by 0.45 μm PTFE micro prep disk (Bio-Rad Laboratories, California, USA) and acidified with 0.07% (vol/vol) H_2SO_4 0.36 N, were injected. Chromatographic conditions were: mobile phase acetonitrile 50% (vol/vol) in ammonium acetate 30 mM, flow rate 1 ml min^{-1}, column temperature 35 °C.

Analysis by gas chromatography with electron capture detector (GC-EDC) was done in a Shimadzu (Kyoto, Japan) 9-A instrument linked to a Shimadzu C-R7A Chromatopac integrator. A Hewlett Packard (California, USA) HP-5 capillary column (30 m × 0.53 mm internal diameter, film thickness 0.88 μm) was used. Carrier N_2 (30 psi), split less 1 : 100, injection temperature 275 °C, oven temperature 210 °C. Gas chromatography-mass spectrometry (GC-MS) analysis were carried out using a Hewlett Packard 5972 mass selective detector and Agilent 5890 Serie II gas chromatograph equipped with a Hewlett Packard HP-5MS capillary column (30 m × 0.25 mm internal diameter, film thickness 0.25 μm). Carrier He (30 psi), split less 1 : 100, injection temperature 250 °C, initial oven temperature 100 °C (5 min), temperature increase 10 °C min^{-1}, final isotherm 275 °C (28 min). Samples of 40 ml were acidified as previously described, extracted with 25 ml ethyl acetate three times and evaporated until 1 ml in a rotary evaporator (1 μl for injection in GC-ECD and GC-MS analysis of underivatized samples). Four hundred microlitres were acetylated with acetic anhydride in pyridine (Gutiérrez et al. 2002) over night at room temperature, other 400 μl were dried under reduced pressure, dissolved in dichloromethane and methylated with diazomethane (Aldrich, 1993) over night at room temperature (1 μl of methylated and acetylated samples for injection in GC-MS).

Chloride release was measured by the HgSCN—$(NO_3)_3Fe$ chromogenic reaction using a double wavelength microplate reader as previously described (Aranda et al. 1999).

Results

Degradation assays in nutritive broth

Table 1 summarizes the abilities of strains to grow in nutritive broth (BNB) and to degrade 246TCP, 245TCP, 24DCP, 4C3MP, 246TBP, 24D or phenol. *R. eutropha* JMP134 was the unique strain able to degrade 24D. All strains were able to degrade 0.1 mM 246TCP and 0.1 mM 246TBP. Phenol was completely degraded by strains JMP134 and PZK, but only partially by strains S32 and S37. The results also showed that 245TCP was inhibitory for all the strains, while no inhibition by 246TCP, 246TBP, phenol, and 24D were observed. On the other hand, 24DCP inhibited strains S32 and S37, indicating that this compound is more toxic for them than 246TCP, and also indicating that phenolic form 2,4-dichloride compound was more toxic than its phenoxyacetic form (24D). The results of cultures in BNB at higher 246TCP concentration showed that all strains were able to tolerate and to degrade up to 0.5 mM 246TCP. However, a lag phase extending with 246TCP at 0.4 and 0.5 mM was observed for strain S32 and S37 (Figures 1a and 1b), but not for *R. eutropha* JMP144 and *Ralstonia* sp. PZK, which required only two days to degrade 0.5 mM 246TCP (data no shown).

Degradation of 246TCP by strains S32 and S37 in mineral salt medium

Strains S32 and S37 were cultured in glucose or maltose as sole carbon and energy source at concentrations ranging from 0.3 to 5.6 mM in MSM supplemented with vitamins. Maximum growths for each substrate concentration were obtained at the third day of incubation. In this time, measurements of OD_{630} reached from 0.1 to 0.7, with a maximum viable cell count of 1.8×10^9 CFU ml^{-1}. The strains did not grow with 246TCP or 246TBP as sole carbon source, and the compounds at concentrations equal or higher than 25 mM were lethal for inocula. Using a mixture of glucose and 246TCP at various concentrations, there was observed that both strains can grow in the presence of 246TCP, but only if a threshold concentration of glucose was also present (Figures 2c and 2d). However, both strains did not grow at the highest concentrations assayed for the mixture of 246TCP and glucose (0.4 mM and 5.33 mM, respectively). The maximum cell densities were obtained at the sixth day of culture when 25 μM of 246TCP was used, independently of glucose concentrations.

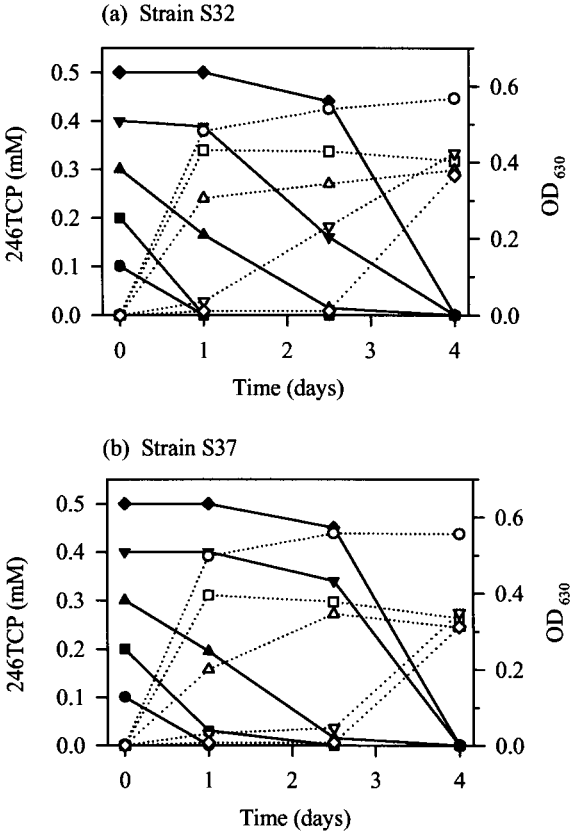

Figure 1. Growth curves and degradation of 246TCP 0.1 (●), 0.2 (■), 0.3 (▲), 0.4 (▼), and 0.5 mM (♦) by strains S32 (a) and S37(b). The growth curves (- - - - -) are presented by open symbol while the degradation curves (——) are presented by closed symbol. No changes in cell morphology were observed at any chlorophenol concentration assayed.

Nevertheless, cultures require 9 days-incubation for maximal growths at higher 246TCP concentrations. The maximal viable counts (data no showed) and OD_{630} achieved were dependent upon glucose concentrations and were no modified by changes in 246TCP concentration. Interestingly, the cells of strain S32 were able to degrade 246TCP 25 μM without glucose (Figure 2a) and in absence of growth, but strain S37 required glucose at 0.33 mM to completely degrade 246TCP 25 μM (Figure 2b) under growing condition. At higher 246TCP concentrations, both strains required higher glucose concentrations to grow and degrade the halophenol. Strain S32 was able to degrade 246TCP up to 0.2 mM with glucose at 5.33 mM (Figure 2a) and the strain S37 was able to degrade 246TCP up to 0.1 mM with glucose at 1.33 mM (Figure 2b). Chloride release correlated with chlorophenol

Table 1. Growths and degradation of phenolic compounds and 24D in nutritive broth

Strain(s)	Compound (mM)						
	245TCP	246TCP	246TBP	24DCP	4C3MP	Phenol	24D
	0.1	0.1	0.1	0.2	0.2	0.2	0.2
Sphingopyxis chilensis S37	−/−*	++/*G*	++/*G*	−/−	−/−	*P*/*G*	−/*G*
Sphingopyxis-like strain S32	−/−	++/*G*	++/*G*	−/−	−/−	*P*/*G*	−/*G*
Ralstonia sp. PZK	−/−	++/*G*	++/*G*	−/*G*	−/*G*	++/*G*	−/*G*
R. eutropha JMP134	−/−	++/*G*	++/*G*	+/*G*	−/*G*	++/*G*	++/*G*

* The right side of the each slash indicates the ability of the strain to grow in BNB supplemented with the compound, while the left side indicates the ability to degrade the compound in this condition.
(++) Total degradation at first day.
(+) Total degradation at second day.
(*P*) Partial degradation.
(*G*) Positive growth.
(−) Negative growth or degradation.

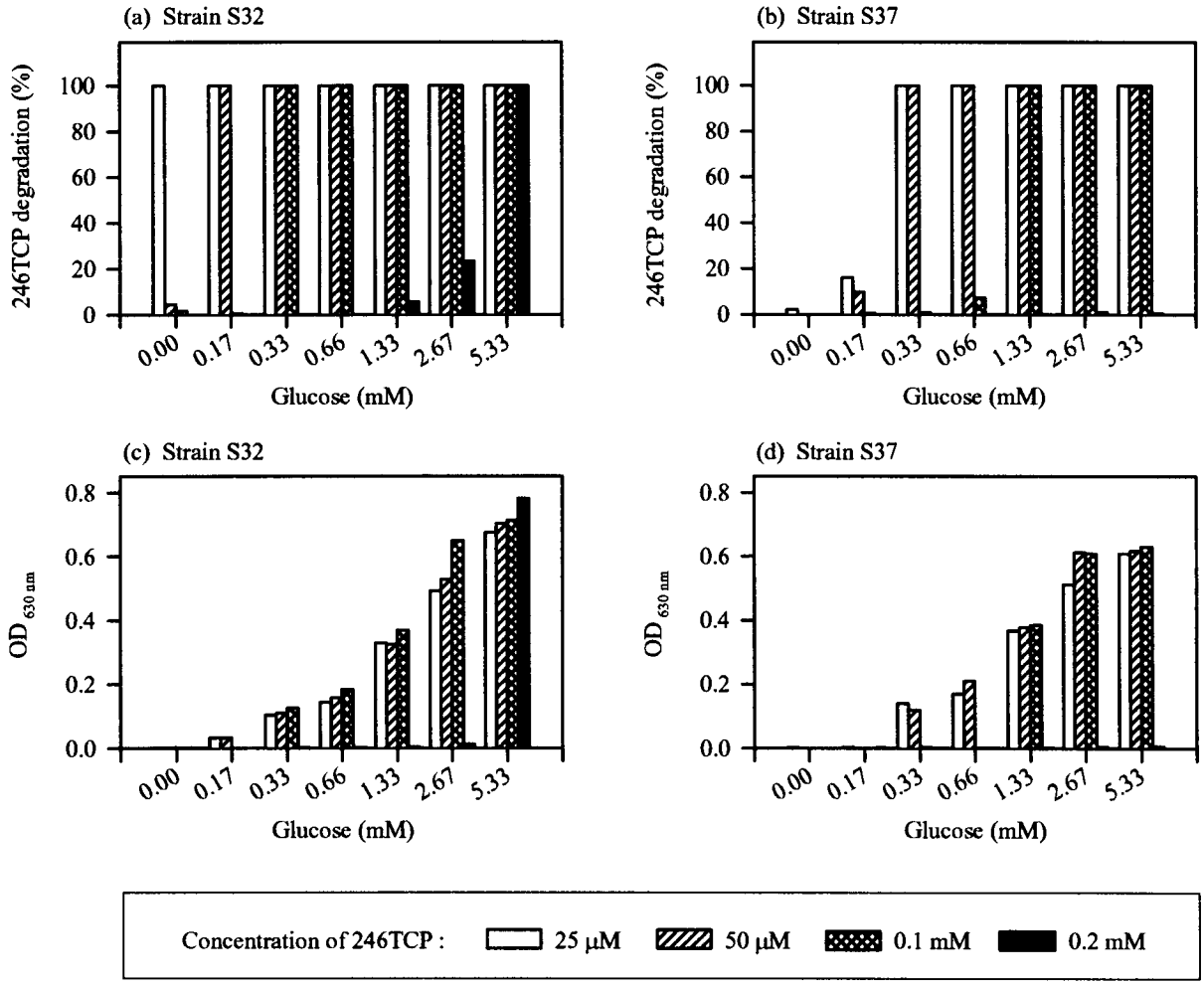

Figure 2. Degradation of 246TCP 25, 50, 100 and 200 μM at ninth day of culture with different glucose concentrations in mineral salt medium by strain S32 (a) and strain S37 (b), and respective growth (c and d).

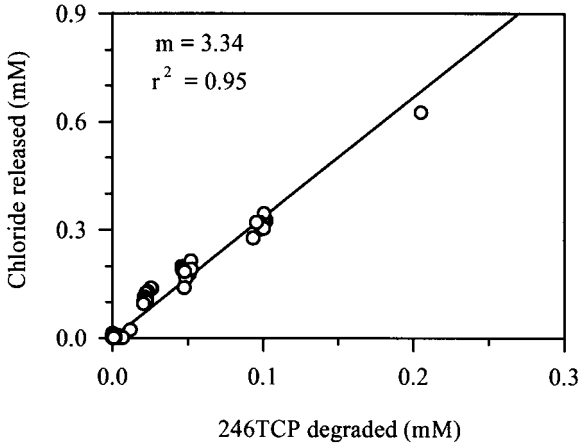

Figure 3. Correlation between chloride release and 246TCP degradation. Values come from all cultures of strains S32 and S37 in mineral salt medium with different combinations of glucose and 246TCP.

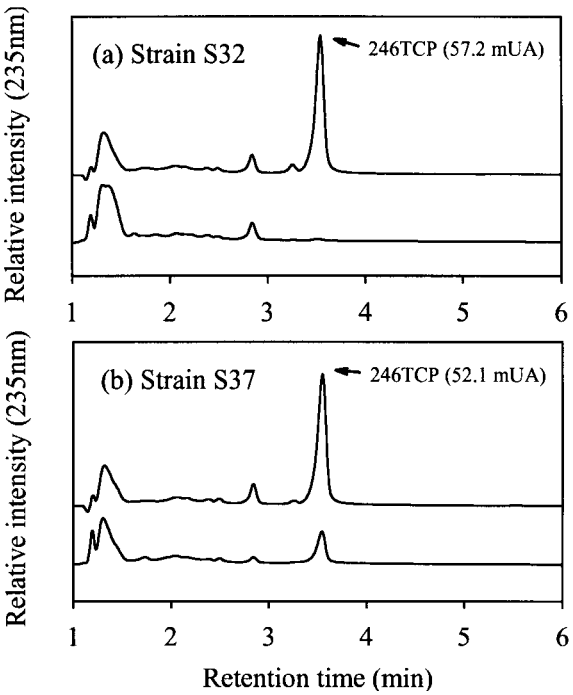

Figure 4. Reversed phase HPLC chromatograms at the beginning (upper ones) and at the third day of incubation (lower ones) with 246TCP 0.1 mM for strain S32 (a) and strain S37 (b) previously grown with glucose 1.33 mM in MSM.

spectral band disappearance, in a ratio close to 3 : 1 (Figure 3).

To further evaluate the possible mineralisation of 246TCP, both strains were grown for 2 days with glucose 1.33 mM in MSM supplemented with vitamins (the values of OD_{630} reached were 0.133 for strain S32 and 0.157 for strain S37) and next incubated with 246TCP 0.1 mM for 3 days. Table 2 shows the result of 246TCP degradation (100% and 75% by S32 and S37 strains respectively) measured by spectral, HLPC, GC-EDC and chlorine analysis. No metabolites were detectable by HPLC (Figure 4) and GC-ECD (data not shown). Gas chromatography-mass spectrometry (GC-MS) analysis performed with derivatized and non-derivatized samples were all consistent with 246TCP mineralisation. Figure 5 shows principal GC-MS results for strain S32, where the mass chromatograph profile of methylated final sample indicated total absence of possible organic acid intermediaries. Besides, possible intermediaries with alcoholic groups were also discarded due to absence of peeks in acetylated samples (data no shown)

Discussion

Phenolic compound assayed could be divided in two groups according to its degradability by the strain assayed: 246TCP, 246TBP and phenol are degradable while 245TCP, 4C3MP and 24DCP are not (except 24DCP by strain PZK). On the other hand, the similar degradative pattern in all strains is an interesting fact that could implicate a similar metabolic pathway, even though S37 strain does not have genes like characterized for 246TCP degradation in *Ralstonia* strains (Padilla et al. 2000).

All strains were able to degrade 0.1 mM 246TCP and 0.1 mM 246TBP with the same kinetics, suggesting that dehalogenase systems involved do not discriminate among chlorinated or brominated substrates. It has already been postulated that bacterial chlorophenols tolerance among bacterial strains isolated from marine environments could be a consequence of a selective pressure exerted by brominated organic molecules, naturally synthesized by marine organisms in pristine seawaters (Martínez et al. 1999). Therefore, the theory of the early appearance of enzymes for brominated substrates dehalogenation in marine sediments bacteria (Gary 1988), followed by the posterior dissemination of the genes involved as a consequence of selective pressure by anthropogenic chloroaromatic compounds, could be further considered to evaluation.

Assays with the strains S32 and S37 in MSM, based on spectral and chloride analysis, demonstrated the metabolic potential for 246TCP aromatic structure

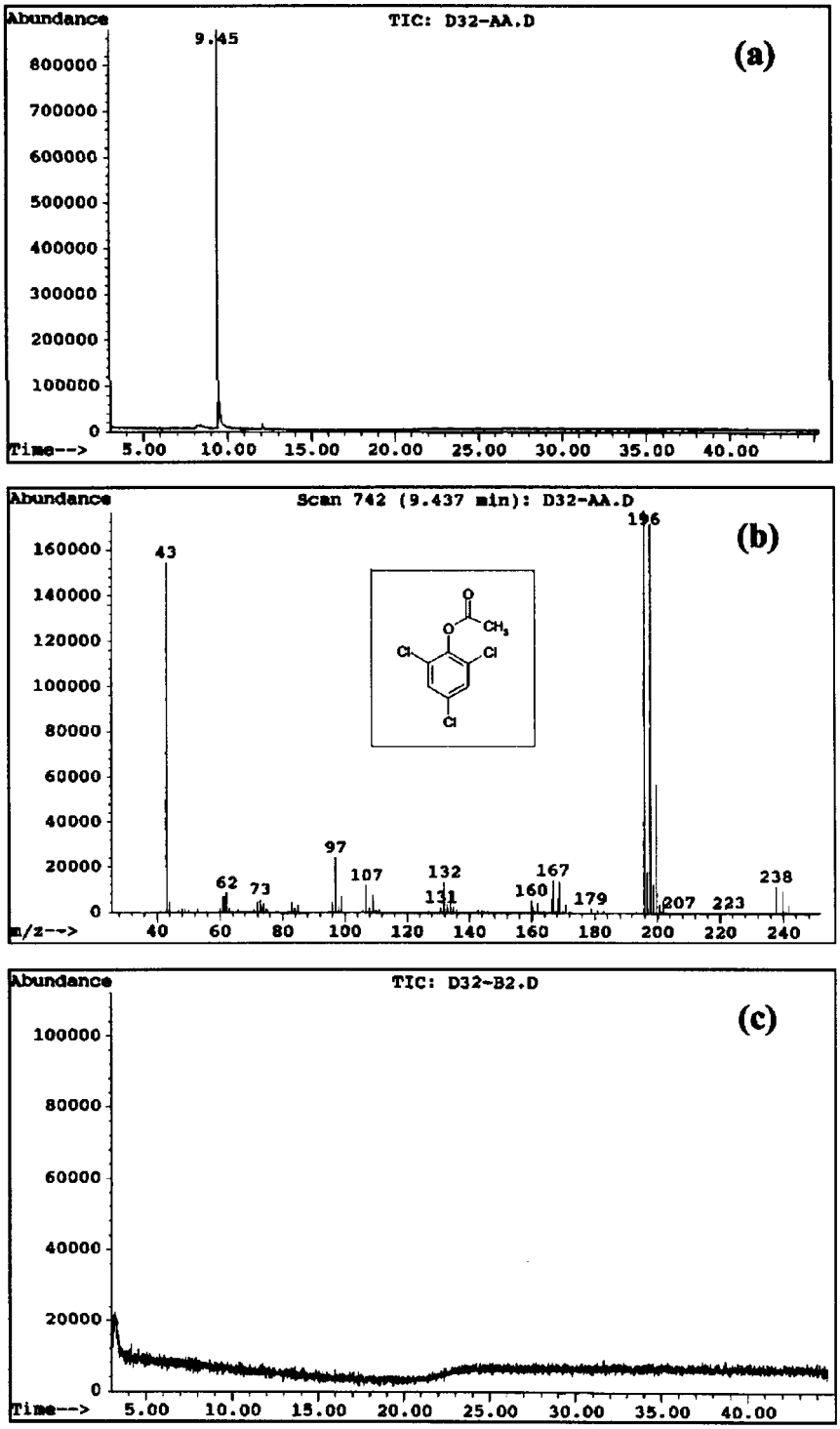

Figure 5. GC-MS analysis of 246TCP degradation by strain S32 previously grown with glucose 1.33 mM in MSM. Panel a: 70-eV mass chromatograph profile of acetylated initial sample. Panel b: mass spectra identifying derivatized 246TCP. Panel c: 70-eV mass chromatograph profile of methylated final sample.

Table 2. Results for 0.1 mM 246TCP degradation, according to the different analytical approaches, by strain S32 and S37 previously grown with glucose 1.33 mM in MSM

Strain reduction (%)	Spectral band reduction (%)	HPLC peak reduction (%)	GC-ECD peak released (%)	Chloride
Sphingopyxis chilensis S37	74.7	76.9	79.3	88 ± 12
Sphingopyxis-like strain S32	100	99.3	98.2	107 ± 14

disappearing and for organic chloride mineralizing, indicating that accumulation of a chlorinated non-aromatic metabolite is not feasible. This activity is different from that observed in the 4-chlorophenol cometabolism by *Pseudomonas putida* ATCC 49451 (Loh & Wang 1998), where the dead end compound 2-hydroxy-chloromuconic semialdehyde was accumulated. The degradation until a non-halogenated and non-aromatic metabolite could implicate a metabolite transfer into the Krebs cycle. This idea is consistent with HPLC, GC-ECD and GC-MS analysis, since possible intermediaries were not detected in samples of 246TCP degraded by both strains.

Two hypotheses could be considered to understand the inability of strains S32 and S37 to grow with 246TCP. One possibility is that the toxic effect of 246TCP produces an energy cell demand for maintenance higher than its catabolic yielding (Rittmann 1992), and the other one is that the implicated catabolic pathways have an intrinsic negative, or approximately zero, energetic yielding due to a possible high requirement of ATP or reductive power in one or more steps.

The extending of the lag period by 246TCP for growing in BNB (or glucose) suggests tolerance mechanism induction (Brözel & Cloete 1993). Other explanation would implicate induction of the chlorophenol metabolic pathway. However, this last idea is until now discarded in accordance with previous evidence that indicated constitutive expression of genes for 246TCP degradation, based on equal degradation activities by resting cell cultures (strain S37) previously grown in R2A broth alone or added with this chlorophenol (data no shown).

Relationship established between 246TCP and glucose in the present work is similar to that established by LaPat-Polasko et al. (1984) for methylene chloride and acetate. These authors used the secondary utilization concept to explain the simultaneous degradation of these two compounds. In this case, methylene chloride and acetate are two compounds that independently can support growth as primary substrate. However, methylene chloride could be also degraded as a secondary substrate, when added at a concentration lower than the minimum one required to support growth, while acetate was used as primary substrate. Secondary utilization means the degradation of a growth substrate, but at a concentration lower than the required for sustaining the bacterial growth, and therefore, this process occurs in the obligate presence of another growth substrate (primary substrate) that sustains the biomass, or occurs while the active biomass degrades itself to give account for energy cell demand. This type of dependence on another growth substrate should be differentiated from real cometabolism, where the non-growth substrate is only partially metabolized. Even though 246TCP is a non-growth and toxic substrate for the strains S32 and S37, our results would suggest secondary utilization instead cometabolism in according to the evidence for mineralisation. Therefore, the use of secondary utilization concept should be expanded, since a toxic compound could be mineralized in the obligate presence of another growth substrate due to two possible reasons: (1) the available concentration of the compound is lower than the minimal one required to support the biomass growth (unique reason until now considered) and (2) the secondary substrate could not support growth independently of its concentrations, because although it could be mineralized, its toxicity gives account for energy demand higher than obtained from its catabolism, or due to an intrinsically low (or negative) pathway energetic yielding.

This secondary utilization approach implies that in the environment could exist bacteria with the ability to mineralize some toxic compound, but depending on other metabolizable compound for growing. For example, in bacterial communities degrading some xenobiotics, from which it has been impossible to isolate a xenobiotic growing bacteria, there should be evaluated the presence of bacteria able to mineralize

the compound without the restriction of growth, and not only bacteria that cometabolizes it.

Other three strains isolated from the same environment than strain S32, S37 and PZK, were able to degrade 246TCP and 246TBP in nutritive broth, but only until 0.1 mM because higher concentrations were inhibitory. These strains (biochemically characterized as *Burkholderia cepacea*) are possible halophenol secondary utilizers, but were not included in this study, due to the absence of a suitable growth condition in mineral salt medium that allow to sustain this definition.

At present, secondary utilization like here reported could be difficult to study, since in some cases the readily metabolizable substrates could inhibit xenobiotic degradation by catabolic repression (Swindoll et al. 1988; Duetz et al. 1996) or by competitive inhibition if key enzymes are shared (Saez & Rittmann 1993; Loh & Wang 1998). In fact, previous experiments with the strain S37 have also shown that high concentrations of glucose decrease 246TCP degradation by resting cell suspensions (Aranda et al. 1999). Two approaches could be considered to facilitate xenobiotic secondary utilization studies: (1) the selection of bacteria with the ability to tolerate some xenobiotic, but without using enrichments culture methodology, for further xenobiotic degradation studies and (2) the use of sequencing reactor systems to decouple bacterial growth with the xenobiotic degradation to avoid possible inhibition of xenobiotic catabolism, similar to that proposed for cometabolic activities (Alvarez-Cohen & McCarty 1991; Segar et al. 1995).

In conclusion, the strains S32 and S37 were able to degrade the non-growth and inhibitory xenobiotic substrate 246TCP. Since there are evidences of mineralisation, the secondary utilization concept was proposed instead of cometabolism. Activities like the ones reported here, may have an important contribution to xenobiotics biodegradation in environments where a mixture of rather easily metabolizable substrates is present (Morita 1988). The understanding of this potential could be useful to improve bioremediation strategies.

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