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Degradation of 2,5-dichlorobenzoic acid by *Pseudomonas aeruginosa* JB2 at low oxygen tensions

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Abstract

From long-term chemostat experiments, variants of *Pseudomonas aeruginosa* JB2 were obtained which exhibited altered properties with respect to the metabolism of 2,5-dichlorobenzoic acid (2,5-DBA). Thus, unlike the original strain JB2-WT, strain JB2-var1 is able to grow in continuous culture on 2,5-DBA as the sole limiting carbon and energy source. Yet, at a dilution rate of 0.07 h^{-1} and a dissolved oxygen concentration of $\leq$ 12 μM, even with this strain no steady states with 2,5-DBA alone could be established in continuous cultures. Yet another strain was obtained after prolonged continuous growth of JB2-var1 in the chemostat. It has improved 2,5-DBA degrading capabilities which become apparent only during growth in continuous culture: a lower apparent K_m for 2,5-DBA and lowered steady-state residual concentrations of 2,5 DBA. Although with this strain steady states were obtained at oxygen concentrations as low as 11 μM, at further lowered concentrations this was no longer possible. In C-limited continuous cultures of JB2-var1 or JB2-var2, addition of benzoic acid (BA) to the feed reduced the amounts of 2,5-DBA degraded, which was most apparent at low oxygen concentrations (< 30 μM). At higher dissolved oxygen concentrations the addition of BA resulted in increasing cell-densities but did not affect the residual steady state concentration of 2,5-DBA. Indeed, whole cell suspensions from chemostat cultures grown on BA plus 2,5-DBA did show a lower apparent affinity for 2,5-DBA than those from cultures grown on 2,5-DBA alone. These results indicate that in environments with low oxygen concentrations and alternative, more easily degradable, substrates the degradation rates of chloroaromatic compounds by aerobic organisms may be negatively affected.

Abbreviations: BA – benzoic acid, 2,5-DBA – 2,5-dichlorobenzoic acid, QO_2^{max} – maximum specific respiration rate

Introduction

Many man-made chlorinated benzoic acids are of environmental concern since their biodegradation has been shown to be slow under aerobic as well as under anaerobic conditions (Häggblom 1992; Mohn & Tiedje 1992). The occurrence of such halogenated benzoic acids in natural environments is due predominantly to the use of herbicides and partial degradation of spilled PCBs (Swanson 1969; Furukawa et al. 1978; Reineke & Knackmuss 1988, Abramowitz 1990). Over the years, an increasing number of microorganisms has been found capable of using various, mostly monohalogenated, benzoic acids either co-metabolically or as true growth-substrates. In addition, several aerobic bacteria, isolated more recently, have been shown capable of using di- or trichlorobenzoic acids, presumably based on the presence of specialized dioxygenases (Hickey & Focht 1990; Hernandez & Focht 1991). Sofar, with respect to the aerobic degradation of chlorinated aromatics, relatively little attention has been payed to the influence of specific growth-limiting conditions, such as the concentration of the target substrate, the concentration of oxygen and the presence of alternative, chlorinated or non-chlorinated, substrates. The oxygen concentration in particular is a

factor which can be expected to have a strong influence on the aerobic degradation of chlorinated aromatic compounds. Under high oxygen concentrations, intermediates like chlorocatechols and chloroprotocatechuates may easily auto-oxidize and polymerize to black-coloured precipitates (Haller & Finn 1979; Crawford et al. 1973). Under conditions of sub-optimal aeration, toxic levels of chlorocatechols were observed in cultures of *Pseudomonas* sp. B13 during growth on 3-CBA (Dorn & Knackmuss 1978). No toxicity, but limitation of the rate of 2,4-dichlorophenoxyacetic acid degradation by an enrichment culture was shown at oxygen tensions below a value of 30 μM (Shaler & Klecka 1986). Also *Pseudomonas* isolates utilizing 3-chlorobenzoate showed a decrease in 3-chlorobenzoate respiration below a dissolved oxygen concentration of 20–30 μM (Haller & Finn 1979). In the observations described above, the low affinity for oxygen of the oxygenases, involved in the degrading pathways of substituted aromatics, is held responsible. Another factor affecting the degradation of chlorinated aromatic compounds is the presence of more than one growth substrate at the same time. It may have different consequences, depending on the nature of the compounds involved, the degrading capacities of the microorganism and the prevailing environmental conditions. In most bacteria, the aerobic conversion of halogenated aromatics has been shown to proceed along pathways used for the metabolism of non-halogenated aromatics. In most early reports on degradation of chlorinated aromatic compounds, organisms have been described which could only use them in cometabolism with a true growth-substrate (Horvath & Alexander 1970, Focht & Alexander 1972, Reber & Tierbach 1980, Reineke 1984, Parsons et al. 1988). In some of these studies, often performed with mixtures with non-halogenated compounds, incomplete breakdown or total intoxication of the aerobic bacteria has been encountered. To explain such phenomena some authors have pointed to the formation of non-degradable end-products or toxic intermediates that may arise during growth on such mixtures (Dorn et al. 1974, Haller & Finn 1979, Bartels et al. 1984, Adriaens & Focht 1991). In some cases very specific interactions between the different halogenated compounds seem to be involved. For example, cultures of *Pseudomonas putida* P111 growing on *ortho*-chlorinated benzoates accumulated dichlorocyclohexadienes and became completely inhibited in the presence of 3,5-dichlorobenzoate (Hernandez et al. 1991). *Pseudomonas aeruginosa* JB2 has been well-characterized as to its ability to grow at the expense of several chlorinated aromatics at high oxygen tensions in batch cultures (Hickey & Focht 1990). However, very little is known on the influence of low oxygen partial pressures and on the presence of non-chlorinated growth substrates. The organism was capable of using 2,5-DBA in an oxygen-limited reactor, and a relatively high (25 μM) apparent Michaelis constant for oxygen with 2,5-DBA as the respired substrate was determined for washed cells of strain JB2 (Gerritse & Gottschal 1992). In the present study, *Pseudomonas aeruginosa* strain JB2 is used as a model organism to address two questions: 1) how does the dissolved oxygen concentration affect growth on and metabolism of 2,5-dichlorobenzoic acid (2,5-DBA), and 2) how does the presence of benzoic acid (as an additional substrate) influence the degradation of 2,5-DBA?

Material and methods

Source and growth of the organism

Pseudomonas aeruginosa JB2 was kindly supplied by Dr. F.K. Higson and Dr. D.D. Focht. The organism is capable of growth on several mono-, di- and trihalogenated benzoic acids (Hickey & Focht 1990). *P. aeruginosa* JB2 was routinely grown at 30° C in a low-chloride mineral salts medium containing the following components (per liter): $MgSO_4 \cdot 7H^2O$ (0.1 g); $Ca(NO_3)_2 \cdot 4H_2O$ (0.04 g); YE (0.01 g); EDTA (1 mg), $FeSO_4 \cdot 7H_2O$ (2 mg), $ZnSO_4 \cdot 7H_2O$ (0.1 mg), $MnCl_2 \cdot 4H_2O$ (0.03 mg), H_3BO_3 (0.3 mg), $CoCl_2 \cdot 6H_2O$ (0.2 mg), $CuCl_2 \cdot 2H_2O$ (0.01 mg), $NiCl_2 \cdot 6H_2O$ (0.02 mg), $Na_2MoO_4 \cdot 2H_2O$ (0.03 mg), $Na_2SeO_3 \cdot 5H_2O$ (0.026 mg) and $Na_2WO_4 \cdot 2H_2O$ (0.033 mg). After autoclaving, the medium was completed by the addition of a $K(NH_4)_2PO_4$ buffer (2.88 g, pH = 7.0) to a final concentration of 25 mM, and varying concentrations of 2,5-dichlorobenzoate (2,5-DBA) from stock solutions, autoclaved separately. For N-limited culture conditions, a KH_2PO_4 buffer (3.40 g, pH = 7.0) was used and a limiting supply of N was added as $(NH_4)_2SO_4$ (33 mg). Batch cultures used for growth measurements were inoculated 1:25 with log-phase cells grown on 2,5-DBA. Continuous growth of strain JB2 was at 30° C in a chemostat-vessel with a working volume of approx. 450 ml, automatically O_2-regulated by stirring rate coupled to continuous O_2 readings from a polarographic oxygen electrode (Ingold, Urdorf, Switzerland). The pH was regulated continuously by

automatic additions of 0.5 N KOH. The air flow-rate over the culture was 300–600 ml h^{-1}.

Analytical and microbiological procedures

Cultures were checked for purity by streaking on Nutrient Broth (BBL, Cockeysville, USA) agar-plates and microscopic observation. Chloride was measured colorimetrically according to the method of Bergman and Sanic (1957) with NaCl as a standard. Benzoates were analyzed as described previously by Gerritse and Gottschal (1992), with a lower detection-limit of 0.01 mM. Turbidity in *P. aeruginosa* JB2 cultures was determined by measuring optical density at 433 nm. Protein was measured according to Lowry et al. (1951) with bovine serum albumin as the standard. Dissolved or cell-carbon were analyzed with a Total-Carbon Analyzer as described by Gerritse et al. (1990). Substrate oxidation kinetics of *P. aeruginosa* JB2 were determined in washed cell-suspensions using the procedures described earlier (Gerritse & Gottschal 1992). The suspensions were made in isotonic $K(NH_4)PO_4$-buffer (25 mM; pH = 7). The values of the maximum specific oxygen consumption rate (QO_2 ^{max}) and the apparent half saturation constant (K_m) were estimated by the direct linear-plot method. The maximum specific consumption rates for benzoic acid ($Q(BA)^{max}$) and 2,5-DBA ($Q(2,5\text{-DBA})^{max}$) were obtained from the QO_2 ^{max} with BA or with 2,5-DBA and the stoichiometry (amount of oxygen consumed/amount of benzoic acid added), determined in washed cell-suspensions.

Chemicals

All chemicals were of analytical grade. 2,5-DBA had a purity of 97–99% (Merck, Germany; Aldrich, Germany).

Results

Growth on 2,5-DBA as the sole carbon and energy source in continuous culture

With the original strain of *Pseudomonas aeruginosa* JB2, designated JB2-WT, we observed good growth with 2,5-DBA in batch culture ($\mu_{max} = 0.085\ h^{-1}$, Fig. 2B; Table 2). However, attempts to obtain steady states in continuous culture at dissolved oxygen concentrations of 120 μM with 2,5-DBA (purchased from two different companies) as the sole growth-limiting carbon and energy source failed initially. However, under NH_4+-limitation (residual concentrations of 2,5-DBA above 1 mM) steady-states were obtained. During growth on mixtures of 2,5-DBA plus benzoate or glucose (2.5 mM each), stable cultures were also obtained with low residual 2,5-DBA concentrations. Removing either of these additional non-chlorinated substrates from the feed of steady-state cultures always resulted in wash-out and inactivation of the culture. Surprisingly, after 2.5 months of continuous operation (140 volume changes) under carbon-limitation at dilution rates between 0.023 and 0.182 h^{-1} with BA as a cosubstrate in the feed, the population of *Pseudomonas aeruginosa* JB2 in the chemostat appeared capable of growth at the expense of 2,5-DBA as the sole limiting growth-substrate. The thus isolated strain, designated JB2-var1, was used in subsequent chemostat experiments with 2,5-DBA as the sole substrate.

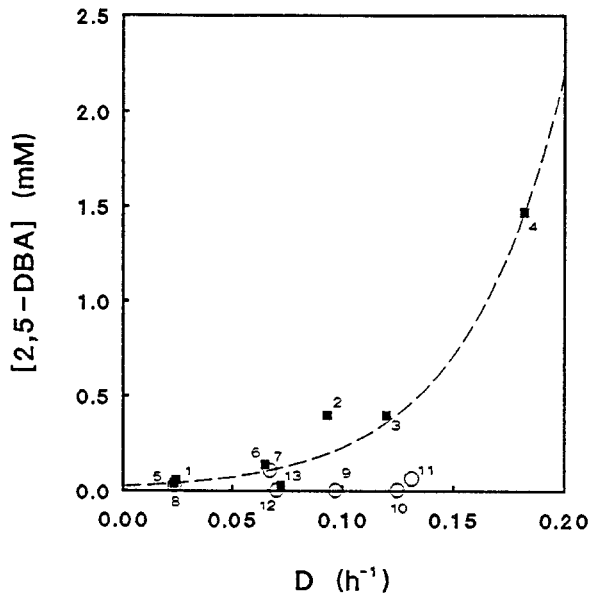

Fig. 1. Steady-state residual concentrations of 2,5-DBA versus dilution rate in an oxygen regulated chemostat ([O_2] in culture liquid = 120 μM) with *Pseudomonas aeruginosa* JB2 strains. Numbers indicate the time-sequence of the different steady states. Nrs 1–6, strain JB2-WT; nrs 7 and 8, strain JB2-var1; nrs 9–13, strain JB2-var2. (■–■), growth on a mixture of BA + 2,5-DBA, 2.5 mM each in the feed; (○–○), growth on 2,5-DBA alone, 2.5 mM in the feed. The dashed line represents best exponential fit to the steady state residual 2,5-DBA concentrations in the strain JB2-WT cultures.

Table 1. Data of steady-state chemostat cultures and kinetic parameters of washed cell suspensions of different *Pseudomonas aeruginosa* JB2 strains, grown on a mixture of 2,5-DBA (2.5 mM) + BA (2.5 mM) or 2,5-DBA (2.5 mM) alone. Cultures were C-limited. Standard deviations between brackets.

Strain[1]	Growth substrate	Dilution rate (h^{-1})	Dissolved oxygen tension (μM)	Chlorine recovery (%)	$C_{cells}/C_{consumed}$ (mmol/mmol)	Residual concentration of 2,5-DBA (mM)	K_m (2,5-DBA) (mM)	QO_2^{max} with 2,5-DBA (mmol $h^{-1}g^{-1}$ protein)	$K_m(O_2)$ with 2,5-DBA μ
WT	2,5-DBA + BA	0.023–0.182	120	87.8 (11.4)	0.21 (0.05)	0.04-1.47	1.0 (0.13)	6-15	30
		0.067	47	112	0.16	0.10	-	-	-
		0.067	26	119	0.17	1.43	1.0	8	27
		0.065	12	98	0.17	2.10	0.8	2	–
var1	2,5-DBA	0.023–0.068	120	95.5 (2.1)	0.22 (0.01)	0.03–0.11	0.09	15	28
		0.070	26	92	0.14	0.22	0.1	20	26
var2	2,5-DBA	0.038–0.131	120	96 (3.1)	0.18 (0.01)	0.01–0.07	0.04 (0.0)	4.8–12	31 (8)
		0.068	11	92	–	0.05	–	–	–
	2,5-DBA + BA	0.072	120	113	–	0.03	0.1	4.2	–
		0.068	11	106	–	1.27	0.07	3.9	–

1: WT = original strain
var1 = variant strain able to grow on 2,5-DBA alone under C-limitation in continuous culture
var2 = similar to var1, with improved apparent K_m (2,5-DBA).

Influence of dilution rate on the metabolism of 2,5-DBA in the presence and absence of BA

At a dissolved oxygen concentration of 120 μM (i.e. approx. 50% air saturation) in continuous culture, *Pseudomonas aeruginosa* strain JB2-WT metabolized 2,5-DBA and BA simultaneously. A plot of the residual concentration of 2,5-DBA versus dilution rate showed a gradual increase of the residual 2,5-DBA concentrations from values as low as 0.04 mM at D = 0.023 h^{-1} to as much as 1.47 mM at a dilution rate of 0.182 h^{-1} (Fig. 1). To compare these values with those in cultures grown without BA, strain JB2-var1 was grown on 2,5-DBA alone. Runs at 0.023 and 0.070 h^{-1} (Fig. 1: nr 8 and 7 vs 1, 5 and 6) resulted in comparable 2,5-DBA residual concentrations, in spite of a lower steady-state biomass. Unexpectedly, at subsequent elevated dilution rates (> 0.09 h^{-1}) a clear drop in the concentration of unused 2,5-DBA was observed (Fig. 1: nr 9, 10, 11). To test whether the lowered residual 2,5-DBA concentrations were the result of a genetic adaptation, the culture was grown again at a lower dilution rate (0.070 h^{-1}). The residual concentration of 2,5-DBA appeared to be much lower than at earlier runs at a comparable dilution rate (0.068 h^{-1}, Fig. 1: nr 13 vs 7). Moreover, when cells of this apparently improved strain of JB2-var1, called JB2-var2, were grown with BA added to the feed, the residual concentration of 2,5-DBA was approx. 4 times lower than the value obtained with strain JB2-WT under the same conditions (dilution rate 0.067–0.070 h^{-1}; Fig. 1: nr 12 vs 7).

Differences in kinetic properties of cells grown in batch and continuous culture

When JB2-var1 was grown in continuous culture fed with a mixture of 2,5-DBA + BA or in batch culture with 2,5-DBA alone, it did not show any differences in growth rate or kinetic parameters from the parent strain JB2-WT. Yet, compared to washed cell suspensions obtained from batch-cultures of strain JB2-var1 grown on 2,5-DBA alone, cells grown on 2,5-DBA alone in the chemostat showed both a decreased K_m (from 0.4 to 0.1 mM) and an increased QO_2^{max} for 2,5-DBA (Table 1, Table 2). The same applies to washed cells of JB2-var2, though under continuous culture conditions expressing a lower apparent K_m for 2,5-DBA than cells of JB2-var1 (Table 1). The μ_{max} on 2,5-DBA of strain JB2-var2, grown under 2,5-DBA limitation alone, was determined by using the wash-out method, and was found to be 0.176 h^{-1}. Yet, if cells taken from this chemostat culture were directly transferred (1:25; v/v) to a batch culture, the μ_{max} on 2,5-DBA appeared to

Table 2. Kinetic parameters of the original strain *Pseudomonas aeruginosa* JB2-WT, grown in batch culture. QO_2^{max} and apparent K_m-values were obtained with washed cell-suspensions.

Growth substrate	μ_{max} (h^{-1})	Benzoate			2,5-DBA		
		QO_2^{max} (mmol $h^{-1}g^{-1}$ protein)	K_m (mM)	$K_m(O_2)$ (μM)	QO_2^{max} (mmol $h^{-1}g^{-1}$ protein)	K_m (mM)	$K_m(O_2)$ (μM)
Glucose	0.35	n.d.	n.d.	n.d.	1.8	n.d.	n.d.
BA	0.30	37	0.05	19	4.3	n.d.	n.d.
2,5-DBA	0.085	1.8	n.d.	n.d.	5.7	0.4	28
BA +	0.24[1]	23	n.d.	n.d.	5.4	n.d.	n.d.
2,5-DBA	0.03[2]	2.8			3.8		

n.d.: not determined.

[1] in presence of benzoate.

[2] after depletion of benzoate.

be only 0.089 h^{-1}, very similar to that of the strains JB2-WT and JB2-var1 (Table 2). It seems that the improved 2,5-DBA growth-capacities of *Pseudomonas* strain JB2-var2 become apparent only during substrate limited growth in continuous culture.

Influence of benzoic acid on the metabolism of 2,5-DBA

Compared to cells of strain JB2-var1 or JB2-var2 grown on 2,5-DBA alone in the chemostat at a dilution rate of 0.023 h^{-1} and 0.068 h^{-1}, cells grown under the same conditions on 2,5-DBA + BA showed a significantly increased K_m and slightly decreased QO_2^{max} for 2,5-DBA (Table 1), resulting in a decreased overall affinity of these cells for 2,5-DBA.

In order to determine the extent to which BA and 2,5-DBA degradation interfered with each other, some basic kinetic parameters were determined in batch cultures of *Pseudomonas aeruginosa* strain JB2-WT. In cultures grown on a mixture of 2,5-DBA + BA, benzoate was consumed at a much higher rate than 2,5-DBA (Fig. 2C). Remarkably, growth at the expense of 2,5-DBA after depletion of benzoate proceeded much slower than in cultures with 2,5-DBA as the sole substrate (Fig. 2B). Yet, even BA-grown cells exhibited maximum substrate respiration capacities (QO_2^{max}) for 2,5-DBA similar to 2,5-DBA-grown cells, while washed cells taken from glucose-grown cultures showed a 2–3 times lower QO_2^{max} for 2,5-DBA compared to cells grown on 2,5-DBA. Cells grown on 2,5-DBA showed only very little respiration capacity for BA (Table 2).

Influence of low oxygen tensions on 2,5-DBA metabolism in the presence and absence of BA

When chemostat cultures of strain JB2-WT, grown with BA + 2,5-DBA, were maintained at different dissolved oxygen concentrations, BA was always completely degraded but an increasing fraction of 2,5-DBA remained unused with decreasing oxygen tension (Table 1). Nevertheless, even at a very low oxygen concentration (12 μM) the culture was substrate-limited, as an additional supply of either 2,5-DBA or BA resulted in an increase of the cell-density. Low oxygen concentrations affected 2,5-DBA metabolism in chemostat cultures in a somewhat different way when BA was not present in the feed. For example, with 2,5-DBA alone at a dissolved oxygen concentration of 12 μM, no steady state could be obtained and total wash-out of strain JB2-var1 occurred, whereas at 26 μM nearly all 2,5-DBA was degraded (Table 1). However, JB2-var2 was able to use all 2,5-DBA completely when grown on 2,5-DBA alone even at a regulated oxygen concentration of 11 μM (Table 1). However, addition of BA to the feed resulted, similar to steady states with strains JB2-WT and JB2-var1, in lower amounts of 2,5-DBA degraded (Table 1). Washed-cell suspensions taken from the various steady states showed that, with respect to strain, presence of benzoate or steady-state oxygen tensions, no significant changes had occurred in the apparent affinity constant for oxygen (with 2,5-DBA as the sub-

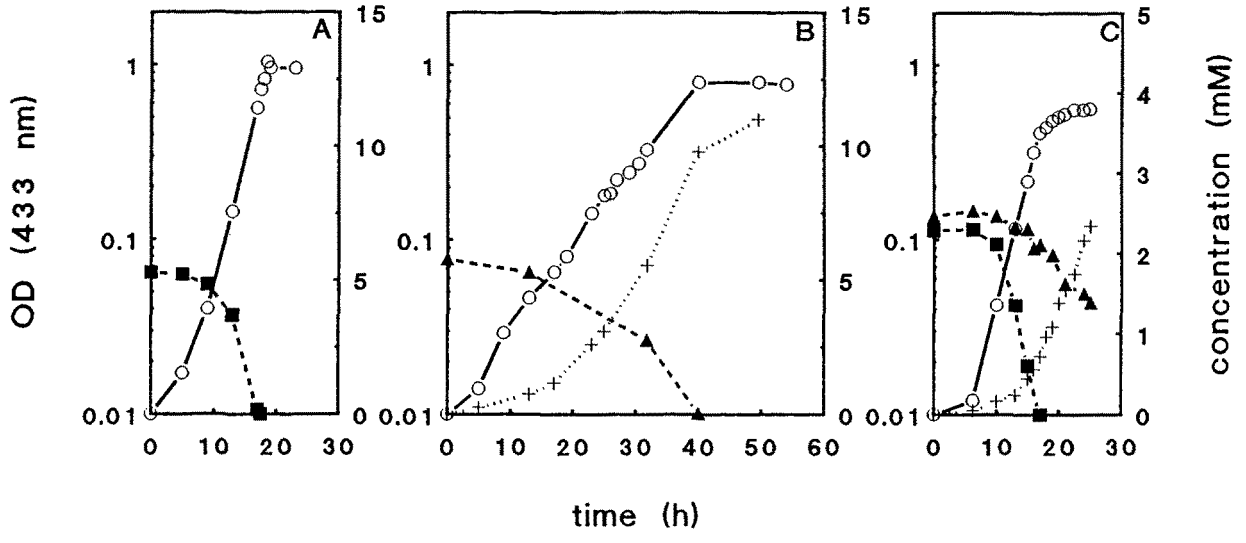

Fig. 2. Growth of *Pseudomonas aeruginosa* JB2-WT in batch culture. A, on 5 mM BA; B, on 5 mM 2,5-DBA; C, on a mixture of BA and 2,5-DBA (2.5 mM each). Cultures were inoculated with log-phase cells from batch cultures grown on 2,5-DBA. (○–○), optical density (E_{433}); (■–■), BA; (▲–▲), 2,5-DBA; (+–+), chloride.

strate). Its value remained approximately 28 μM, a figure comparable to that obtained with batch-cultured cells (Table 2).

Discussion

Growth of the original strain of *Pseudomonas aeruginosa* JB2 under conditions of 2,5-DBA limitation in the chemostat appeared not possible. Growth of this strain JB2-WT over extended periods of time under dual BA plus 2,5-DBA limitation resulted in an adapted variant, JB2-var1, able to grow on 2,5-DBA alone in continuous culture. After prolonged continuous growth of this strain under 2,5-DBA limitation alone, yet another variant was obtained, strain JB2-var2. This strain expressed a decrease in the apparent K_m for respiration of 2,5-DBA (from approx. 0.1 to 0.04 mM). Apparently both strains have become insensitive to the potentially toxic intermediates derived from 2,5-DBA. Possibly, the relative toxicity (Topp et al. 1988) of such as yet undetected intermediates for strain JB2-WT may have decreased thanks to the additional carbon from glucose or benzoate in 2,5-DBA limited chemostats. *Acinetobacter calcoaceticus* strain Bs5 has been shown to be dependent on a readily available energy source such as succinate or pyruvate for the oxidation of chlorinated benzoates. Its failure to achieve steady states in continuous culture in the presence of 3-chlorobenzoate seemed to be related to the build-up of toxic concentrations of chlorocatechols (Reber & Tierbach 1980). The presence of benzoic acid suppressed the degradation of 2,5-DBA in the chemostat as well as in batch culture. Washed cells taken from C-limited continuous cultures fed with 2,5-DBA showed a higher apparent K_m for 2,5-DBA and a lower maximum specific 2,5-DBA respiring capacity when BA was present than when absent in the feed of the chemostat. Moreover, the residual 2,5-DBA concentration in the culture growing on BA plus 2,5-DBA (S_r = 2.5 mM BA plus 2.5 mM 2,5-DBA) was not lower than in the culture growing on 2,5-DBA alone (S_r = 2.5 mM). This contrasts mixed substrate studies such as those of Lendenmann et al. (1992), who measured lowered residual concentrations of the individual growth substrates in continuous cultures of *Escherichia coli* if glucose and galactose were present simultaneously. The results obtained with *Pseudomonas* JB2 in chemostat culture suggest a suppression of the 2,5-DBA degrading pathway during growth with BA as a cosubstrate. This is further supported by the fact that in batch culture, 2,5-DBA was degraded at a high rate only after most of the BA was depleted, even though during the first growth-phase both BA and 2,5-DBA degrading pathways were already fully induced. The suppressive effect of BA is even more apparent at lower oxygen

concentrations. In chemostat cultures of *Pseudomonas aeruginosa* JB2-var1, the variant strain capable of 2,5-DBA limited growth in continuous culture, a steady state ($D = 0.07\ h^{-1}$) with nearly complete consumption of 2,5-DBA (residual concentration 0.22 mM) could be established at an oxygen concentration of 26 μM (approx. 10% air saturation). At this same oxygen concentration in the presence of BA, steady-state cultures of strain JB2-WT could also be obtained though with higher residual 2,5-DBA concentrations (1.43 mM) if compared with growth at elevated oxygen concentrations (120 μM). The influence of BA as a second substrate is demonstrated most elegantly in steady-state cultures of strain JB2-var2. Even though this improved strain is capable of 2,5-DBA limited growth at very low oxygen tensions (11 μM) the presence of BA in such cultures results in significantly increased residual 2,5-DBA concentrations (0.05 $\rightarrow$ 1.27 mM). Perhaps the presence of BA as a second substrate is responsible for steeper oxygen gradients inside the cell due to a higher total flux of respirable organic matter together with the 5–7 times higher respiratory capacity for BA. The resulting lower intracellular oxygen concentration could have prevented the induction of dioxygenases required for 2,5-DBA metabolism. Indeed, it has been reported that the induction of catechol 1,2-dioxygenase in *Pseudomonas putida* is strongly inhibited at lower oxygen concentrations in the culture (Viliesid & Lilly 1992). For the biodegradation of halogenated aromatic compounds in environments with low oxygen concentrations in the presence of more easily degradable substrates, microorganisms require high affinities for oxygen in order to allow complete degradation of chloroaromatics. Strain JB-var2 with improved 2,5-DBA degrading capacities under both high and reduced oxygen concentrations is an example of this. Apparently, even in short-term experiments in the lab, chloroaromatics degrading organisms can adapt to such low environmental substrate concentrations. Therefore it seems likely that enrichments on chlorobenzoates under low-oxygen conditions would yield cultures well adapted to the metabolism of such compounds at depressed oxygen concentrations. The original organism used in the present study has been obtained from air saturated batch cultures (Hickey & Focht 1990). Therefore it is not surprising that 'improved' variants were obtained during prolonged cultivation under low substrate and oxygen concentrations. To fully appreciate the nature and the implications of such adaptations these variants need further characterization at both the physiological and the genetic level.

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