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Induction of the halobenzoate catabolic pathway and cometabolism of *ortho*-chlorobenzoates in *Pseudomonas aeruginosa* 142 grown on glucose-supplemented media

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Abstract

The aerobic cometabolism of *ortho*-substituted chlorobenzoates by *Pseudomonas aeruginosa* strain 142 growing on glucose-supplemented medium was analyzed. The strain, which can use 2-chlorobenzoate (2-CBA) and 2,4-dichlorobenzoate (2,4-DCBA) as sole carbon and energy sources, showed high rates of 2-CBA metabolism in glucose-fed cells. In contrast, 2,4-DCBA was metabolized only after extended incubation of the full grown culture and depletion of glucose. In addition to the *ortho*-dehalogenation (ohb_{142}) genes encoding the α and β subunits of the oxygenase component of a 2-halobenzoate dioxygenase, strain 142 harbours a closely related *ohbABCDFG* gene cluster previously identified in *P. aeruginosa* JB2 (ohb_{JB2}). The genes for the chlorocatechol *ortho*-catabolic pathway were identified and sequenced in this strain, showing a near complete identity with the *clc*ABD operon of the pAC27 plasmid. Relative quantification of mRNA by RT-PCR shows a preferential induction of ohb_{142} by 2-CBA, which is abolished in glucose-grown cultures. The alternate ohb_{JB2} and *clc* genes were expressed preferentially in 2,4-DCBA grown cultures. Only ohb_{JB2} appears to be expressed in the presence of the carbohydrate. Detection of chlorocatechol-1,2-dioxygenase activity in 2,4-DCBA plus glucose grown cultures suggests the presence of an alternate system for the *ortho*-cleavage of chlorobenzoates. The recruitment of elements from two halobenzoate dioxygenase systems with different induction patterns, together with a chlorocatechol degradative pathway not repressed by carbon catabolite, may allow *P. aeruginosa* 142 to cometabolize haloaromatics in carbohydrate grown cultures.

Abbreviations: CBA: chlorobenzoate; CDO: catechol 1,2-dioxygenase; DCBA: dichlorobenzoate; MM: mineral medium; MMG: mineral medium supplemented with glucose

Introduction

Chlorobenzoates are central intermediates in the degradation of polychlorinated biphenyls and benzoate herbicides. A number of organisms can use different chloro- substituted benzoates as sole energy and carbon source ((Fetzner 1998) and references therein). Although *ortho*-substituted chlorobenzoates appear to be particularly recalcitrant to biodegradation, several bacterial strains can complete the degradation of these compounds (Engesser & Schulte 1989; Reineke & Knackmuss 1988). All these organisms can use 2-chlorobenzoate (2-CBA) as sole carbon and energy source, but differences are observed in the range of metabolizable dichlorobenzoates (DCBA). The mechanism proposed for the initial reaction of 2-CBA metabolism in *Pseudomonas aeruginosa* (Romanov & Hausinger 1994), *Burkholderia cepacia* 2CBS (Fetzner et al. 1989; Fetzner et al. 1992) and *P. putida* P111 (Hernandez et al. 1991) involves a halobenzoate 1,2-dioxygenase which would catalyze hydroxylation, dehalogenation and decarboxylation of 2-CBA to yield catechol. Such activ-

ity on *ortho*-dichlorobenzoates would conversely yield chlorocatechols as intermediate products. Chlorocatechols may then be channelled through the modified *ortho*-cleavage pathway to tricarboxylic acid cycle intermediates.

A three-component chlorobenzoate 1,2-dioxygenase has been partially purified from *P. aeruginosa* 142 (Romanov & Hausinger 1994), and the two subunit of the dioxygenase component were cloned and expressed in *E. coli* (Tsoi et al. 1999). Catechol 1,2-dioxygenase (CDOI), chlorocatechol 1,2-dioxygenase (CDOII), and (chloro)muconate cycloisomerase activities also have been reported in this strain (Romanov & Hausinger 1994). *P. aeruginosa* 142 can only use 2-CBA and 2,4-DCBA as growth substrates; however, other dichlorobenzoates, such as 2,5- and 2,6-DCBA, were shown to be transformed *in vitro* (Romanov & Hausinger 1994) or oxidized by resting cells (Tsoi et al. 1999). The inability to mineralize these compounds may be related to the lack of induction of key metabolic reactions, the appearance of non-metabolizable intermediates, or the lack of suitable systems for uptake.

Bacterial growth on halogenated aromatics as sole energy and carbon source is often suboptimal, showing slow growth rates, long adaptation lag periods and low maximum cell densities. Cells using a conventional growth substrate, such as glucose, would be expected to prevent the induction of large regulons for the catabolism of halogenated compounds by carbon catabolite repression (Duetz et al. 1994). However, several examples of co-metabolism have been reported when the medium contains a substrate which can induce the metabolic pathway (Adriaens & Focht 1991; Janke & Fritsche 1985; Kohler et al. 1988). A possible application of co-oxidation is the degradation of non-growth substrates by cultures grown using an alternate carbon source. Cells growing on ethene have been shown to degrade the non-substrates dichloroethene and vinyl chloride (Koziollek et al. 1999). Furthermore, cometabolic degradation may be facilitated when glucose is used for cell growth and regeneration of necessary co-substrates NADH or NADPH (Wang & Loh 1999).

In the study that follows, we have studied the metabolism of representative *ortho*-chlorobenzoates by *P. aeruginosa* strain 142 growing in glucose-supplemented media. The ability to transform different chlorobenzoates, individually and as substrate mixtures, the expression of the halobenzoate dioxygenase and chlorocatechol *ortho*-cleavage operons, and the induction of CDOI and CDOII activities were tested under fast-growing conditions. The results obtained give insight in the induction of the pathways for the metabolism of these substrates.

Materials and methods

Bacterial strains, media and cultural conditions

Cultures of *P. aeruginosa* 142 (Romanov et al. 1993) were grown aerobically at 37 °C either on 50 ml of mineral media (MM) (Hareland et al. 1975) or the same medium containing glucose 10 mM (MMG). Both MM and MMG were supplemented when required with 2 mM (chloro)benzoates. All cultures were started by adding 0.25 ml of a previous culture, grown on MM supplemented with 2-CBA to 100 ml of fresh medium in 500 ml Erlenmeyer flasks. Growth curves were monitored spectrophotometrically at 550 nm.

Analytical procedures

Inorganic chloride was monitored colorimetrically (Bergman & Sanik 1957). The presence of metabolites in culture supernatants was determined by HPLC. Separations were carried out using a C18 reverse-phase column (Supelcosil LC318) on a Smart (Pharmacia) system equipped with peak μ detector. Before injection, 100 μl of supernatant was treated with 10 μl of 5M H_2SO_4 to stop microbial activity and adjusted to pH = 4. The mobile phase was acidified acetonitrile : water 40 : 60 with a flow of 0.5 ml/min. Mono- and dichlorobenzoates were monitored in the eluate at 214 nm and compared to the peaks obtained with standard solutions.

Enzyme assays

Cell extracts were prepared from 100 ml cultures grown to late exponential phase. Cells were washed twice with 0.1 M tris-acetate pH = 8 and resuspended in 1.5 ml of the same buffer supplemented with phenylmethylsulphonylfluoride 50 μg/ml and dithiothreitol 0.3 mM. The cells were sonicated, debris was discarded by centrifugation at 100000 $\times$ *g* for 30 min, and catechol 1,2-dioxygenase activity was monitored in the supernatants as accumulation of *cis,cis*-muconate in the presence 10 mM EDTA (Romanov & Hausinger 1994).

DNA isolation and amplification

Bacterial DNA was obtained following the method described by Wilson (Ausubel et al., 1991) from 200 ml cultures. Polymerase chain reactions (PCR) reactions were carried out in reaction mixtures of 50 μl containing about 100 ng of total DNA. Amplification was carried out using Dynazyme® DNA polymerase following the manufacturer instructions, and a Perkin-Elmer Gene-amp PCR System 2400. The PCR program was composed of 30 cycles of the following profile: 30 s at 94 °C, annealing between 50 °C and 60 °C depending on the melting temperature of the primers in use, and extension at 72 °C for 1 min. The products obtained were further purified by filtration through Chroma-spin-100 columns (Clontech)

DNA sequencing and accession numbers

Both strands of the amplified fragments were sequenced either directly using the same oligonucleotides as primers or after subcloning into a pGEM-T vector (Promega), using Applied Biosystems sequencing kits and 377 automatic sequencer. The sequences were deposited at Genbank, accession number AF 161263.

RNA isolation and reverse transcriptase-PCR

Total RNA was purified from a 1.5 ml-sample of cultures grown to late exponential using a Quiagen™ RNAeasy isolation column. Residual DNA was removed by incubation with 50 units of RNase-free DNase for 30 min at 25 °C, and precipitation of RNA with 3 M LiCl. Pellets were resuspended in 50 μl of 0.1 mM EDTA in DEPC treated water. Total RNA was quantified after staining with SYBRgreenII™ (Molecular Probes) using a Perkin-Elmer LS-50B fluorimeter, and its integrity was checked by electrophoresis in 1.5% agarose gels. Single-tube reverse transcriptase-PCR reactions were carried out using Sigma-Aldrich Enhanced Avian RT-PCR kit. For each transcript to be analyzed dilutions containing 20, 10, 5, 2.5, and 1.25 ng of total RNA were used as templates. RT-PCT reactions using primers specific for 16S rRNA were carried out as positive controls. A sample of 20 ng RNA lacking reverse transcriptase was included as control for DNA contamination. The reaction volume was 50 μl, and contained 25 pmol of each primer. The primers pairs used were: *clcA* 5′TGGATAAACGAGTTGCCGAGG / 5′CGATGATGGTGCTGTTCAGGA (177 bp amplicon), $ohbA_{142}$ 5′GAACACCGATAGTCCGCTGAAC / 5′GGTGAACAGCTCTGGCAACTCT (157 bp amplicon), and $ohbA_{JB2}$ 5′GAACGTTCCGTCATCATGACG / 5′CGCCTTTAAGGCTGTAGTTCCA (157 bp amplicon). Tubes were incubated 45 min at 45 °C for reverse transcriptase extension, followed by denaturation for 3 min at 94 °C. PCR was then performed for 30 cycles under the following conditions: 94 °C 20 s, 55 °C 30 s, 68 °C 30 s, with a final 5 min extension at 68 °C. After separation on agarose 1.5% gels and ethidium bromide staining, a digital image was obtained with a Kodak System 120, and the density of the bands was quantified using the public domain NIH Image program. Only the measurements showing a linear correlation with the amount of RNA loaded in the RT-PCR reaction after 30 cycles were considered. The mean density of reference bands were used to standardize the values obtained.

Results and discussion

Degradation of chlorobenzoates in glucose-supplemented media

The ability of *P. aeruginosa* 142 to metabolize chlorobenzoates in mineral medium supplemented with glucose was tested by monitoring chloride release and substrate disappearance during exponential growth. All cultures were started by dilution of a preinoculum grown on 2-CBA as sole carbon source. Table 1 compares data obtained from different MM-chlorobenzoate grown cultures. *P. aeruginosa* 142 was able to dehalogenate and transform 2-CBA in glucose grown cultures, suggesting that the 2-halobenzoate 1,2-dioxygenase activity is induced in glucose grown cells. As neither 2CBA nor other metabolites could be detected by HPLC in the full grown cultures supernatants (not shown), the relatively low values of chloride released in 2CBA grown cultures may be ascribed to insolubilization of the reaction product or differences in the substrate-uptake/chloride-release rates.

Growth on 2,4-DCBA as sole carbon source was very inefficient: inoculated cultures took, on the average, four days to reach 0.2 OD, and appearance of cell aggregates was common. Degradation of 2,4-DCBA on glucose supplemented media seemed to be more efficient, although a significant amount of substrate remained in the culture supernatants, even following incubation for two days after the growth ceased.

Table 1. Growth and release of chloride by cells cultured on mineral medium supplemented with different chlorobenzoates, each at 2 mM initially.

Carbon source during Growth	Final OD 525 nm	[Cl] (mM)	Remaining substrate (%)
2-CBA	0.8	1.1	ND
2,4-DCBA	0.2	1.0	80
Glucose + 2-CBA	>2	1.5	ND
Glucose + 2,3-DCBA	>2	0.5	ND
Glucose + 2,4-DCBA	>2	1.4	45
Glucose + 2,5-DCBA	>2	ND	100
Glucose + 2,6-DCBA	>2	0.3	ND

ND: not detected.

Growth on glucose supplemented media yielded consistently higher ratios of chloride released vs. initial chlorobenzoate concentration than MM cultures. While transformation in MMG could not be detected for 2,5-DCBA or *meta*-substituted dichlorobenzoates, limited degradation of 2,3- and 2,6-DCBA was observed in glucose supplemented cultures.

The progress of disappearance of chlorobenzoates on MMG was monitored for the substrates that allowed growth on mineral media: 2-CBA, and 2,4 DCBA. As shown in the Figure 1, degradation of 2-chlorobenzoate started in early exponential phase, while a time lag of approximately 5 hours was observed in glucose-free cultures. The chloride accumulation both on MM and MMG correlates with the cultures growth rate. In consequence, dehalogenation in the presence of glucose is faster and the final chloride concentration is higher than in cultures grown on MM, suggesting that the conversion of the chlorobenzoate to (chloro)catechols remains induced under these conditions. A culture of *P. aeruginosa* 142 growing on MM supplemented with 2 mM 2-CBA needs approximately 20 h to reach a chloride concentration of 1 mM in the medium; when grown on MMG under the same conditions, the chloride concentration increases up to 1.2 mM Cl- in 5 h. *P. aeruginosa* strain 142 offers therefore the possibility of using a fast-growing culture to metabolize 2-CBA efficiently.

Transformation of 2,4-DCBA in the presence of glucose (Figure 2) shows a diauxic-like kinetics: slow decrease of substrate during exponential growth, and a sharp decrease after the culture reaches saturation. This may indicate that 2,4-DCBA is transformed only after the glucose supply is depleted.

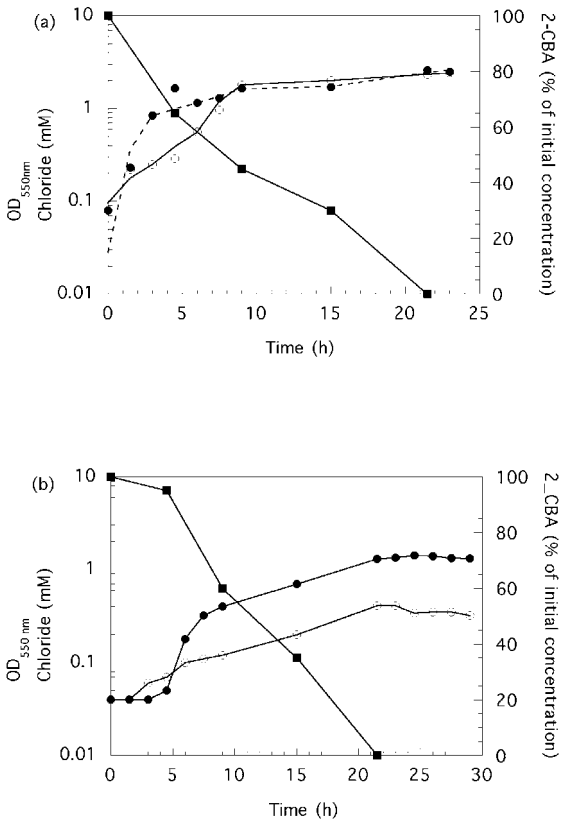

Figure 1. Cell growth, chloride release kinetics and substrate disappearance in MM (a) and MMG (b) cultures supplemented with 2-CBA. Symbols: ○, Optical density; ●, chloride; ■, remaining 2-CBA.

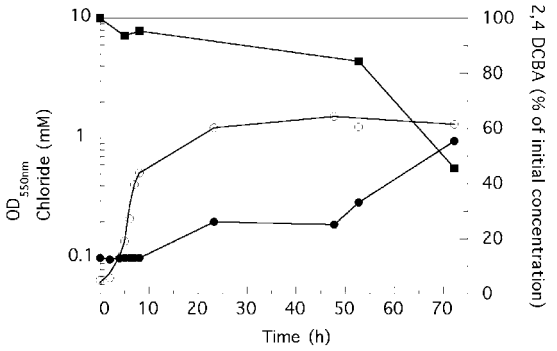

Figure 2. Cell growth, chloride release kinetics and substrate disappearance in MMG cultures supplemented with 2,4-CBA. Symbols: ○, Optical density; ●, chloride; ■, remaining 2,4-DCBA.

Induction of chlorobenzoate metabolic genes

In *P. aeruginosa* JB2 a gene cluster *ohbABCDEFGH* encoding the peptides of a putative three component 2-halobenzoate dioxygenase, ABC-type transport proteins and LysR-type transcription regulators, is located 12.5 kbp downstream of the *clc* operon (Genebank AF164958). The presence of both JB2 *ohbABCDEFG* (ohb_{JB2}, AF087482) and 142 *ohbAB* (ohb_{142}, AF121970) was confirmed in strain 142 by PCR amplification and sequencing of a DNA fragment using primers specific for each gene (not shown). The gene *ohbH*, encoding a putative Lys-R type regulator in JB2, was not found in strain 142. *P. aeruginosa* 142 contains therefore at least two *ohb* operons encoding the α and β subunits of the oxygenase component of three component aromatic dioxygenases closely related, one of which has been shown to be involved in the *ortho*-dehalogenation of 2-CBA (Tsoi et al. 1999).

The induction of the ohb_{142}, *clc*, and ohb_{JB2} operons under different growth conditions was measured by semi-quantitative RT-PCR. As shown in Figure 3, ohb_{142} was induced in 2-CBA- and 2,4-DCBA-grown cultures, while no expression was detected in glucose supplemented cultures, or when benzoate was used as carbon source. Although some expression is detected in 2-CBA, ohb_{JB2} appeared to be induced preferentially by 2,4-DCBA or benzoate. In addition, ohb_{JB2} does not appear to be repressed by glucose. From these results it can be inferred that, when grown on 2CBA, *P. aeruginosa* 142 expresses preferentially the α and β subunits of the oxygenase component encoded by ohb_{142} genes. Although both *ohb* operons are induced by 2,4-DCBA, two observations suggest that this substrate is not metabolized by the dioxygenase components encoded by ohb_{JB2}: (i) *P. aeruginosa* JB2 can not use 2,4-DCBA as sole carbon source and (ii) the inefficient metabolization of 2,4-DCBA by strain 142 in spite of the high expression detected in glucose supplemented medium. The ohb_{142} operon does not include the ferredoxin or reductase genes required to build the three component halobenzoate dioxygenase. As some induction of the ohbJB2 is observed in 2-CBA grown cells, the α and β subunits of the dioxygenase component encoded by ohb_{142} may interact with the ferredoxin and reductase component encoded by ohb_{JB2}. The presence of a complete ohb_{JB2} system in strain 142 should, in theory, allow the metabolization of the same range of chlorobenzoates used by JB2, including 3-CBA, 2,3- and 2,5-DCBA. However, no induction of either *ohb* genes was observed

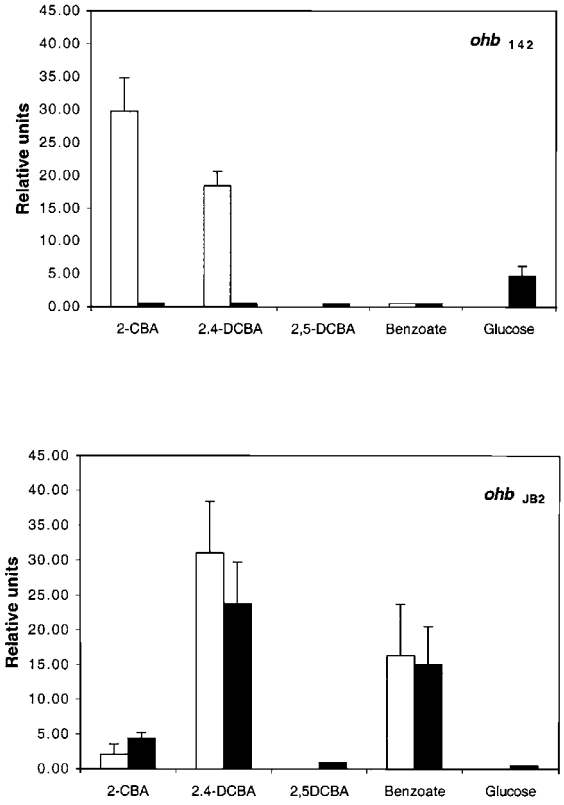

Figure 3. Expression of operons involved in *ortho*-halobenzoate metabolism measured by semi-quantitative RT-PCR. Bars represent the mean density of bands obtained after RT-PCR of total RNA isolated from cultures grown on the indicated substrates, in the absence (light) or the presence (dark) of glucose. Values were standardized using reference bands. Error bars represent the standard deviation from linearity in the RNA:RT-PCR product ratio.

when 2,5-DCBA was added to MMG grown cultures. This indicates that the differences in substrate range between strains 142 and JB2 are probably due to the lack of induction of the *ohb* systems in strain 142 by these compounds. The absence of *ohbH* in 142, a putative regulator gene, may be related to this last possibility.

Chlorocatechol metabolic genes of P. aeruginosa 142

To verify if the genes for the metabolism of chlorocatechols by *ortho*-ring fission were present in this strain, we carried out PCR assays using oligonucleotide primers designed to amplify conserved regions of the pAC27 *clc* operon (Chatterjee & Chakrabarty 1982). Preliminary sequence results (not shown), indicated the presence of a gene highly homologous to *clcA* of plasmid pAC27. To obtain the complete DNA se-

Table 2. Sequence similarities between the *clc*ABCD cluster of strain 142 and related genes

Gene	Strain or plasmid	Amino acid identity
Catechol 1,2 dioxygenase II (*clc*A)	pAC27	98.8
	P. aeruginosa JB2	98.8
	pJP4	59.0
Chloromuconate cycloisomerase (*clc*B)	pAC27	98.9
	P. aeruginosa JB2	99.7
	pJP4	64.1
Dienelactone hydrolase (*clc*D)	pAC27	96.6
	P. aeruginosa JB2	95.3
	pJP4	54.3

Table 3. Changes found in the amino acid sequence of the *P. aeruginosa* 142 putative chlorocatechol *ortho*-ring cleavage gene products, after comparison to the pAC27 plasmid *clcABD* genes.

Enzyme	Sequence change	
	Amino acid	Position
Chlorocatechol 1,2 dioxygenase (ClcA) 260 aa	R → S	107
	L → S	113
	K → T	258
Chloromuconate cycloisomerase (ClcB) (371 aa)	C → V	42
	H → R	115
	P → S	209
	E → G	321
Dienelactone hydrolase (ClcD) 236 aa	H → Y	14
	A → G	65
	Q → R	76
	R → A	79
	K → N	154
	H → R	166
	S → P	210
	R → T	224

quence of *clcA* and the downstream genes, a new set of primers was synthesized and used in PCR reactions. After sequencing of the resulting fragments, the genetic organization of the *clc* cluster of *P. aeruginosa* 142 was determined and found to be similar to that of plasmids pAC27, pJP4, and *P. aeruginosa* JB2 strain (Genebank AF164958). The similarity to the equivalent genes is shown in Table 2, and indicates that the *clc* cluster is nearly identical to that of pAC27. This confirms that strain 142 harbours the genes required for the *ortho*-cleavage of chlorobenzoates. The amino acid changes found after comparison to the pAC27 clcABD genes are compiled in Table 3. The limited extent of changes suggests that the enzyme activities and the genetic regulation of *clc* operon of strain 142 should be equivalent to that of *P. putida* plasmid pAC27. The complete sequence has been deposited in Genbank AF 161263.

Expression of the ortho-cleavage pathway

After oxygenolytic dehalogenation of 2CBA, degradation in strain 142 has been proposed to proceed through the *ortho*-cleavage pathway initiated by a CDOI activity. We have unsuccessfully looked for a *catABC* or *meta*-ring-fission operons in strain 142. However, the high rate of 2-CBA metabolism and detection of CDOI activity shown in Table 4 supports the presence of a *ortho* catechol dioxygenase I pathway in this strain. Benzoate and 2-chlorobenzotate induce a CDOI activity, while 2,4-DCBA induced low levels of both CDOI and CDOII activities. 2,4-DCBA should produce 4-chlorocatechol as intermediate (see Figure 4), which would be channelled through a modified *ortho*-cleavage pathway by a catechol 1,2-dioxygenase II (CDOII) activity. The high activity of CDOI found in MMG agrees with the observed ability for 2-CBA degradation in this medium. Compared to cultures grown on MM, a two to seven-fold increment in CDOI and CDOII activities were recorded in glucose-fed cells. This unexpected result may be related to the existence in this strain of an additional gene regulation network for the *ortho*-cleavage pathway, like quorum- sensing regulators (Swift et al. 1996), which may be triggered by the high population density reached in MMG.

As compared to the *ohb* genes, low levels of *clcABD* mRNA expression (Figure 5) were detected in cultures grown on 2,4-DCBA. In agreement with data collected in *P. putida* (McFall et al. 1997), showing that expression of the *clcABD* operon is repressed by fumarate, a TCA cycle intermediate, no *clc* mRNA was detected in glucose-supplemented cultures. This repression, however, is not detected for CDOII activity in cell extracts. The disagreement between mRNA detection and enzyme activity for the *clc* genes suggest the presence of an alternate gene system yielding CDOII activity, which may remain induced when glucose is present. The cumbersome growth of strain 142 on 2,4-DCBA can not be then completely explained by the low *clcABD* induction. Specificity of the halobenzoate 1,2-dioxygenase for

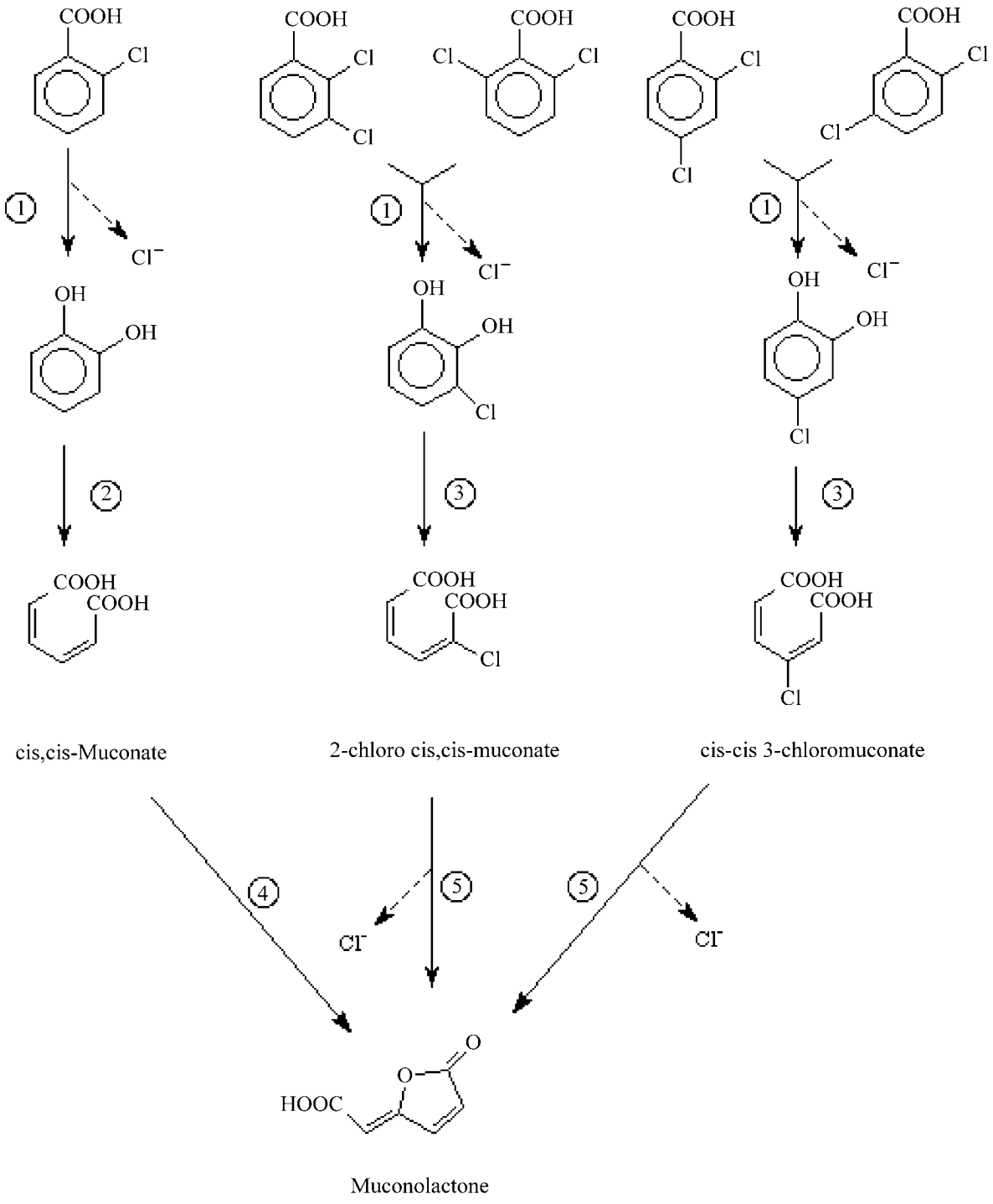

Figure 4. Degradative pathways for the *ortho*-substituted chlorobenzoates. Enzymes: 1: halobenzoate 1,2-dioxygenase; 2: catechol 1,2-dioxygenase I; 3: catechol 1,2-dioxygenase II; 4: muconate cycloisomerase; 5: chloromuconate cycloisomerase.

Table 4. Catechol and chlorocatechol 1,2-dioxygenase activities in cell extracts of *P. aeruginosa* 142

Carbon source during growth	Activity (nmol/min/mg)		
	CDOI	CDOII	
	Catechol	3-chlorocatechol	4-chlorocatechol
2-CBA	8	ND	ND
2,4-DCBA	4	4	1
BA	792	ND	6
Glucose	ND	ND	ND
Glucose + 2-CBA	60	ND	4
Glucose + 2,4-DCBA	20	24	11
Glucose + BA	1,543	ND	160

ND: not detected.

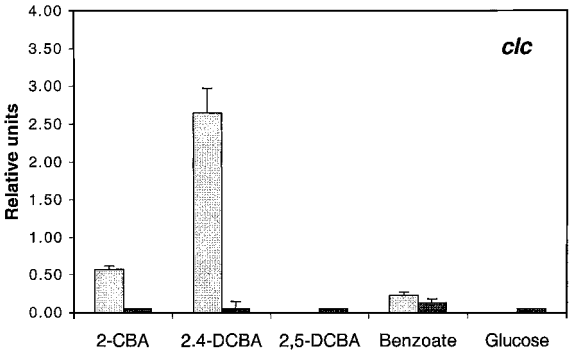

Figure 5. Expression of operons involved in *ortho*-chlorocatechol metabolism measured by semi-quantitative RT-PCR. Values are shown as in Figure 3.

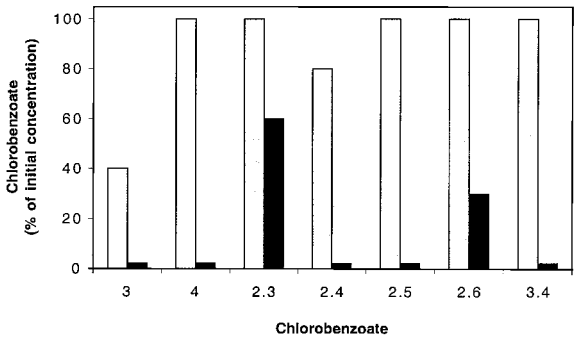

Figure 6. Transformation of 2-CBA (dark columns) and other mono- and di-chlorobenzoates (light columns) by *P. aeruginosa* 142 when both are present in glucose-supplemented growth medium. Substrates were identified from HPLC chromatograms of the culture supernatants and percents calculated from samples collected before inoculation and after completion of cell growth.

other chlorobenzoates (Romanov & Hausinger 1994), or inefficient uptake of 2,4-DCBA may contribute to such behaviour.

Degradation of chlorobenzoate mixtures in glucose supplemented media

A possible application of chlorobenzoate cometabolism is the degradation of non-growth substrates by cultures grown on glucose and induced by 2-CBA. On the other hand, the presence of other chlorobenzoates in the medium may eventually inhibit the transformation of 2-CBA, as it has been shown for the metabolism of *ortho*-chlorobenzoates in *P. putida* P111, which is inhibited when 3,5-DCBA is present (Hernandez et al. 1991). To test this possibility, *P. aeruginosa* 142 cells were grown on MMG supplemented with 2-CBA in the presence of different mono- and di-chlorobenzoates. The extent of the degradation was monitored as disappearance of the substrates from the media. Under these conditions, only the ohb_{JB2} system would be expressed. The results, shown in the Figure 6, indicate that 3-CBA is partially consumed by 2-CBA induced cells, while 2,3- and 2,6-DCBA had an inhibitory effect on 2-CBA metabolism, and 4-CBA, 2,5-DCBA and 3,4-DCBA did not affect the degradation of 2-CBA. Transformation of 3-CBA only in the presence of 2-CBA supports the hypothesis of lack of induction of *ohb* genes in 142 by this substrate. The inhibition observed when 2,3- or 2,6-DCBA are present may be explained by interference with the uptake system or with the Ohb_{JB2} dioxygenase subunits. In spite of the limited success for degradation of halobenzoate mixtures by the wild-type strain 142, future engineering of the intricate network of haloben-

zoate catabolic activities revealed in this work would allow the extension of its co-metabolic ability.

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