Growth medium composition-determined regulatory mechanisms are superimposed on CatR-mediated transcription from the pheBA and catBCA promoters in Pseudomonas putida

Andres Tover, Eve-Ly Ojangu and Maia Kivisaar

Expression of the phenol degradation pathway in Pseudomonas putida strain PaWB85 requires coordinated transcription of the plasmid-borne pheBA operon encoding catechol 1,2-dioxygenase and phenol monooxygenase, respectively, and the chromosomally encoded catechol degradation catBCA operon. Transcriptional activation from the pheBA and catBCA promoters is regulated by CatR and the catechol degradation pathway intermediate cis,cis-muconate. Here it is shown that physiological control mechanisms are superimposed on this regulatory system. Transcriptional activation from the pheBA and catBCA promoters is growth-phase-regulated in P. putida cells grown on rich medium (LB medium). CatR-mediated transcription from these promoters is silenced on rich medium until the transition from exponential to stationary phase. A slight positive effect (threefold) of stationary-phase-specific sigma factor σS on transcription from the pheBA promoter was observed. Expression of the catBCA promoter was not influenced by the activity of this sigma factor. In contrast to rich growth medium, transcription from the pheBA and catBCA promoters in minimal medium containing a mixture of glucose and sodium benzoate was rapidly induced in exponential culture. It was shown that the presence of amino acids in the culture medium causes exponential silencing of the pheBA and catBCA promoters. The possibility that a hypothetical repressor protein could be involved in physiological control of transcription from the pheBA and catBCA promoters is discussed.

Keywords: pheBA and catBCA operons, exponential silencing of transcription

INTRODUCTION

Expression of catabolic pathways in Pseudomonas is inhibited by a number of growth conditions that adjust the activity of specific promoters to a given metabolic and physiological status (Cases & de Lorenzo, 1998). The best studied examples of growth-condition-dependent expression of aromatic compound degradation genes in soil pseudomonads are catabolic operons of TOL plasmid pWW0 (Cases & de Lorenzo, 2000; Marques et al., 1994, 1999; Ramos et al., 1997 and references cited therein) and dimethylphenol degradation genes, dmp, from megaplasmid pVI150 (Sze & Shingler, 1999). The strong repressive effect of LB medium or amino acids has been observed for the promoters of these operons (Cases et al., 1996; de Lorenzo et al., 1993; Sze & Shingler, 1999; Sze et al., 1996). The physiological down-regulation of the Pu promoter of TOL plasmids is a complex process controlled by two or more independent mechanisms (Cases & de Lorenzo, 2000). For example, PtsN-mediated carbon source inhibition (Cases et al., 1999) and the control of the activity of σ^{34} (Cases et al., 1996) are involved in regulation of transcription from Pu. Promoter Po of the dmp operon drives the expression of the dimethylphenol degradation pathway very similarly to that of Pu for the upper pathway of TOL plasmids (Sze et al., 1996). The stimulating effect of (p)ppGpp on transcriptional activation was recently demonstrated for Po (Carmona et al., 2000). At the same time, this signal molecule, which triggers the stringent response to

Abbreviations: CCM, cis,cis-muconate; β-Gal, β-galactosidase.
amino acid starvation, had only a moderate effect on the activity of the very similar promoter Pu (Carmona et al., 2000).

In Pseudomonas species, phenolic compounds are transformed by different enzymes to central intermediates, such as protocatechuate and (substituted) catechols (Harayama & Timmis, 1989). Typically, unsubstituted compounds, such as benzoate, are metabolized by an ortho-cleavage pathway (Fig. 1). The genes for benzoate metabolism, including ortho-pathway genes, are chromosomally encoded in P. putida (Harwood & Parales, 1996). The catBCA operon encodes three enzymes of the ortho-pathway required for benzoate catabolism, namely muconate lactonizing enzyme I, muconolactone isomerase and catechol 1,2-dioxogenase, respectively (Houghton et al., 1995). The induction of this operon, which is σ^A-independent, requires a LysR family transcriptional activator, CatR, and an inducer molecule, cis,cis-muconate (CCM), an intermediate of the ortho-pathway (Rothmel et al., 1990, 1991). The pheB and pheA genes originating from plasmid DNA of Pseudomonas sp. EST1001 encode catechol 1,2-dioxogenase and phenol monooxygenase, respectively (Kivisaar et al., 1990). When the pheBA operon is introduced into P. putida, the bacteria acquire the ability to degrade phenol (Kivisaar et al., 1991, 1990). The pheBA promoter resembles the catBCA promoter and is also activated by CatR (Kasak et al., 1993; Parsek et al., 1995). Comparative studies of the interaction of CatR with the promoters of the pheBA and catBCA operons have revealed that the CatR-mediated activation mechanism is well conserved, despite the different origins of these operons (Parsek et al., 1995; Tover et al., 2000).

In this study we have investigated (i) how the physiological status of the cells controls expression of the pheBA and catBCA promoters in P. putida and (ii) the similarity of these control mechanisms on transcription from these promoters. We show that transcription from the pheBA and catBCA promoters is strongly influenced by the composition of the growth medium (rich versus minimal medium). The presence of amino acids in the growth medium is one component that causes exponential silencing of both the pheBA and the catBCA promoters.

**METHODS**

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. Escherichia coli TG1 (Carter et al., 1985) was used for DNA cloning procedures. E. coli was incubated at 37 °C and P. putida at 30 °C. E. coli was transformed with plasmid DNA as described by Hanahan (1983). P. putida was electrophoresed according to the protocol described by Sharma & Schimke (1996). Antibiotics were added at the following final concentrations: ampicillin at 100 μg ml^{-1} for E. coli; carbenicillin at 1500 μg ml^{-1} and kanamycin at 50 μg ml^{-1} for P. putida.

**Growth conditions and β-galactosidase (β-Gal) assay.** To investigate the role of rich medium on transcription from the pheBA and catBCA promoters, P. putida cells were grown in LB medium (Miller, 1992). Cells were grown overnight in LB medium and diluted into fresh medium to obtain an OD_{600} of 0.02. After 2 h cultivation sodium benzoate was added at a final concentration of 2.5 mM to induce the transcription of the pheBA–lacZ and catBCA–lacZ transcriptional fusions. The inducer molecule, CCM, required for CatR-dependent transcriptional activation is an intermediate of the benzoate degradation pathway. Because P. putida uses sodium benzoate as a carbon source, this compound was added again after 5

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**Fig. 1.** (a) The β-ketoadipate pathway by which phenol and benzoate are converted to catechol and to the common metabolic intermediates succinate and acetyl-CoA. (b) Schematic representation of the promoter region of the pheBA and catBCA operons. The bracketed sequences represent nucleotides protected by CatR from cleavage by DNaseI (Chugani et al., 1997; Parsek et al., 1995). The protected regions are designated as RBS (recognition binding sites) and ABS (activation binding sites). Bold italic type designates the T and A residues of the conserved T–N_{11}–A sequence of the LysR family regulatory proteins binding motif of the RBS. The catBCA promoter has a G instead of the conserved T. The nucleotides of the interrupted inverted repeat within the RBS are underlined. The nucleotides of the −35 and −10 hexamers of the promoters are in bold type and boxed. The transcriptional start sites are denoted by asterisks.
and 12 h cultivation (at a final concentration of 5 mM) to
maintain the source of the inducer in the growth medium.
Samples for the β-Gal assay were taken from cultures
grown for 5, 12 and 24 h.

To investigate the regulation of transcription from the
pheBA and catBCA promoters in minimal medium, P. putida
cells were grown overnight in MOPS medium (Neidhardt et al.,
1974) either with 0.5%CAA or amino acids at a final
concentration of 200 ng each amino acid ml⁻¹. The overnight
bacterial culture was diluted with fresh medium (1:20, v/v)
grown to exponential phase and then diluted again in minimal
medium containing 0.2% glucose. The OD₆₀₀ of the diluted
culture was 0.1. Sodium benzoate was added at a final
concentration of 10 mM at the beginning of the experiment
and 1 ml samples were taken at different time points during
growth to assay the β-Gal activity in cell suspensions.

To investigate the possible effect of overexpression of the
CatR protein on the transcription from the pheBA and catBCA
promoters, the culture was grown overnight in LB medium
and thereafter diluted into fresh LB medium as indicated above.
To induce the expression of CatR, IPTG (at a final
concentration of 0.5 mM) was added to the growth medium.
When the culture reached the exponential growth phase,
it was diluted again into fresh LB medium containing 0.5 mM
IPTG. At this step, 5 mM sodium benzoate was added for the
induction of transcription from the pheBA and catBCA
promoters.

A β-Gal assay with the cell lysates was carried out as specified
by Miller (1992). In all cases at least three independent
measurements were made. Protein concentration in crude
lysates was measured by the method of Bradford (1976). β-Gal
measurement in cell suspensions was performed by
modification of the standard protocol of Miller (1992). The
amount of Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄,
10 mM KCl, 10 mM MgSO₄ and 50 mM β-mercaptoethanol) in
a test tube was 16 ml. ONGP (2-nitrophenyl-β-D-galacto-
pyranoside; 400 µl of a 50 mg ml⁻¹ solution), 100 µl 0.002%
SDS and 100 µl chloroform were added into the reaction
mixture. Finally, bacteria were added into the test tube and
mixed well. The β-Gal reaction was stopped by the addition of
1 ml 1 M Na₂CO₃. Data of at least three independent
experiments are presented in all figures.

Cloning procedures. Construction of the pheBA
promoter–lacZ transcriptional fusion (plasmid pZ-pheBA)
was described previously (Tover et al., 2000). For the cloning
of the catBCA promoter–lacZ promoter region from the P. putida
chromosome was amplified by using oligonucleotides cat35
(5'-GGCGTCG-CAGCCGGGCGCC3') and AFG62 (5'-AGCGCGCGGG-
CTCGAGACGCTG/PstI). CAGAGC3'), complementary to
the upstream and downstream regions of the catBCA
promoter, respectively. The PCR amplification product
was cleaved with PstI and cloned into pBluescript SK (+) cleaved
with PstI and EcoRV. The sequence of the catBCA promoter
was verified by DNA sequencing. Subsequently, the catBCA

Table 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or construction</th>
<th>Source of reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>supE hsdD5 thi (lac–proAB) F' (traD36 proAB’ lacZΔM15)</td>
<td>Carter et al. (1985)</td>
</tr>
<tr>
<td>S17-1 aprI</td>
<td>Tp’ Sm’ recA thi pro (r− m−) RP4::2 Tc::Mu::Km Tn7 aprI</td>
<td>Miller &amp; Mekalanos (1988)</td>
</tr>
<tr>
<td>C118 aprI</td>
<td>Δ ara–leu araD Δ lacX74 galE galK phoA20 thi-1 rpsE rpoB argE (Am) recA1 aprI phage lysogen</td>
<td>Herrero et al. (1990)</td>
</tr>
<tr>
<td>P. putida</td>
<td></td>
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</tr>
<tr>
<td>PaW85</td>
<td>Tn4652</td>
<td>Bayley et al. (1977)</td>
</tr>
<tr>
<td>PKS34</td>
<td>Tn4652 rpoS::Km'</td>
<td>Ojangu et al. (2000)</td>
</tr>
<tr>
<td>PaW85 CatR⁺</td>
<td>PaW85 catR gene under control of Ptac promoter and lacI° repressor, Km'</td>
<td>This study</td>
</tr>
<tr>
<td>Plasmids</td>
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<tr>
<td>pBluescript SK(+)</td>
<td>Cloning vector (Ap')</td>
<td>Stratagene</td>
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<tr>
<td>pZ-pheBA</td>
<td>pheBA promoter cloned into pKTlacZ</td>
<td>This study</td>
</tr>
<tr>
<td>pZ-catBCA</td>
<td>catBCA promoter cloned into pKTlacZ</td>
<td>This study</td>
</tr>
<tr>
<td>pUTmini-Tn5 Km2</td>
<td>Delivery plasmid for mini Tn5 Km2</td>
<td>de Lorenzo et al. (1993)</td>
</tr>
<tr>
<td>pUC18Not</td>
<td>pUC18 with NosI restriction sites in multicloning region (Ap')</td>
<td>Herrero et al. (1990)</td>
</tr>
<tr>
<td>pBRlacIrac</td>
<td>Ptac promoter and lacI° repressor in 2.2 kb NruI–EcoRI fragment from plasmid pMMB208 cloned into EcoRV/EcoRI-cleaved pBR322</td>
<td>Ojangu et al. (2000)</td>
</tr>
<tr>
<td>pKZAHf</td>
<td>pKR1300 derivative containing catR gene</td>
<td>Rothmel et al. (1990)</td>
</tr>
<tr>
<td>pBRlacIrac-catR</td>
<td>catR in 1 kb EcoRI–HindIII fragment cloned into pBRlacIrac to obtain lacI°-P-tac-catR</td>
<td>This study</td>
</tr>
<tr>
<td>pUCP tacR</td>
<td>lacI°-P-tacR expression cassette cloned into pUC18Not</td>
<td>This study</td>
</tr>
<tr>
<td>pUT-lacIrac-catR</td>
<td>lacI°-P-tacR expression cassette cloned from pUCP tacR into pUT-miniTn5 Km2</td>
<td>This study</td>
</tr>
<tr>
<td>pV1363</td>
<td>relA gene of E. coli overexpressed from P BAD</td>
<td>Sze &amp; Schingler (1999)</td>
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promoter was inserted using BamHI- and XhoI-generated ends into the promoter–probe vector pKTlacZ to obtain plasmid pZ-catBCA. For the construction of *P. putida* CatR overexpression strain PaWCatR\(^{+}\), the catR gene was cloned from plasmid pKR\(^{+}\)HRAF (Rothmel *et al.*, 1991) by using HindIII- and EcoRI-generated ends to the vector pBRlacItac (Ojangu *et al.*, 2000) cleaved with same enzymes (pBRlacItac-catR in Table 1). The CatR expression cassette lac\(^{+}\)-Ptac-catR was inserted into pUC18Not (Herrero *et al.*, 1990) using EcoRI- and BamHI-generated ends. Thereafter, the lac\(^{+}\)-Ptac-catR sequence was inserted into the NotI site of pUTmini-Tn5 Km2 (de Lorenzo *et al.*, 1990) and pUTlacItac-catR was selected in *E. coli* C118 zipr (Herrero *et al.*, 1990). The lac\(^{+}\)-Ptac-catR expression cassette was inserted into the chromosome of *P. putida* strain PaW85 (Bayley *et al.*, 1977) by the method of random insertion using *E. coli* S17-1 zipr (Miller & Mekalanos, 1988) as donor strain. *P. putida* PaW85CatR\(^{+}\) was selected at 30 °C on glucose/kanamycin plates. The expression of CatR in PaW85CatR\(^{+}\) was verified by Western blot analysis using polyclonal antibodies against *P. putida* CatR protein.

**RESULTS AND DISCUSSION**

**Growth-phase-dependent activation of transcription of the pheBA and catBCA operons**

The promoters of the pheBA and catBCA operons were cloned into plasmid pKTlacZ carrying the lacZ reporter gene and \(\beta\)-Gal activity was measured to examine transcription from these promoters. *P. putida* PaW85 cells containing lacZ transcriptional fusion plasmids pZ-pheBA and pZ-catBCA were grown in rich medium (LB) to the late stationary phase. \(\beta\)-Gal activity was measured in cells sampled from different time points during culture (Fig. 2). Sodium benzoate was always added into the growth medium to induce transcription from the pheBA and catBCA promoters. Results presented in Fig. 2 show that transcription from the pheBA and catBCA promoters remained at a low level during the first 9 h of growth. The level of expression of \(\beta\)-Gal activity was remarkably elevated in stationary-phase cells and it remained high in stationary-phase cultures during the 24 h period of the study. This indicated that the pheBA and catBCA promoters are stationary-phase-inducible and down-regulated during exponential growth.

**Transcription from the pheBA and catBCA promoters in a \(\sigma^{5}\)-deficient background**

Use of an *in vitro* transcription assay has demonstrated that the only requirements for transcription from the pheBA and catBCA promoters are purified CatR, CCM and RNA polymerase-\(\sigma^{5}\) holoenzyme (Ez\(^{5}\)) (Chugani *et al.*, 1997). Stationary-phase-specific sigma factor \(\sigma^{5}\), encoded by rpoS, activates transcription of several genes in stationary phase or otherwise stressed bacteria (Hengge-Aronis, 1999). Analysis of \(\sigma^{5}\)- and \(\sigma^{5}\)-promoter specificity has revealed that Ez\(^{5}\) and Ez\(^{5}\) recognize many promoter sequences equally well *in vitro*, but differentially control genes *in vivo* (reviewed by Hengge-Aronis, 2000). The exponential silencing of transcription from the pheBA and catBCA promoters gave us the idea to investigate the role of \(\sigma^{5}\) on transcription from these promoters.

We have previously constructed a *P. putida* \(\sigma^{5}\)-deficient strain, PKS54, that is a derivative of the wild-type strain PaW85 (Ojangu *et al.*, 2000). Plasmids pZ-pheBA and pZ-catBCA, containing the pheBA–lacZ and catBCA–lacZ transcriptional fusions, respectively, were introduced into the rpoS-deficient strain, PKS54, and into the wild-type strain, PaW85. The level of expression of \(\beta\)-Gal activity measured in exponential- and stationary-phase cells of PKS54 was compared with that estimated in PaW85. The results presented in Fig. 2 show that in the case of the pheBA promoter, \(\beta\)-Gal activity remained approximately threefold lower in the \(\sigma^{5}\)-deficient strain than in the wild-type strain in stationary-phase cultures (Fig. 2a). At the same time, lack of expression of \(\sigma^{5}\) in *P. putida* cells did not affect transcription from the catBCA promoter (Fig. 2b). The sequence of the −10 region of the catBCA promoter (CAATCCT) shows more similarity to the consensus CTATACT proposed for the promoters recognized by Ez\(^{5}\) than that of the pheBA promoter (CTAGCCT). Based on *in vivo* experiments presented in this paper we
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cannot state that the pheBA promoter is recognized by σ8. However, the nucleotide sequence of the −10 region of the promoter is only one component that determines σ8-dependent transcription. There is increasing evidence that additional regulators play a crucial role in establishing sigma factor specificity at stress-inducible promoters (Hengge-Aronis, 1999). Moreover, coming back to the sequence determinants, we have recently shown on fusion promoters generated in a starving population of P. putida that not only the −10 hexameric sequence, but also sequence downstream from the −10 hexamer is important for σ8-dependent transcription (Ojangu et al., 2000).

The positive effect of σ8 observed in the case of the pheBA promoter can give P. putida cells a little advantage to use phenol as a single source of carbon and energy under stressful conditions. However, this effect is insufficient to account for the inhibition of the pheBA promoter during exponential growth. Moreover, transcription from the catBCA promoter was not influenced by the presence of σ8 in P. putida cells. It is obvious, therefore, that stationary-phase-specific transcription from the pheBA and catBCA promoters must be regulated by some other mechanism than σ8-mediated control.

Modulation of pheBA and catBCA promoter activity by growth phase does not operate through the amount of regulator protein CatR

Transcriptional activation from the pheBA and catBCA promoters requires the presence of CatR and an inducer molecule CCM (Kasak et al., 1993; Parsek et al., 1995). Therefore, we examined whether the amount of the regulator protein CatR would be limiting in transcriptional activation from the pheBA and catBCA promoters in exponentially growing cells. To study the expression of the catR gene during the growth cycle, the promoter of the catR gene was cloned into plasmid pKTlacZ to generate a catR–lacZ transcriptional fusion. The activity of this fusion was compared in the wild-type P. putida strain PaW85 and in its rpoS-deficient mutant PKS54. In both strains, the level of β-Gal expression increased in stationary-phase cells when compared to that observed in exponentially growing cells (Fig. 3). Therefore, although catR promoter activity remained at a very low level during all growth phases of the bacteria, the intracellular amount of CatR (undetectable by Western blot analysis) may be somewhat increased in stationary-phase cells.

To study whether exponential silencing of the pheBA and catBCA promoters could be related to the amount of CatR in cells, we constructed a P. putida strain enabling artificial modulation of the expression of the catR gene. For that purpose, the catR gene was cloned under the control of inducible P tac promoter and the lacIq repressor. The catR expression cassette was introduced into the chromosome of P. putida PaW85. Overexpression of CatR in the presence of 0.5 mM IPTG was verified by using Western blot analysis with polyclonal antibodies against CatR (data not shown). We found that overexpression of CatR does not affect transcription from the pheBA and catBCA promoters in stationary-phase cells. No transcription from these promoters could be detected in exponentially growing cells (sampled from 2-h-old cultures), irrespective of the addition of IPTG into the growth medium. Therefore we concluded that the exponential silencing of the pheBA and catBCA promoters is not determined by the cellular amount of CatR.

Exponential silencing of the pheBA and catBCA promoters is dependent on the nature of the culture medium

The role of growth medium in the regulation of gene expression has been shown in many cases. For example, growth medium composition (either rich or minimal) determines the level of transcription from the Pm promoter of the operon (dmp) encoding dimethylphenol degradation (Sze & Shinger, 1999; Sze et al., 1996), from the Pu promoter of the TOL plasmid pWW0 (Cases et al., 1996; de Lorenzo et al., 1993) and from the PalkB promoter from the Pseudomonas oleovorans alkane degradation pathway (Yuste et al., 1998). We found that transcription from the pheBA and catBCA promoters was rapidly activated when bacteria were grown in MOPS minimal medium (Fig. 4). Measurement of β-Gal activity in cells sampled at different time points of an exponentially growing culture of P. putida PaW85 allowed us to detect the enzyme activity as early as 20 min after the addition of sodium benzoate.

When 0.5% CAA was added to minimal medium, the activation of transcription from the pheBA and catBCA promoters was delayed, similar to that observed in cells grown in LB medium (compare Figs 2 and 4). In the

Fig. 3. Effect of the growth phase of bacteria and σ8 on transcription from the catR promoter. β-Gal activity (filled symbols) was measured either in the cell lysates of P. putida strain PaW85 (wild-type; ●, ○) or in rpoS-deficient strain PKS54 (▲, △) harbouring the catR–lacZ fusion in plasmid pKTlacZ. Bacteria were grown in LB medium to stationary phase in the presence of sodium benzoate. The growth curves of P. putida cells are indicated by open symbols. The standard deviation is shown in the figure.
RelA and promoter was demonstrated using overexpression of (p)ppGpp for activation of transcription from the Po-limiting (Sze & Schingler, 1999). The requirement of silenced in rich medium until amino acids become dmp the dimethylphenol (Fig. 4). The occurrence of the partial silencing effect by different groups of amino acids on the transcription from the pheBA/promoter is very low during the first 4 h of cultivation (Fig. 4a). The transcription from the pheBA promoter was lower than that observed with catBCA promoter. The β-Gal activity was 12 ± 2.5 Miller units when all amino acids were added into the growth medium and it was approximately half of that observed without amino acids: 178 ± 18 and 199 ± 50 Miller units in the presence of 5 and 15 amino acids, respectively. This also indicates that the physiological control on the pheBA and catBCA promoters mediated by the presence of amino acids might be different from the mechanism related to stringent response. The occurrence of the partial silencing effect by different groups of amino acids on the transcription from the pheBA and catBCA promoters excludes the possibility that one particular amino acid could mediate this effect.

At this stage the mechanism by which the presence of amino acids causes repression of these two promoters is unclear. Data presented in Fig. 4 and results obtained in experiments with different sets of amino acids show that specific groups of amino acids could cause partial silencing of transcription from the pheBA and catBCA promoters. We were not able to demonstrate the positive role of (p)ppGpp on transcriptional activation from the pheBA and catBCA promoters. The absence of an effect of (p)ppGpp on transcription from these promoters was confirmed by experiments where the (p)ppGpp level was artificially increased either by adding 1 mM serine hydroxamate into the growth medium or by using RelA overexpression plasmid pVIS36 (Sze & Schingler, 1999) in P. putida cells carrying the pheBA-lacZ transcriptional fusion in the chromosome (data not shown).

In the (p)ppGpp-dependent Po regulatory system, distinct groups of amino acids were not able to mediate the delay in transcription (Sze & Schingler, 1999). We found that specific groups of amino acids could cause partial silencing of transcription from the pheBA and catBCA promoters. Bacteria were grown in MOPS minimal medium containing glucose for 60 min in the presence of sodium benzoate. No amino acids or a different number of amino acids (all 20, 15 or 5) were added into the growth medium. The sets of amino acids were designed according to their biosynthetic pathways. The third group consisted of 5 amino acids (Asp, Asn, Glu, Gln and Ser) that are precursors for several other amino acids, and the second group contained the other 15 amino acids. In the absence of amino acids, the level of β-Gal activity was 950 ± 44 Miller units in the case of the pheBA promoter and 397 ± 50 Miller units in the case of the catBCA promoter. In the case of the pheBA promoter, no expression of β-Gal activity could be detected when all amino acids were added, but the presence of 5 and 15 amino acids allowed partial expression of this promoter: the β-Gal activities were 91 ± 3 and 196 ± 20 Miller units, respectively. The expression of amino acids on transcription from the catBCA promoter was lower than that observed with the pheBA promoter. The β-Gal activity was 12 ± 2.5 Miller units when all amino acids were added into the growth medium. The occurrence of the partial silencing effect by different groups of amino acids on the transcription from the pheBA and catBCA promoters excludes the possibility that one particular amino acid could mediate this effect.

Transcription from the σ^54-dependent Po promoter of the dimethylphenol (dmp)-degradation pathway is silenced in rich medium until amino acids become limiting (Sze & Schingler, 1999). The requirement of (p)ppGpp for activation of transcription from the Po promoter was demonstrated using overexpression of RelA and E. coli (p)ppGpp-deficient mutants (Sze & Schingler, 1999). We were not able to demonstrate the positive role of (p)ppGpp on transcriptional activation from the pheBA and catBCA promoters. The absence of an effect of (p)ppGpp on transcription from these promoters was confirmed by experiments where the
differences of the target DNA. However, despite the minor differences in the expression level of the pheBA and catBCA promoters under certain growth conditions, the general mechanisms for physiological control of these promoters seem to be well conserved to coordinate the expression of the hybrid plasmid-chromosom-encoded pathway for phenol degradation in *P. putida*.

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