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Carbon Catabolite Repression of Phenol Degradation in *Pseudomonas putida* Is Mediated by the Inhibition of the Activator Protein PhlR

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Enzymes involved in (methyl)phenol degradation of *Pseudomonas putida* H are encoded by the catabolic operon (*phlA-L*) on plasmid pPGH1. Transcription of this operon by the σ^{54} (RpoN)-containing RNA polymerase is positively controlled by the gene product of the divergently transcribed *phlR* in response to the availability of the respective substrate. Additionally, phenol degradation is subject to carbon catabolite repression induced by organic acids (e.g., succinate, lactate, and acetate) or carbohydrates (e.g., glucose and gluconate). Analysis of *lacZ* fusion to the catabolic promoter and quantified primer extension experiments indicate that carbon catabolite repression also occurs at the transcriptional level of the catabolic operon. In this study, it is furthermore shown that carbon catabolite repression is a negative control. Titration of the postulated negative controlling factor was exclusively observed when extra copies of functional *phlR* gene were present in the cell. We therefore conclude that PhlR is the target and that carbon catabolite repression of phenol degradation occurs by interfering with the activating function of PhlR.

Bacterial genes encoding carbon catabolic enzymes are often regulated in response to the availability of the respective substrate. However, if a rapidly metabolizable carbon source is additionally present in the growth medium, then synthesis of the peripheral catabolic enzymes can be reduced. This general regulatory phenomenon is called carbon catabolite repression (CR). It has been well described for enteric bacteria and more recently for some species of *Bacillus* and other gram-positive genera. Although glucose is the preferred carbon source in these two groups of bacteria, metabolic processes involved in CR and the molecular mechanisms of repression of the target genes are quite different: in enteric bacteria, uptake of glucose via the phosphotransferase system leads indirectly to a low level of cyclic AMP (cAMP). Therefore, cAMP-cAMP receptor protein-mediated activation of genes responsible for the degradation of secondary carbon sources does not take place if glucose is available (for a recent review, see reference 25). In *Bacillus* cells, glycolysis is involved in signal transduction and mediates activation of catabolite control protein (CcpA) (16), which negatively controls target genes (29). CR has also been observed in several *Pseudomonas* species. In this genus, the preferred carbon source is usually an organic acid like succinate, citrate, and acetate. In the presence of one of these substrates, the level of activity of the enzymes necessary for the transport and/or catabolism of glucose, amides, histidine, and some others is reduced (17, 22, 24, 27, 28). Although *Pseudomonas aeruginosa* mutants defective in CR have been isolated (33) and the corresponding gene (*crc*) has been identified, cloned, and analyzed (19), both the metabolic process(es) involved in signal transduction and the molecular mechanism of CR remained unknown.

Unusual plasmid-encoded degradative pathways may also be subject to CR, and thus, CR may impede biodegradation of aromatic pollutants both in nature and under artificial conditions. Recently, a carbon source-dependent inhibition of the activity of the upper pathway operon and *xylS* of TOL plasmid pWW0 was described (14). The results demonstrate that this inhibition is provoked by another set of carbon sources as described for the chromosomal genes: the availability of glucose led to strong inhibition, whereas in the presence of succinate, the activity of TOL plasmid operons was not reduced. In continuous culture, however, inhibition of TOL genes was also found when succinate was in excess (8).

Plasmid pPGH1 of *Pseudomonas putida* H encodes enzymes allowing the degradation of (methyl)phenol (11). The genes encoding catabolic enzymes are organized in a single large operon. This operon is activated by the product of *phlR* in the presence of the substrate and is transcribed under the control of an RpoN-dependent promoter (3, 13).

In the present paper, we demonstrate that transcription of the catabolic *phl* genes is reduced as well in the presence of an organic acid as in the presence of glucose. Furthermore, we present data which indicate that CR of the *phl* genes is mediated by a negative controlling factor which inhibits the activator PhlR.

MATERIALS AND METHODS

Bacterial strains, plasmids, and transposons. The bacterial strains, plasmids, and transposons used in this study are listed in Table 1.

Media and growth conditions. Minimal medium (agar) was either M9 medium (21) or phosphate ammonium salt (PAS) medium (4). Carbon sources were added to a final concentration of 40 mM, except phenol, which was used at 2.5 mM. Selective media contained antibiotics at the following concentrations: piperacillin, 100 μg/ml; kanamycin, 500 μg/ml; rifampin, 250 μg/ml; and nalidixic acid, 1,000 μg/ml.

Biochemical characterization of phenol catabolism. Phenol hydroxylase activity was determined by measuring phenol consumption in vivo as described previously (13), β-Galactosidase activity was determined as described by Miller (21).

Conjugative transfer. To introduce plasmids or transposons into *P. putida* strains, a filter mating procedure was used as described previously (13). The mobilizing donor was S17-1λ-pir (5).

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TABLE 1. Strains, plasmids, and transposons used in this study

Strain, plasmid, or transposon	Relevant characteristic(s)[a]	Source or reference
Strains		
E. coli		
DH5α	*hsdR recA thi-1 relA1 gyrA96*	10
S17-1-λ-pir	Sm^r Tp^r *thi hsdR* chr::RP4-2 (Tc::Mu Km::Tn*7*) λ-pir lysogen	5
P. putida		
KT 2440	mt-2 (*hsdR*)	2
PG5	KT2440 (Rif^r Nal^r)	12
PG314	H, plasmidless, Rif^r Nal^r	Laboratory collection, Greifswald, Germany
PG320	H(pPGH1) (Phl$^+$) Rif^r Nal^r	13
PG320-C902	Insertion mutant (Phl$^-$) of PG320	13
Plasmids		
pMMB67EH/HE	Ap^r expression vector	9
pMMB67EHΔ	*lacI*^{q}, P*tac* deletion derivative of pMMB67EH generated by *Eco*RI-*Pvu*II digestion and religation	This paper
pKOK5	Ap^r Km^r *lacZ* (promoterless)	18
pUT	Ap^r R6K *oriV* RP4*oriT*, mini-Tn*5* transposon delivery plasmid	6
pTZ18/19R	Ap^r expression vector	20
pPGH1	Phl$^+$ Hg^r	10
pPGH11	Phl$^+$ Ap^r Km^r Tc^r R68.45-pPGH1 hybrid plasmid	11
pPGH500	*phlA-phlE*, *phlR* and the common promoter-operator region inserted in pMMB67EHΔ (Fig. 4a)	This paper
pPGH512	Complete catabolic promoter and *phlR* inserted as *Xcm*I-*Acc*III fragment into pMMB67EHΔ (Fig. 6A)	This paper
pPGH514	*phlR* inserted as *Bcl*I-*Acc*III fragment into pMMB67EHΔ (Fig. 6A)	This paper
pPGH515	5′-truncated *phlR* inserted as *Bgl*II-*Acc*III inserted into pMMB67EHΔ (Fig. 6A)	This paper
pPGH517	Complete catabolic promoter and 3′-truncated *phlR* inserted as *Xcm*I-*Sac*I fragment into pMMB67EHΔ (Fig. 6A)	This paper
Transposons		
Mini-Tn*5* Km1	Km^r	6
Mini-Tn*5* *lacI*^{q}/P*trc*	Km^r *lacI*^{q} P*trc* IPTG-dependent promoter-out Tn*5*	5
Mini-Tn*5* phl1	*phlA-phlF phlR* and the common promoter-operator region inserted in mini-Tn*5*Km (Fig. 4A)	This paper
Mini-Tn*5* *lacI*^{q}/P*trc* phl3	Complete catabolic promoter, *phlA* and *lacZ* fused to *phlB* inserted in mini-Tn*5*Km *lacI*^{q}/P*trc* with Km deleted (Fig. 5A)	This paper
Mini-Tn*5* *lacI*^{q}/P*trc* phl4	Truncated catabolic promoter, *phlA* and *lacZ* fused to *phlB* inserted in mini-Tn*5*Km *lacI*^{q}/P*trc* with Km deleted (Fig. 5A)	This paper

[a] Tc, tetracycline; Km, kanamycin; Ap, ampicillin; Rif, rifampin; Nal, nalidixic acid; Sm, streptomycin.

DNA isolation, analysis, and manipulation. Cloning vectors and recombinant plasmids were isolated with Qiagen spin columns (Qiagen GmbH, Hilden, Germany) according to the manufacturer's recommendation. Restriction digests were performed as recommended by the supplier. Phosphatase treatment, ligation, transformation, and gel electrophoresis were performed as outlined by Sambrook et al. (26). Primary clonings were performed in *Escherichia coli* DH5α (10) with pTZ18/19R (20).

Primer extension analysis. To isolate RNA, cells were grown overnight in M9 medium containing phenol and were transferred into fresh M9 medium containing phenol, phenol and pyruvate, phenol and glucose, or phenol and succinate. After 5.5 h of growth, cells were harvested. In addition, one culture pregrown in succinate was inoculated into fresh M9-succinate medium and also cultivated for 5.5 h. At that time, all of the cultures grew logarithmically. Cultures had an optical density at 600 nm (OD_{600}) of 0.254 on phenol, 0.457 on phenol and pyruvate, 0.470 on phenol and glucose, 0.600 on phenol and succinate, and 0.732 on succinate. Total RNA was isolated according to the method of Völker et al. (31) without the lysozyme treatment and the extraction with diethyl ether. The amount of isolated RNA was determined by UV light absorption and controlled in agarose gels stained with ethidium bromide (26). Ten micrograms of total RNA of each culture was subjected to primer extension. Primer extension analyses were performed with Moloney murine leukemia virus reverse transcriptase as described elsewhere (32). Primer 1 (complementary to positions 280 to 309; numbering according to the sequence deposited under GenBank accession number X80765) for *phlA* and primer 2 for *phlR* (complementary to positions 237 to 266; GenBank accession number X91145) labeled at their 5′ ends with γ-^{32}P were used for primer extension analysis. Sequence ladders of *phlA* and *phlR* promoter regions were generated on a single-stranded template DNA with primer 1 and primer 2, respectively. Signal intensities were quantified with the PhosphorImager (Molecular Dynamics, Sunnyvale, Calif.) and ImageQuaNT 4.0 software.

RESULTS

Phenol hydroxylase activity is reduced in the presence of organic acids and glucose. Carbon CR of phenol degradation of *P. putida* H was examined with two different experimental approaches. (i) Induction of phenol hydroxylase was achieved (growth in M9 medium containing phenol as sole source of carbon and energy), and then the second carbon source was added (repression experiment). Or (ii) cells were pregrown in M9 medium containing a preferred carbon source, and phenol hydroxylase was induced by addition of phenol to a logarithmically growing culture (induction experiment).

Phenol hydroxylase activities were dramatically reduced after addition of succinate or glucose to a culture growing on phenol. Addition of pyruvate led to less strong reduction (Fig. 1A). In the reverse situation, in cells which grew on succinate or glucose, nearly no induction of phenol hydroxylase could be detected as long as cells grew logarithmically (Fig. 1B). Only when cells entered the stationary phase did phenol hydroxylase activity significantly increase. Induction of phenol hydroxylase in cells growing on pyruvate seems to be rather enhanced or at least unaffected. CR was also observed when other organic acids (e.g., lactate and acetate) or carbohydrates (e.g., gluconate) were available (data not shown).

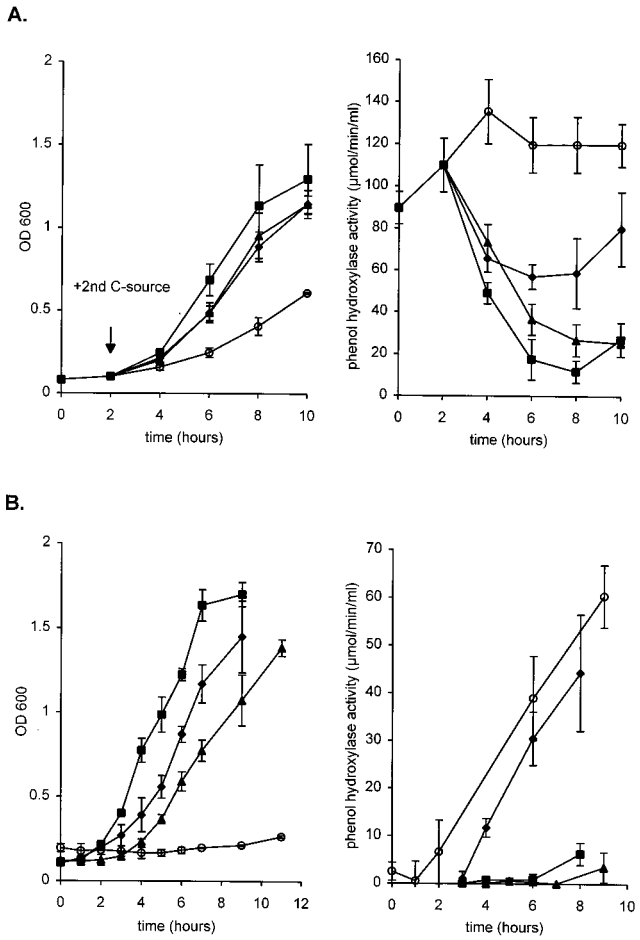

FIG. 1. Carbon CR of phenol hydroxylase. (A) Growth of PG320 (left) and phenol hydroxylase activity (right). The cultures were pregrown in M9 medium containing phenol (○) as the sole source of carbon and energy. At 2 h, a second carbon source (pyruvate [◆], succinate [■], or glucose [▲]) was added. Mean values and standard deviations of four independent experiments are shown. (B) Growth of PG320 (left) and induction of phenol hydroxylase (right) in cultures growing on various carbon sources. Cells were pregrown in M9 medium containing pyruvate (◆). At time zero, cells were harvested and inoculated into M9 medium containing phenol (○), pyruvate (◆), succinate (■), or glucose (▲) as the carbon source. To induce phenol degradation, phenol was added after 3 h (to the pyruvate and succinate culture) or after 4 h (to the glucose culture). Mean values and standard deviations of four independent experiments are shown.

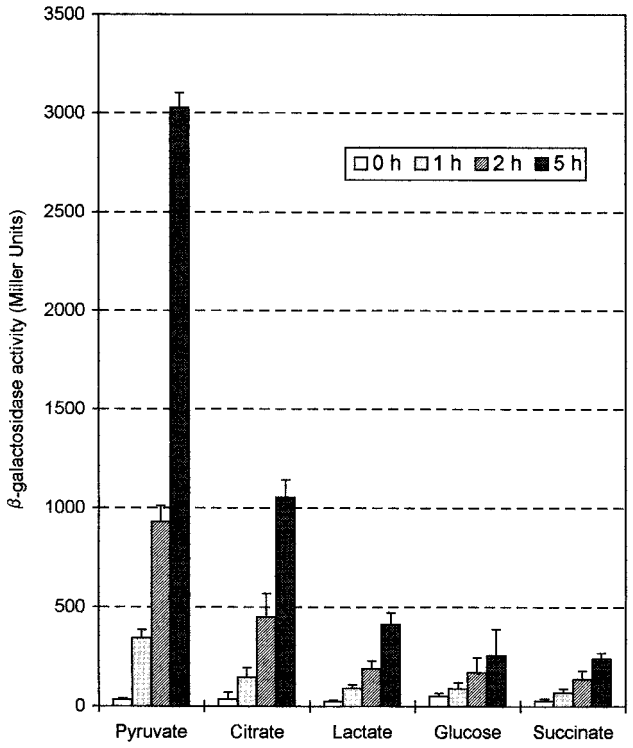

FIG. 2. Dependence of β-galactosidase expression in PG320-C902 on the presence of various carbon sources. Cells were pregrown overnight in M9 medium containing pyruvate as the carbon source. Cells were harvested and transferred into M9 medium containing various carbon sources. To induce the catabolic promoter, phenol was added to logarithmically growing cultures (time zero). The means of four independent experiments and error bars are shown.

Phenol degradation genes can be introduced into *P. putida* KT2440, a derivative of mt-2, the original host of TOL plasmid pWW0. CR of the phenol catabolic genes was also observed in this strain, and like in the original host, the availability of both organic acids and glucose or gluconate induces CR (described below [data not shown]).

Carbon CR occurs at the transcriptional level of the catabolic operon. To test whether the reduced phenol hydroxylase activity reflected the decreased transcription of the catabolic operon, *lacZ* fusions to the catabolic promoter were analyzed. The fusions were generated via replacement of the wild-type sequence of pPGH1 by the homologous sequence mutated by the insertion of a promoter-probe *lacZ* cassette (18) as described previously (13). Figure 2 shows the induction of *lacZ* by phenol in cells of PG320-C902 grown on various carbon sources. The expression of β-galactosidase in the presence of a certain carbon source corresponded to that of phenol hydroxylase observed in the wild-type strain under related conditions.

To confirm that transcription of the catabolic operon but not the transcription of *phlR* is affected by CR, total RNA was subjected to primer extension analysis (Fig. 3A). The quantification of the corresponding cDNA products of the catabolic and of the *phlR* mRNA (Fig. 3B) indicates that exclusively the transcription of the catabolic operon was reduced in the presence of a second carbon source. The low constitutive level of transcription of *phlR* did not vary significantly depending on the carbon source.

Carbon CR is a negative control. To study the molecular mechanism which mediates CR of the catabolic operon, we constructed derivatives containing a 7-kb fragment of pPGH1 carrying phenol hydroxylase genes and *phlR* with their common promoter-operator region (Fig. 4A). The fragment was cloned in pMMB67EHΔ, a medium-copy RSF1010-derived vector (10 to 12 copies per cell), and introduced into the foreign strain PG5, a derivative of KT2440 devoid of any phenol degradation genes. In addition, these genes were inserted into mini-Tn*5* (6) and integrated via transposition into the chromosome of the same strain. In this way, we could analyze CR of phenol degradation in strains which differed only in regard to the copy number of the target genes. In the strain carrying one copy of target genes (PG5::mini-Tn*5* phl1) phenol hydroxylase activity was reduced in the presence of a second carbon source such as that observed in the original host, strain PG320, carrying the genes on pPGH1 (one copy per cell [Fig. 4B]). In contrast, in the medium-copy-number situation [PG5(pPGH500)],

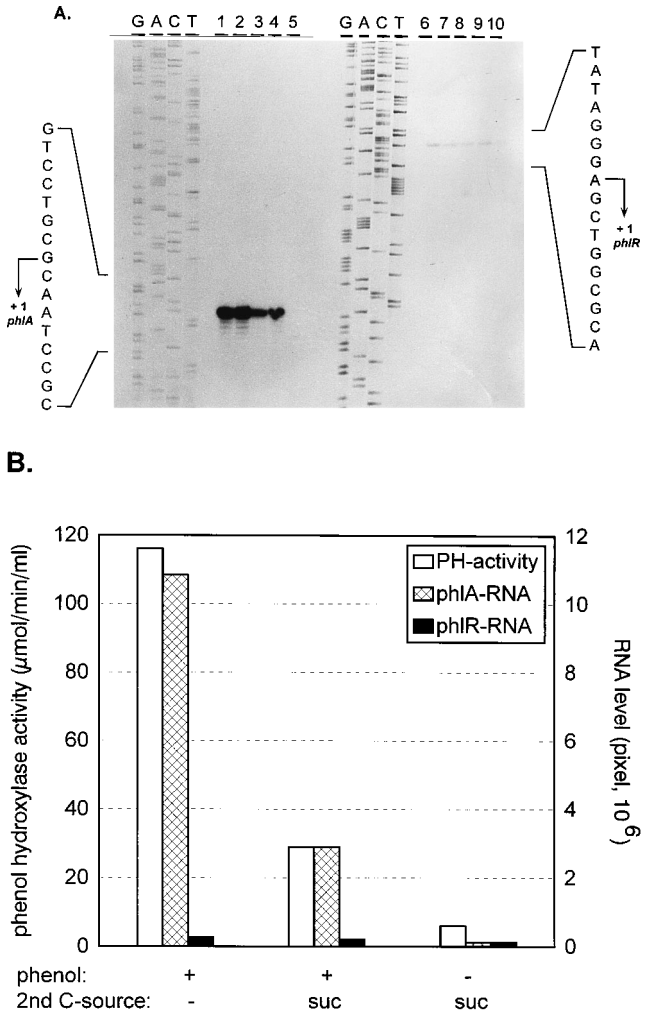

FIG. 3. Effect of various carbon sources on the mRNA synthesis of *phl* genes in PG320. (A) Transcription start points of the catabolic promoter (left) and of *phlR* (right). RNA was isolated from cells grown on phenol (lanes 1 and 6), phenol and pyruvate (lanes 2 and 7), phenol and glucose (lanes 3 and 8), phenol and succinate (lanes 4 and 9), or succinate (lanes 5 and 10). (B) Correlation of the quantified cDNA products of *phlR* and *phlA* mRNAs (autoradiogram in panel A) to the phenol hydroxylase activities determined from the corresponding cultures. PH, phenol hydroxylase; suc, succinate.

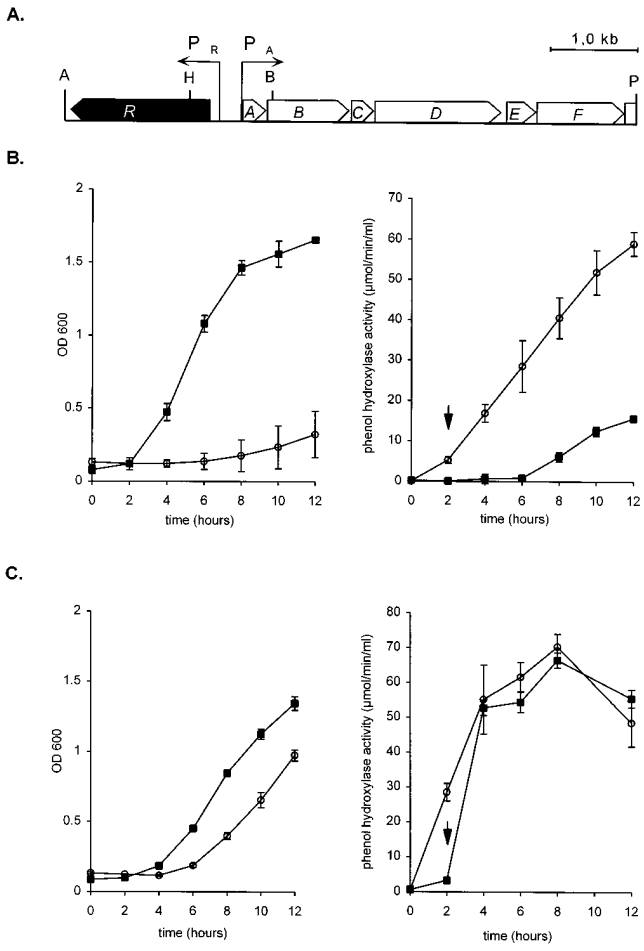

FIG. 4. Effect of the copy number of *phl* genes on their carbon CR. (A) Genetic and physical map of the *phl* gene fragment (phl1) inserted in pMMB67EHΔ (pPGH500) or mini-Tn*5*Km. Genes *A* to *F* code for phenol hydroxylase, and gene *R* codes for the activator. The catabolic operon is transcribed under the control of P_A, and *phlR* is transcribed from P_R. Restriction sites: A, *Acc*III; B, *Bam*HI; H, *Hin*dIII; P, *Pst*I. Not all restriction sites of a certain enzyme are shown. (B) Growth of KT2440::mini-Tn*5* phl1 (left) and induction of phenol hydroxylase (right) with phenol (○) or succinate plus phenol (■). Cells were precultured as described in the legend to Fig. 1B. To induce phenol degradation in the culture growing on succinate, phenol was added at 2 h as indicated by the arrow. Mean values and standard deviations of five independent experiments are shown. (C) Growth of KT2440(pPGH500) (left) and induction of phenol hydroxylase (right) with phenol or succinate and phenol. Cultivation was as described for panel B. Mean values and standard deviations of four independent experiments are shown.

phenol hydroxylase activity in the presence of a second carbon source was as high as that observed in its absence (Fig. 4C). CR of phenol hydroxylase encoded by the hybrid plasmid pPGH11 (12) (about five copies per cell) shows an intermediary state of CR (data not shown). These results indicate that CR of the phenol catabolic genes of *P. putida* is mediated by a negative controlling factor which can be titrated by multiple copies of its target. This negative controlling factor seems to be not specific to *phl* genes, because CR of phenol degradation does not differ in *P. putida* H and mt-2 and does function in the foreign host which exclusively carries the phenol hydroxylase genes and *phlR* of the original host.

Carbon CR is mediated by inhibition of PhlR. Negative control of gene expression can occur directly, by true repression, or—in the case of genes which must be activated—by interference with activation. It is to be expected that true repression only depends on corresponding *cis* sequences and that it operates independently on the promoter which drives transcription. In contrast, interference with activation should only be detectible if activation is necessary for gene expression.

To discriminate between the two modes of negative control, a *lacZ* fusion to the catabolic promoter was cloned into mini-Tn*5* *lacI*^{q}/P*trc* (5) (Fig. 5A) and introduced into *P. putida* PG314, generating PG314-phl3. Because PG314 lacks *phlR*, *lacZ* can only be transcribed under the control of the transposon-born P*trc* after induction by IPTG (isopropyl-β-D-thiogalactopyranoside). In this strain, β-galactosidase activity was not affected by CR, although the complete catabolic promoter was present (Fig. 5B). In a deletion derivative (PG314-phl4), transposon-born P*trc* overlaps the −24 region of catabolic promoter (P_A). Also in this derivative, expression of β-galactosidase is not repressed in the presence of another carbon source (Fig.

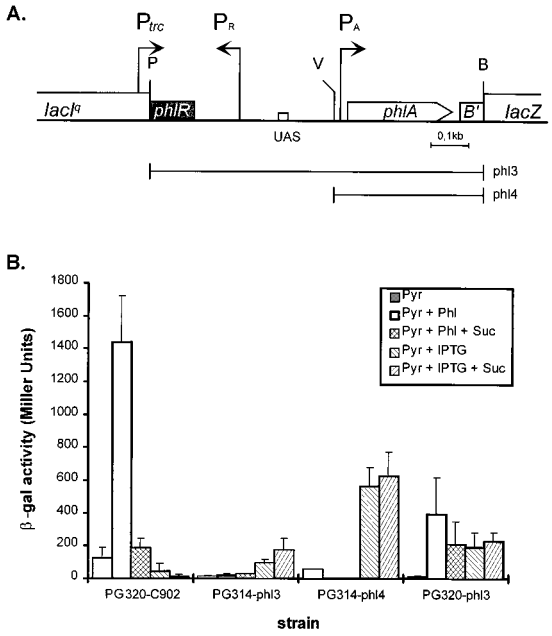

FIG. 5. Dependence of carbon CR of phenol degradation on the activation by PhlR. (A) Genetic and physical map of the *phl* gene fragments inserted into mini-Tn*5* *lacI*^{q}/P*trc* derivatives and in PG320-C902. Cells pregrown on pyruvate were inoculated into M9 medium containing pyruvate (Pyr) or succinate (Suc). To induce β-galactosidase, phenol (Phl) or IPTG was added at time zero to logarithmically growing cultures. β-Galactosidase activity determined at 4 h is shown. Experiments were performed at least four times. Strain PG320-phl4 was tested two times. Error bars are shown. Restriction sites: P, *Pst*I; V, *Pvu*I; B, *Bam*HI.

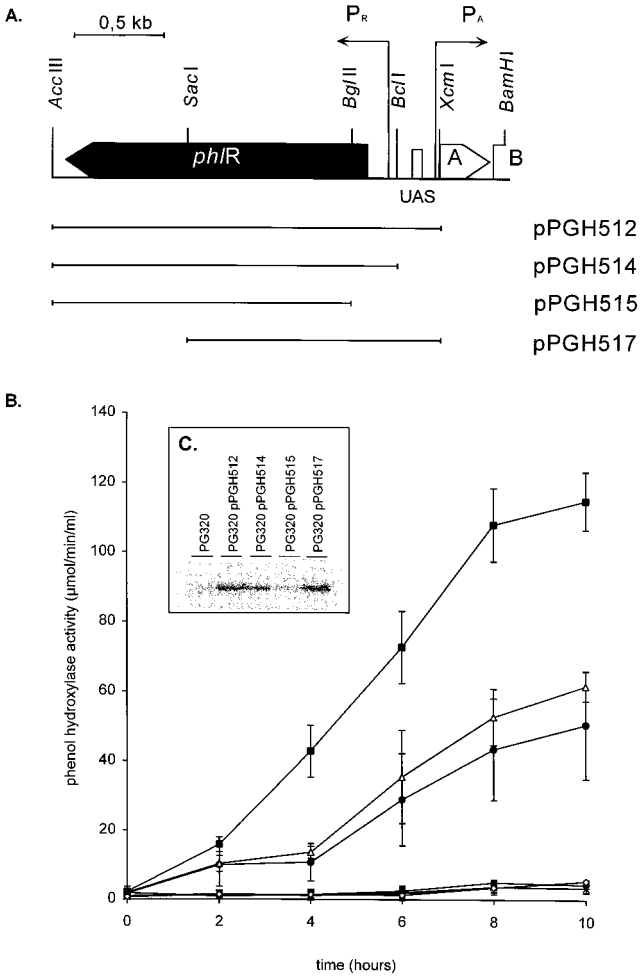

FIG. 6. Effect of extra copies of the catabolic promoter and/or *phlR* on carbon CR of the catabolic operon on pPGH1. (A) Genetic and physical map of the *phl* gene fragment on pPGH512 and derivatives thereof. (B) Expression of phenol hydroxylase of PG320, PG320(pMMB67EHΔ), PG320(pGH512), and derivatives thereof growing in M9 medium with succinate and phenol. Precultivation was performed as described in the legend to Fig. 1B. Phenol was added at time zero. Mean values and standard deviations of four independent experiments are shown. ◆, PG320; ●, PG320(pPGH512); △, PG320(pPGH514); ×, PG320(pPGH515); ◇, PG320(pMMB67EHΔ); ■, P6320 growing in M9 medium with phenol. (C) Quantitative primer extension analysis of *phlR* mRNA. Ten micrograms of total RNA of the indicated strains was harvested 2 h after induction. Cells were observed in mid-logarithmic growth phase (OD_{600}, ≈0.5).

5B). After transfer of pPGH1 into PG314-phl3, generating PG320-phl3, *lacZ* can be expressed alternatively either under the control of the promoter of the catabolic operon (in *trans* activated by PhlR encoded by pPGH1) or under the control of P*trc*, depending on the induction conditions. Results presented in Fig. 5B indicate that exclusively transcription under the control of the catabolic promoter is affected by CR. Therefore, we suppose that CR of phenol degradation is mediated by interference with substrate-induced, PhlR-mediated activation of the catabolic genes. This might occur either by competition for upstream activator sequence, responsible for binding of PhlR, or by inhibition of the activator protein PhlR itself. To confirm this result and to identify the target of the CR controlling factor, several recombinant plasmids were constructed that contained the catabolic promoter and/or *phlR* (or truncated *phlR*) (Fig. 6A). The medium-copy-number plasmid pMMB67EHΔ was used as the vector. The recombinant plasmids were introduced into strain PG320, which carries pPGH1. Phenol hydroxylase was induced by addition of phenol to a culture growing on succinate. The results presented in Fig. 6B indicate that only in strains PG320(pPGH512) and PG320 (pPGH514) was induction of phenol hydroxylase observed. These two strains contain plasmids carrying the complete, functional *phlR* gene either together with the upstream activator region (UAS) of the catabolic operon responsible for binding of PhlR (pPGH512) or without it (pPGH514). CR was not affected in PG320 carrying pPGH517. This plasmid contains the UAS, but *phlR* is truncated at its 3′ end. Quantitative primer extension analysis with the RNA of these strains proves the correlation of the amount of *phlR* mRNA to the *phlR* gene dosage (Fig. 6C). These results demonstrate that the CR controlling factor is only titrated in strains carrying extra copies of *phlR*. We therefore suppose that PhlR protein is the target and that the interaction of PhlR with the CR controlling factor interferes with the activating capability of PhlR.

DISCUSSION

We have demonstrated that phenol degradation in *Pseudomonas putida* is subject to carbon catabolite repression. The results presented indicate that the level of activity of phenol catabolic enzymes was reduced as well in the presence of organic acids, as previously described for chromosomally encoded enzymes of *P. aeruginosa* and *P. putida* (17, 22, 24, 27, 28), as in the presence of carbohydrates, which were found to inhibit TOL plasmid-encoded toluene and xylene degradation (14). All repressive carbon sources were rapidly metabolizable substrates, but not all of the

rapidly metabolizable carbon sources (e.g., pyruvate) repress phenol degradation under all conditions. The results presented are taken as evidence that carbon CR of phenol degradation is mediated by a negative controlling factor which inhibits the *phl* gene-specific activator PhlR. The main arguments are as follows. (i) The levels of phenol hydroxylase activity observed in a strain carrying 10 to 12 copies of the corresponding genes were as high in the presence of organic acids or glucose as they were in their absence. Therefore, CR must be mediated by negative control. (ii) If catabolic genes can be transcribed alternatively from the native or from a (regulated) foreign promoter, exclusively transcription from the native promoter is affected by CR. Thus, CR is not a true repression but rather interferes with phenol-induced, PhlR-mediated activation. (iii) PhlR must be the target for the negative controlling factor, because exclusively in the presence of recombinant plasmids carrying a functional activator gene is CR of the catabolic genes reduced. CR mediated via PhlR inhibition would also explain the reduced CR when bacterial cultures enter the stationary phase. Exactly at that time, increasing β-galactosidase activity was observed in a strain carrying *lacZ* fused to the *phlR* promoter (3).

The lack of transcription of the catabolic operon due to the inhibition of PhlR is the dominant mechanism of CR of phenol degradation. This could be demonstrated by the correlation of phenol hydroxylase activity, β-galactosidase activity of the *lacZ* fusion to the catabolic promoter, and the amount of *phlA* mRNA determined in primer extension experiments. Obviously, this mechanism is sufficient to explain the results in induction experiments. However, in the repression experiment, the rapid decrease in phenol hydroxylase activity after addition of a preferred carbon source might indicate that additional posttranscriptional events contribute to the observed CR.

A negative control via inhibition of an activator is the third known mechanism which is applied to reduce the catabolism of a secondary carbon source if a preferred one is available. On the basis of the current knowledge about CR in *Pseudomonas* species, it is quite difficult to decide whether this mode of action is a general CR regulatory mechanism of target genes. The observation that phenol degradation is affected by CR as well in the original host of *phl* genes, *P. putida* H, as in the foreign strain mt-2, which carries only the well-known *phl* genes of strain H, argues against a special mechanism limited to the *phl* genes. On the other hand, the different phenotypic properties of CR of various genes led us to assume that more than one mechanism is realized.

CR mediated by inhibition of the specific activator of a certain gene or operon requires positive control of the gene's or operon's transcription. Although most catabolic pathways affected by CR are inducible by their substrate, the precise regulatory mechanism, if known at all, differs: the *xyl* upper pathway operon of TOL plasmid is like the *phl* catabolic operon of pPGH1 transcribed from an RpoN-dependent promoter and requires activation by XylR (1). Genes encoding carbohydrate catabolic enzymes of *P. aeruginosa* are most likely transcribed under the control of the vegetative promoter and seem to be (among other things) positively controlled by HexR (30). Expression of the amidase operon of *P. aeruginosa* depends on AmiR-mediated transcription antitermination (7). Although the mode of action of these proteins is different, all of them must bind to nucleic acids. The general mechanism of their inhibition might operate through masking of the region responsible for binding. In *P. aeruginosa*, one type of CR mutants (Crc^-) has been isolated so far (33). These mutants exhibited a pleiotropic loss of CR of multiple pathways. This mutant phenotype hints at only one common step of CR. It would be interesting to analyze the CR of phenol degradation in such a mutant.

There are also major differences in the regulation of genes or operons encoding carbon catabolic enzymes with respect to the effect of organic acids and glucose in the growth medium: expression of the TOL plasmid-localized *xyl* upper pathway operon is catabolite repressed, especially in the presence of glucose, as is that of the catabolic *phl* genes in the presence of both glucose and organic acids. Genes responsible for glucose catabolism and uptake, however, are induced by glucose (or its metabolites) and are catabolite repressed, like most of the chromosomal CR-sensitive genes, in the presence of organic acids. If CR of these pathways is mediated by the same factor, physiological processes which lead to synthesis or functionalization of this element should differ. There are at present no hints of a certain physiological reaction which could be involved in signal transduction. Because all of the carbon sources which provoke CR were taken up by special transport systems, the phosphotransferase system can be excluded. Furthermore, in *P. aeruginosa* and *P. putida*, the cellular cAMP level does not vary appreciably with the carbon source as it does in the enteric bacteria (24, 27).

Genes encoding histidine utilization enzymes are candidates for another CR regulatory mechanism. Although induced by the substrate, these genes of *P. putida* are negatively controlled (15) but nevertheless catabolite repressed if additional succinate is available (24). Furthermore, in the CR mutant of *P. aeruginosa*, histidine catabolism is still CR sensitive (23).

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