

Effects of Dichloroethene Isomers on the Induction and Activity of Butane Monooxygenase in the Alkane-Oxidizing Bacterium “*Pseudomonas butanovora*”

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We examined cooxidation of three different dichloroethenes (1,1-DCE, 1,2-*trans* DCE, and 1,2-*cis* DCE) by butane monooxygenase (BMO) in the butane-utilizing bacterium “*Pseudomonas butanovora*.” Different organic acids were tested as exogenous reductant sources for this process. In addition, we determined if DCEs could serve as surrogate inducers of BMO gene expression. Lactic acid supported greater rates of oxidation of the three DCEs than the other organic acids tested. The impacts of lactic acid-supported DCE oxidation on BMO activity differed among the isomers. In intact cells, 50% of BMO activity was irreversibly lost after consumption of ~20 nmol mg protein⁻¹ of 1,1-DCE and 1,2-*trans* DCE in 0.5 and 5 min, respectively. In contrast, a comparable loss of activity required the oxidation of 120 nmol 1,2-*cis* DCE mg protein⁻¹. Oxidation of similar amounts of each DCE isomer (~20 nmol mg protein⁻¹) produced different negative effects on lactic acid-dependent respiration. Despite 1,1-DCE being consumed 10 times faster than 1,2-*trans* DCE, respiration declined at similar rates, suggesting that the product(s) of oxidation of 1,2-*trans* DCE was more toxic to respiration than 1,1-DCE. Lactate-grown “*P. butanovora*” did not express BMO activity but gained activity after exposure to butane, ethene, 1,2-*cis* DCE, or 1,2-*trans* DCE. The products of BMO activity, ethene oxide and 1-butanol, induced *lacZ* in a reporter strain containing *lacZ* fused to the BMO promoter, whereas butane, ethene, and 1,2-*cis* DCE did not. 1,2-*trans* DCE was unique among the BMO substrates tested in its ability to induce *lacZ* expression.

Chlorinated ethenes (CEs), such as perchloroethene (PCE) and trichloroethene (TCE), are common groundwater contaminants that have been linked to liver and kidney damage and are suspected carcinogens (1). Although the microbially driven process of reductive dechlorination can effectively reduce the concentrations of both PCE and TCE under anaerobic groundwater conditions (5, 23, 27, 28), partially dechlorinated products, such as dichloroethenes (DCEs), often persist and disperse in groundwater plumes (35). Recent evidence has emerged for the existence of bacteria that will grow aerobically on 1,2-*cis* DCE as a sole C source, yet their enrichment is difficult and growth is extremely slow (8). In contrast, it is well documented that hydrocarbon-oxidizing microorganisms are able to degrade DCEs via reductant-driven cooxidative mechanisms (4, 6, 9, 15, 17, 19, 38, 39). Unfortunately, the need for the natural hydrocarbon to serve both as enzyme inducer and source of reductant results in competition between the natural substrate and CEs, and cooxidation efficiency is compromised (6, 11, 21, 24, 25, 37).

In this connection, several studies have shown that a combination of TCE and nonhydrocarbon substrates simultaneously induce the genes for toluene oxygenases and provide reductant for TCE oxidation by some toluene-degrading bacterial strains (26, 30, 36, 41). Conceptually, this observation has some appeal for bioremediation because it implies that oxyge-

nases could be induced by CEs in the absence of a growth-supporting substrate and that cooxidation might be driven by alternative electron donors without interference from hydrocarbon substrates. The potential of DCEs to act as inducers of hydrocarbon-oxidizing systems has rarely been examined, and the results are equivocal. McClay et al. (30) showed that 1,2-*cis* DCE was a poor inducer of toluene oxygenase activity in *Pseudomonas mendocina* KR1, and 1,2-*trans* DCE did not induce at all. Neither compound significantly induced toluene monooxygenase in *Pseudomonas stutzeri* OX1 (33). In contrast, both 1,2-*cis* and 1,2-*trans* DCEs induced suboptimal levels of alkene monooxygenase activity in *Xanthobacter* sp. strain Py2, whereas short-chain-length alkanes and chloroalkanes did not (10). We thought it would be interesting to determine if DCEs induced butane monooxygenase (BMO) in the butane-oxidizing bacterium “*Pseudomonas butanovora*.” Butane-oxidizing bacteria degrade a wide range of unsaturated and saturated aliphatic chlorinated hydrocarbons individually and in complex mixtures (15, 17, 24, 25). Furthermore, because short-chain-length organic acids, such as acetic, propionic, butyric, and lactic acids, are metabolized effectively by butane-utilizing bacteria and are likely to be produced as fermentative end products under conditions where DCEs are produced, we thought it would be worthwhile to examine the efficacy of fatty acids as electron donors for BMO-dependent DCE oxidation. As the project evolved, it became clear that the response of “*P. butanovora*” to different DCE isomers pointed to the potential usefulness of the compounds for interrogating BMO operon regulation that extends beyond the practical implications of the work to DCE bioremediation.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. "*P. butanovora*" (ATCC 43655) is an aerobic gram-negative bacterium that grows on *n*-alkanes (C₂ to C₉). The 16S rRNA gene nucleotide sequence of "*P. butanovora*" indicates that this bacterium is phylogenetically affiliated with the *Rhodocyclus* group, and its closest relatives belong to the genus *Thauera* rather than to the genus *Pseudomonas* (2). No formal attempt has been made to publish an official classification paper at this time. "*P. butanovora*" was grown in 750-ml flasks sealed with butyl rubber stoppers and containing 200 ml of a mineral salts medium consisting of (per liter) 2.7 g (NH₄)₂HPO₄, 4.3 g Na₂HPO₄, 4.2 g KH₂PO₄, 0.5 g MgSO₄ · 7H₂O, 0.06 g CaCl₂ · 2H₂O, and 1 ml trace element solution (16) adjusted to pH 7.1. When butane served as the growth substrate, the gas (99% Airgas; Randor, Pennsylvania) was sterilized by passage through a 0.2- μ m-pore-size filter and supplied as an overpressure of 7% volume/total volume (about 10% headspace) of the gas. When organic acids served as the carbon source for growth, a concentration of 10 mM was routinely used. Cultures were routinely grown at 30°C with constant circular shaking (180 rpm) for 2 days. Once cultures reached an optical density at 600 nm of about 0.7 (stationary phase), cells were harvested by centrifugation (10 min at 6,000 rpm and 10°C) and washed three times in 50 mM NaK phosphate buffer (pH 7.1). Harvested cells, kept on ice as a concentrated cell suspension, remained active for >3 h. Fresh cells were grown and harvested as needed.

Ethene oxidation assay. Ethene-dependent ethene oxide (EtO) production is a convenient measure of BMO activity (16, 34). Vials (10 ml) sealed with butyl rubber stoppers and aluminum crimp caps served as the reaction chambers for this assay. Each vial contained 1 ml of whole-cell suspension (0.35 mg of cellular protein) and the specified electron donor, and 20% (vol/vol) ethene gas was added as overpressure to start the assay. The vials were agitated (150 rpm) at 30°C in a covered water bath shaker. Ethene oxide formation was followed by gas chromatography with a Shimadzu GC-8A equipped with a flame ionization detector and a 120-cm Porapak Q column (Alltech Associates, Inc.). The column temperature was maintained at 120°C, and the injector and detector were maintained at 200°C as previously described (17). Protein concentrations in the cell suspensions were determined using the biuret assay (13) and were 0.35 mg protein per vial.

Cooxidation of DCEs. The cooxidation of DCEs was followed by measuring their disappearance from the headspace by gas chromatography. Abiotic controls, which did not receive "*P. butanovora*," and butane-grown "*P. butanovora*" pretreated with acetylene, a mechanism-based inhibitor of BMO (16), were used to ensure that the disappearance of DCEs was due to BMO activity. Vials (10 ml) containing phosphate buffer (900 μ l) and the specified electron donor were prepared. Aliquots of DCE saturated solutions were injected into vials that had been sealed with Teflon-lined butyl rubber stoppers. DCEs were allowed to equilibrate between the gas and aqueous phases by shaking the vials at 150 rpm and 30°C for 30 min prior to the start of the assay. Assays were initiated by the injection of 100 μ l (0.5 mg protein) of concentrated cell suspension.

Effects of DCE cooxidation on BMO activity. Vials were prepared as described above. Accurate assessment of time-dependent inhibition of BMO required the prompt interruption of the cooxidation reaction. To accomplish this, butane was flushed into the headspace of the vial. Conceptually, this prevented the continued cooxidation of the DCE during the washing procedure by purging DCE out of the vial while providing the natural substrate to compete for the BMO active site. Two 60-ml syringes were used; the first syringe was kept empty with the plunger depressed, and the other syringe was filled with butane. Both syringes were inserted into the vial, and pulling back the plunger of the empty syringe pulled the butane from the second syringe through the vial. The cells were washed three times by centrifugation, resuspended in phosphate buffer, and tested for residual BMO activity using the ethene oxidation assay.

Inhibition of O₂ uptake by DCEs. Lactate-dependent O₂ uptake was followed using a Clark style O₂ electrode in a glass reaction vial (YSI model 5300 biological oxygen monitor; Yellow Springs Co., Yellow Springs, OH). The temperature of the reaction chamber was maintained at 30°C. Phosphate buffer (1.75 ml; 50 mM) was allowed to equilibrate with air for 5 min before the start of each assay, and the reaction chamber was washed 20 times with water and twice with phosphate buffer between assays to remove residual carbon sources and DCEs. The reaction chamber was capped, and additions were made in the following order: butane-grown "*P. butanovora*" cells (0.2 mg protein), sodium lactate (2 mM final concentration), and the appropriate cosubstrate (5 nmol in the electrode chamber). This order of addition allowed the sequential determination of resting-cell O₂ consumption, lactate-dependent O₂ consumption, and the effects of the cosubstrate on O₂ consumption. Controls were treated in the same manner, except that cells were first treated with acetylene to inactivate BMO.

TABLE 1. Influences of exogenous sources of reductant on initial rate of ethene, 1,1-DCE, 1,2-*cis* DCE, and 1,2-*trans* DCE cooxidation in butane-grown "*P. butanovora*"

Reductant source	Initial rate of oxidation ^a (nmol min ⁻¹ mg protein ⁻¹)			
	Ethene	1,1-DCE	1,2- <i>cis</i> DCE	1,2- <i>trans</i> DCE
Buffer	3.6 (1)	4.0 (1.0)	0.9 (1.0)	1.6 (1.0)
Butyrate	13.2 (3.7)	22.0 (5.5)	6.8 (7.6)	2.0 (1.3)
Propionate	3.4 (0.9)	10.0 (2.5)	5.9 (6.6)	0.4 (0.3)
Acetate	17.0 (4.7)	16.4 (4.1)	8.5 (9.4)	3.8 (2.8)
Formate	0.7 (0.2)	3.6 (0.9)	1.4 (1.6)	1.2 (0.7)
Lactate	20.1 (5.6)	28.4 (7.1)	11 (12.2)	4.5 (2.8)

^a Values in parentheses represent the increase (*n*-fold) above the endogenous (buffer) rate.

Induction of BMO by DCEs. "*P. butanovora*" was grown in mineral salts medium containing 10 mM lactate, a repressor of BMO (34). Sealed 10-ml vials containing 900 μ l of medium and various concentrations of the test compounds (10 to 100 μ M) were allowed to reach equilibrium by shaking them at 30°C for 30 min. Aliquots of washed (to remove lactate) uninoculated cells (100 μ l) were injected into the vials and incubated for 4 h. Positive (butane) and negative buffer controls were included. After 4 h, cells were harvested by centrifugation and tested for BMO activity using the ethene oxidation assay described above.

Induction of the *lacZ* reporter strain. A reporter strain of "*P. butanovora*" (*bmoX::lacZ::kan*) was constructed with a bicistronic expression system in which kanamycin resistance is constitutive and the BMO promoter controls β -galactosidase (*lacZ*) expression. The *lacZ* transcriptional fusion provided multiple in-frame transcriptional stop codons immediately upstream of the BMO structural genes. The reporter strain was grown in the same growth medium as the wild-type cells, except that kanamycin sulfate (20 μ g/ml) was added to the medium. Inductions were performed in 10-ml vials with 1 ml of the reporter strain culture diluted with growth medium to an optical density at 600 nm of 0.3. All vials were sealed with butyl rubber stoppers and aluminum crimp caps. Inductions were 2 or 4 h in length and were initiated by the addition of cells. β -Galactosidase activity was determined by adding 2 μ l sodium dodecyl sulfate (10%), 2 μ l β -mercaptoethanol, 4 μ g orthonitrophenyl β -D-galactopyranoside (ONPG), and 2 μ l chloroform. The vials were incubated at 36°C, and the reactions were stopped by the addition of 200 μ l 5% (wt/vol) calcium carbonate. The amount of nitrophenyl product formed was determined colorimetrically and is reported in Miller units (31).

Analytical methods. Analytical-high-performance liquid chromatography grade PCE, TCE, 1,2-*trans* DCE, 1,2-*cis* DCE, and 1,1-DCE were acquired from Sigma-Aldrich (St. Louis, Mo.). PCE, TCE, 1,2-*cis* DCE, 1,2-*trans* DCE, and 1,1-DCE saturated solutions were prepared by agitating two-phase solutions (aqueous/chlorinated ethene) for 24 h on an orbital shaker. The saturated aqueous phase was used to aliquot chlorinated ethenes into the reaction vials. Concentrations of chlorinated ethenes in the aqueous phase were assumed to follow their unitless Henry's constants (14).

RESULTS

BMO-dependent cooxidation of ethenes by different exogenous electron donors. Organic acids supported ethene cooxidation by butane-grown "*P. butanovora*" at different rates, with lactate and acetate being superior to butyrate, while formate and propionate did not increase BMO activity above endogenous background levels (Table 1). 1,2-*trans* DCE was transformed the most slowly of the three DCEs tested, and the superiority of both lactate and acetate over the other electron donors was definitely evident. 1,1-DCE was transformed the most rapidly, and lactate clearly supported the highest rate of oxidation. Furthermore, the organic acids showed differences in their abilities to sustain linear rates of ethene oxidation. For example, when butyrate served as the exogenous electron donor, the linearity of ethene oxidation could not be maintained for greater than 5 min. In contrast, lactate-supported cooxida-

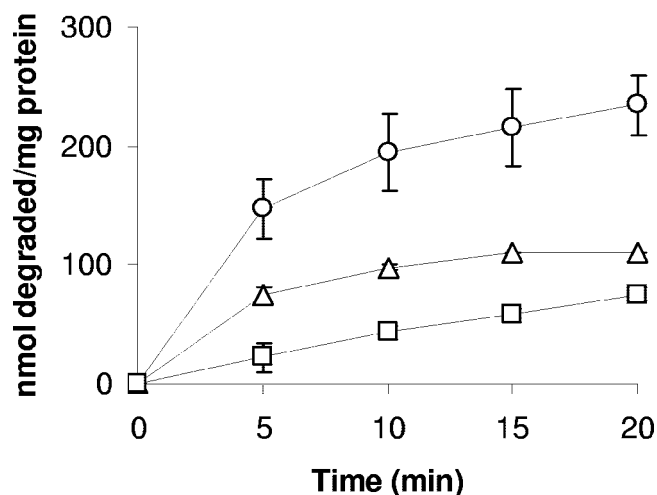


FIG. 1. Time course of 1,2-*cis* DCE (Δ), 1,2-*trans* DCE (\square), and 1,1-DCE (\circ) degradation by butane-grown "*P. butanovora*." The concentration of each DCE at the start of the assay was 25 μ M aqueous phase. Lactate (5 mM) served as the exogenous electron donor. The data points represent the means of three replicates, and the error bars represent standard deviations.

tion remained linear for at least 20 min (data not shown). Lactate was chosen to be the exogenous electron donor for the remainder of the study.

Properties of DCE cooxidation by butane-grown "*P. butanovora*." The kinetics of lactate-driven oxidation varied among the DCEs. The initial rates of oxidation of 1,1-DCE and 1,2-*cis* DCE were highest and also decreased rapidly with time, while the rate of 1,2-*trans* DCE oxidation was lower and remained constant for approximately 15 min (Fig. 1). Distinctly different time-dependent irreversible loss of BMO activity was observed upon exposure of butane-grown "*P. butanovora*" to each of the DCEs (Fig. 2). In the case of 1,1-DCE, one half of BMO activity was irreversibly lost within 0.5 min of exposure and after oxidation of approximately 25 nmol mg protein⁻¹. In the case of 1,2-*trans* DCE, one half of BMO activity was lost after 5 min of incubation, during which a similar amount (20 nmol 1,2-*trans* DCE mg protein⁻¹) was oxidized. The products of 1,2-*trans* DCE and 1,1-DCE must be equally toxic to BMO activity despite being produced at 10-fold-different rates. Decreases in cooxidation activity caused by damage to the BMO enzyme per se versus damage to cellular processes, such as reductant generating potential or loss of membrane integrity, could not be distinguished.

In the case of 1,2-*cis* DCE, however, BMO activity declined to 50% of initial activity after transformation of a much larger amount (120 nmol mg protein⁻¹) over 15 min.

More evidence was obtained for different oxidation-induced cytotoxic effects among the DCEs by measuring lactic-acid-supported O₂ consumption (Fig. 3). Cells were allowed to oxidize an amount of each DCE (25 nmol mg protein⁻¹) that was significantly less than the maximum transformation capacities of these compounds (i.e., 200, 130, and 90 nmol mg protein⁻¹ of 1,1-DCE, 1,2-*cis* DCE, and 1,2-*trans* DCE, respectively). After exposure to either 1,1-DCE or 1,2-*trans* DCE, lactic-acid-dependent O₂ consumption followed similar kinet-

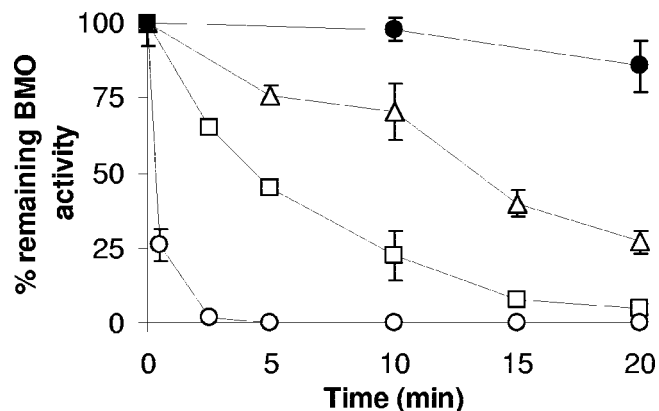


FIG. 2. Time course of the irreversible loss of BMO activity during the consumption of 1,2-*cis* DCE (Δ), 1,2-*trans* DCE (\square), and 1,1-DCE (\circ) by butane-grown "*P. butanovora*." Incubations were performed as described in the legend to Fig. 1, except that 50 μ M DCE was used to ensure that the substrate was not limiting. Cells that did not receive DCEs served as positive controls (\bullet). The data points are the means of three replicates, and the error bars represent standard deviations.

ics of decline despite the fact that these amounts of 1,1-DCE and 1,2-*trans* DCE were consumed in <0.5 min and 5 min, respectively. In contrast, lactic-acid-dependent O₂ consumption was much less sensitive to 1,2-*cis* DCE oxidation. Cells oxidized the aliquot of 1,2-*cis* DCE in about 2.5 min and retained the majority of their lactate-dependent O₂ uptake for about 14 min before it declined abruptly (Fig. 3). These data indicate that (i) lactate-supported cellular respiration is generally less sensitive than BMO activity to the product(s) of 1,1-DCE oxidation; (ii) despite being produced at a lower rate, the product(s) of 1,2-*trans* DCE oxidation is a potent inhibitor of both cellular respiration and BMO activity; and (iii) given a long enough incubation, a small amount of the product(s) of 1,2-*cis* DCE oxidation is toxic to lactic-acid-driven cellular respiration, while BMO activity is less sensitive. In all three cases, cell viability had declined by >90% (as measured by dilution plating onto R2A agar) after 15 min of incubation, indicating

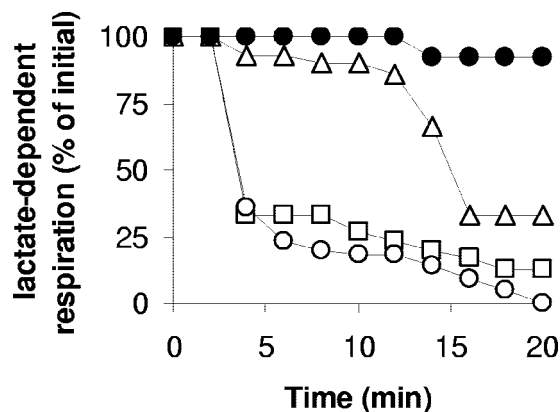


FIG. 3. Time course of lactate-dependent O₂ respiration by butane-grown "*P. butanovora*" during the consumption of 25 μ M of 1,2-*cis* DCE (Δ), 1,2-*trans* DCE (\square), and 1,1-DCE (\circ). The values are expressed as percentages of the initial rate of respiration. Cells treated with acetylene to inactivate BMO served as positive controls (\bullet).

TABLE 2. Induction of BMO activity in wild-type "*P. butanovora*" and β -galactosidase activity in the *lacZ*-BMO fusion in response to various substrates and products of BMO

Compound	Concn (μ M)	BMO activity in wild type (% of butane control)	LacZ activity in reporter strain (Miller units) ^c
Butane	50	100	0
1-Butanol	50	>10 ^b	263 \pm 68
Butyrate	50	0	0
Ethylene	517 ^a	24 ^c	0
Ethylene oxide	8,000 ^a	ND ^d	89 \pm 13
PCE	50	0	0
TCE	50	0	0
1,2- <i>trans</i> DCE	50	47	84 \pm 36
1,2- <i>cis</i> DCE	50	25	0
1,1-DCE	50	0	0

^a Gas added as 10% headspace.

^b 1-Butanol is consumed during the 4-h incubation, and the extent of induction of BMO relative to butane could not be accurately determined in wild-type cells.

^c Ethene oxide accumulation was measured during a 4-h incubation of lactate-grown cells in the presence of ethene.

^d ND, not determined. Ethene oxide, in the absence of ethene, inhibits BMO activity in wild-type cells, and induction of BMO could not be accurately quantified in wild-type cells.

^e Results indicate the increase in β -galactosidase above the level of the buffer control. Background levels of β -galactosidase were typically about 50 Miller units.

that oxidation of an amount of each DCE much lower than their respective transformation capacities was sufficient to incapacitate cell division by the majority of cells (data not shown).

Induction of BMO activity in wild-type "*P. butanovora*" and of LacZ expression in a reporter strain by CEs. 1,2-*trans* DCE and 1,2-*cis* DCE induced 47 and 25%, respectively, of the BMO activity of butane-exposed cells over a 4-h incubation (Table 2). In contrast, neither PCE, TCE, nor 1,1-DCE induced measurable expression of BMO activity. A minimum concentration (20 μ M) of DCE was required to induce the BMO activity, which could not be induced further by increasing the DCE concentration. In the case of the *lacZ* reporter strain, neither butane, ethene, nor most chlorinated substrates, including 1,2-*cis* DCE, was able to induce *lacZ* expression. In contrast, the stable products of BMO activity, 1-butanol and ethene oxide, effectively induced *lacZ* expression (Table 2). 1,2-*trans* DCE was the only substrate that induced *lacZ* expression in the reporter strain. Increases in *lacZ* activity occurred more rapidly in response to 1-butanol than to 1,2-*trans* DCE (Fig. 4). However, over a 4-h incubation, 75 to 100 μ M 1,2-*trans* DCE induced the same amount of *lacZ* activity in the reporter strain as did the same concentrations of 1-butanol.

DISCUSSION

Many studies have been carried out over the past 15 years on the cooxidation of CEs by hydrocarbon-oxidizing bacteria, and the subject has been thoroughly investigated and reviewed (4, 12, 29, 35). Nonetheless, this study uncovered some novel findings, including the unexpected superiority of lactic acid metabolism to supply reductant to BMO, and it illustrated the potential of DCEs to uncover new insights into the regulation of the BMO operon.

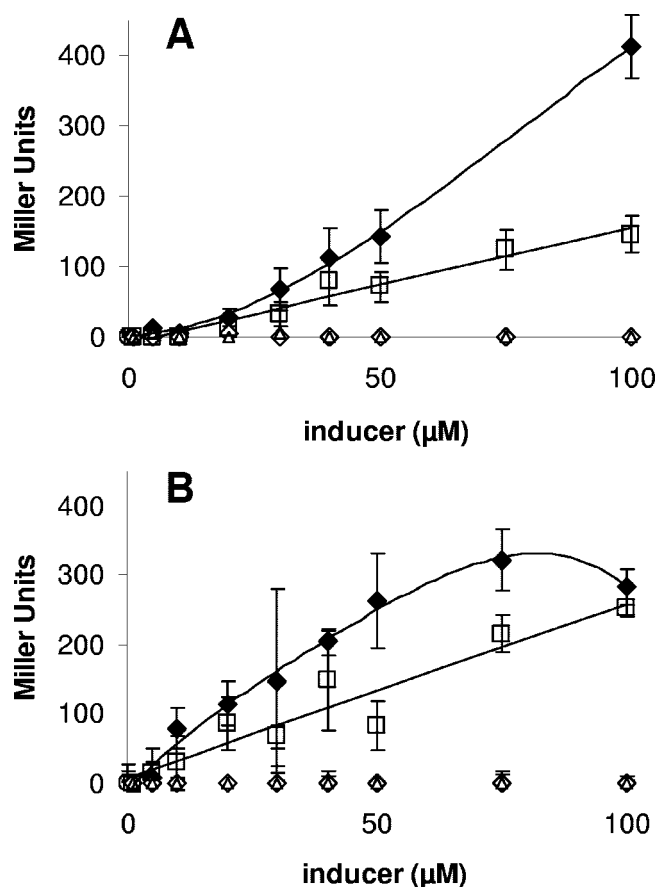


FIG. 4. Induction response of *lacZ* in the reporter strain of "*P. butanovora*" to increasing concentrations of 1-butanol (\blacklozenge), 1,2-*cis* DCE (\triangle), 1,2-*trans* DCE (\square), and 1,1-DCE (\circ). β -Galactosidase (*lacZ*) activities were determined at time intervals of 2 h (A) and 4 h (B) as described in Materials and Methods. The data points are the means of three replicates, and the error bars represent standard deviations.

The use of exogenous electron donors has allowed researchers to examine the substrate range and kinetic parameters of cooxidation while avoiding the competitive effects that occur when natural substrates are provided as electron donors (6, 7, 16, 17, 18). For example, formic acid has been used widely as an electron donor in whole-cell kinetic studies of CE cooxidation by methanotrophic bacteria (6, 7, 38, 39), and by analogy, initial studies carried out on butane-oxidizing bacteria have routinely used butyric acid as an electron donor (16, 17). Nonetheless, our data show that while various organic acids supported BMO activity in "*P. butanovora*," differences exist in the maximum rates obtained. Lactic acid consistently supported greater rates of oxidation than did the other organic acids, and the choice of butyric acid as an electron donor might have compromised interpretation of previously published data from our own laboratory that indicated 1,2-*trans* DCE was not cooxidized by butane-grown "*P. butanovora*" (17).

Although it remains unclear why lactic acid is a superior reductant for driving cooxidation in "*P. butanovora*," several possibilities should be considered. First, enzymes involved in generating reductant from butyric acid might be extremely

sensitive to toxic products of the cooxidation process. In this context, it is interesting that pyruvate, a known antioxidant and the first product of lactic acid metabolism, offset some of the damage that occurred during TCE cooxidation in *Burkholderia cepacia* G4 (41). Second, it is possible that downstream products of alkane oxidation (alcohols, aldehydes, and fatty acids) are structurally similar enough to the natural substrates to make them inhibitors of BMO. Both a bacterial aromatic oxygenase and a peroxidase were irreversibly inactivated either during the catalytic oxidation of phenylpropionaldehyde and phenylbutyraldehyde (32) or by aliphatic fatty acids in general (20). If a similar phenomenon occurs in BMO, it would provide a mechanism to explain why the aliphatic fatty acids, in particular, did not sustain BMO activity.

Finally, it was interesting that relative to lactic acid, propionate did not support BMO-dependent oxidation activities in butane-grown cells as well as it did in propane-grown cells (D. M. Doughty, D. J. Arp, P. J. Bottomley, unpublished observations). Although alkane substrates, such as butane and propane, appear to be oxidized to their respective aliphatic acids by the same enzyme (3), different downstream pathways are probably involved in metabolism of these fatty acids and will influence their efficacy as electron donors for cooxidation by BMO. Further studies are under way to examine the roles of "downstream" propionate and butyrate metabolism in supporting and regulating butane- and propane-oxidizing activities in "*P. butanovora*."

Although methane monooxygenase (MMO) has been shown to oxidize the different isomeric forms of DCE (6, 22, 38, 39), our observations indicate that BMO responded differently to the oxidation of DCEs than did MMO. For example, 1,2-*trans* DCE was oxidized at a lower rate by BMO than either 1,1-DCE or 1,2-*cis* DCE, resulting in a much smaller amount of 1,2-*trans* DCE turnover being required to inactivate BMO than 1,2-*cis* DCE. These data contrast with an earlier report from studies carried out on *Methylosinus trichosporium* OB3b, in which 1,2-*cis* DCE oxidation inactivated MMO and reduced cell viability more rapidly than did oxidation of 1,2-*trans* DCE (39). The different sensitivities of *M. trichosporium* OB3b to DCEs was attributed to differential activity of MMO toward the epoxides of the *cis* and *trans* DCEs. Further studies are required to compare in more detail the effects of 1,2-*trans* DCE, 1,2-*cis* DCE, and their epoxides on BMO and MMO.

We also obtained evidence for the potential of 1,2-DCE isomers as tools for dissecting BMO gene regulation. Although it was shown previously that butane, 1-butanol, and butyraldehyde are positive effectors of BMO transcription in "*P. butanovora*" (34), it was not possible to unequivocally separate substrate from product induction. The same problem was encountered with *Xanthobacter* sp. strain Py2, in which both 1,2-*cis* and 1,2-*trans* DCEs partially induced ethene monooxygenase activity (10). The lack of butane-dependent induction of *lacZ* in the reporter strain of "*P. butanovora*," coupled with the ability of 1-butanol to induce *lacZ*, implies that BMO gene expression is up regulated in response to the products of alkane oxidation. This idea is consistent with the fact that ethene oxide is an inducer of *lacZ* while ethene is not and that 1,2-*cis* DCE is an inducer of the wild type but not of the *lacZ* reporter strain. Our data illustrated, however, that 1,2-*trans* DCE behaved uniquely by inducing as much *lacZ* expression as the

natural product, 1-butanol. At this time, however, we cannot discount the possibility that induction by 1,2-*trans* DCE represents another undefined form of gene regulation, as was suggested for toluene monooxygenase induction by PCE and TCE in *P. stutzeri* OX1 (33). Further work on the regulation of the BMO operon by substrates and products of BMO activity is under way in our laboratory.

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