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An AraC/XylS Family Member at a High Level in a Hierarchy of Regulators for Phenol-Metabolizing Enzymes in *Comamonas testosteroni* R5

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Comamonas testosteroni **strain R5 expresses a higher level of phenol-oxygenating activity than any other bacterial strain so far characterized. The expression of the operon encoding multicomponent phenol hydroxylase (mPH), which is responsible for the phenol-oxygenating activity, is controlled by two transcriptional regulators, PhcS and PhcR, in strain R5. In this study, we identified a third transcriptional regulator for the mPH operon (PhcT) that belongs to the AraC/XylS family. While the disruption of *phcT* in strain R5 significantly reduced the expression of the mPH operon, it did not eliminate the expression. However, the disruption of *phcT* in strain R5 increased the expression of *phcR*. The phenol-oxygenating activity was abolished by the disruption of *phcR*, indicating that PhcT alone was not sufficient to activate the expression of the mPH operon. The disruption of *phcS* has been shown in our previous study to confer the ability of strain R5 to express the mPH operon in the absence of the genuine substrate for mPH. PhcT was not involved in the gratuitous expression. Strain R5 thus possesses a more elaborate mechanism for regulating the mPH operon expression than has been found in other bacteria.**

The expression of a bacterial catabolic pathway for aromatic compounds is often controlled by one or more transcriptional regulatory proteins (21), and sometimes, one transcriptional regulator controls the expression of another transcriptional regulator (24).

The expression of multicomponent phenol hydroxylase (mPH) (9, 11, 12, 19, 20, 36, 37) is generally thought to be controlled by a regulator of the XylR/DmpR subclass within the NtrC-type family of transcriptional regulators, resulting in the expression of phenol-metabolizing enzymes only in the presence of the pathway substrate or its structural analog (2, 12, 14, 18, 19, 22, 26, 30–33, 37). The regulators of this subclass are activated by direct interaction with an effector molecule which is normally the substrate for the catabolic pathway the regulators control (29).

Comamonas testosteroni R5 has been shown to exhibit an exceptionally high level of activity for phenol oxygenation (42). We have cloned a DNA fragment encoding mPH (*phcKLMNOP*) and its cognate transcriptional activator (*phcR*) of the XylR/DmpR subclass from strain R5 (37). This work (37) and an electrophoretic analysis in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (our unpublished data) indicated that the high activity of strain R5 was due to the high level of mPH expression, leading us to investigate its transcriptional mechanism. PhcR caused the expression of the mPH operon even in the absence of the genuine substrate for mPH, but this gratuitous expression was repressed by a member of the GntR family of transcriptional regulators named PhcS (38). This GntR family member for regulating the mPH operon has also been identified for *C. testosteroni* TA441. For strain TA441, the regulator named AphS repressed the transcription of the mPH operon even in the presence of phenol, which prevented strain TA441 from growing on phenol (1). In the present study, we found one open reading frame, named *phcT*, downstream of *phcS*. The physiological role of PhcT on the expression of phenol-metabolizing enzymes in strain R5 was studied.

(This work is taken from a thesis submitted by Maki Teramoto to Ochanomizu University, Tokyo, Japan, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.)

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. The culture media used in this study were Luria-Bertani (LB) medium (25), M9 medium (3), and an inorganic medium called MP containing (per liter) 2.75 g of K_2HPO_4, 2.25 g of KH_2PO_4, 1.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgCl_2 \cdot 6H_2O$, 0.1 g of NaCl, 0.02 g of $FeCl_3 \cdot 6H_2O$, and 0.01 g of $CaCl_2$ (pH 6.8 to 7.0). The *Escherichia coli* strains were grown at 37°C, while the *C. testosteroni* strains were grown at 30°C, unless otherwise stated. When required, the media were supplemented with the following antibiotics at the indicated concentrations: tetracycline, 12 μg/ml; ampicillin, 100 μg/ml; carbenicillin, 500 μg/ml; and chloramphenicol, 20 μg/ml (*E. coli*) or 80 μg/ml (*C. testosteroni*).

Genetic techniques. Plasmid isolation, restriction endonuclease digestion, and transformation of the *E. coli* strains were conducted by the methods of Sambrook et al. (25). The *C. testosteroni* strains were transformed by the method of Chakrabarty et al. (6).

Nucleotide sequencing and computer analysis. To determine the nucleotide sequence of the 2.5-kb *Xba*I-*Sal*I fragment downstream of *phcS* (Fig. 1), subfragments were cloned into the multicloning site of pBluescript II KS(−). The nucleotide sequences of the subfragments were determined in both orientations by using M13 primers (Takara), a DNA sequencing kit (Dye Terminator Cycle Sequence; Perkin-Elmer) and a model 377 DNA sequencer (Perkin-Elmer) according to the manufacturer's instructions. The templates for the dideoxy chain-termination reactions were prepared by Wizard minipreps (Promega). The DNA sequence data were aligned by using version 1.7 of ClustalW (39).

Construction of the *phcT* and *phcR* knockouts and of the knockout defective in

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)[a]	Reference or source
Strain		
C. testosteroni		
R5	Phl$^+$, wild type, contains Phc mPH genes	42
R5T	Phl$^+$, Δ*phcT*::Tc^r of R5	This study
R5TS	Phl$^+$, Δ(*phcT*−*phcS*)::Tc^r of R5	This study
R5R	Phl$^-$, Δ*phcR*::Tc^r of R5	This study
E. coli		
DH5α	Host strain for DNA manipulation	Toyobo
S17-1	Host strain for plasmid mobilization	34
Plasmid		
pBluescriptIIKS(−)	Ap^r, cloning vector	Toyobo
pROR501	Ap^r, 11.1-kb *Xba*I-*Sal*I fragment of pLAFRR501 cloned into *Nhe*I-*Sal*I-cleaved pRO1614	38
pMT5059	Ap^r, pBR322 derivative carrying multiple cloning sites and an *Not*I site	41
pMT5056	Ap^r Tc^r, pBR322 derivative carrying a Tc^r gene cartridge flanked by *Eco*RV and *Pvu*II sites	41
pMT5071	Cm^r, plasmid containing an *Not*I-flanked mobilization cassette (the cassette contains the Cm^r gene, *sac*B, and the Mob region)	40
pSK1	Ap^r, 3.9-kb *Kpn*I-*Sma*I fragment of pROR501 carrying the *phcT* and *phcS* genes cloned into pMT5059	38
pSK01T	Ap^r Tc^r, 1.7-kb *Eco*RV fragment of pMT5056 carrying the Tc^r gene cloned into a blunted *Sac*II-*Sal*I site of pSK1	This study
pSK02T	Ap^r/Cb^r Tc^r Cm^r, *Not*I fragment carrying the mobilization cassette of pMT5071 cloned into pSK01T	This study
pSK01TS	Ap^r Tc^r, 1.7-kb *Pvu*II fragment of pMT5056 carrying the Tc^r gene cloned into a blunted *Sac*II-*Apa*I site of pSK1	This study
pSK02TS	Ap^r/Cb^r Tc^r Cm^r, *Not*I fragment carrying the mobilization cassette of pMT5071 cloned into pSK01TS	This study
pBS2	Ap^r, 4.0-kb *Bgl*II-*Sac*II (blunted) fragment of pROR501 carrying the *phcS* and *phcR* genes cloned into a *Bgl*II-*Nru*I site of pMT5059	38
pBS01R	Ap^r Tc^r, 1.7-kb *Pvu*II fragment of pMT5056 carrying the Tc^r gene cloned into a *Pvu*II-*Pvu*II site of pBS2	This study
pBS02R	Ap^r/Cb^r Tc^r Cm^r, *Not*I fragment carrying the mobilization cassette of pMT5071 cloned into pBS01R	This study
pRC50	Tc^r Cm^r IncP, *lacZ* promoter-probe vector	38
pUC18	Ap^r, cloning vector	Takara
pUC18Pr	Ap^r, 2.8-kb *Eco*RV-*Nhe*I fragment of pROR501 cloned into a *Hin*cII-*Xba*I site of pUC18	This study
pRC50Pr	Tc^r Cm^r, 2.8-kb *Hin*dIII-*Bam*HI fragment of pUC18Pr cloned into pRC50; *phcR*::*lacZ* transcriptional fusion	This study
pRC50Pk	Tc^r Cm^r, *phcKL*::*lacZ* transcriptional fusion	38
pUC18Pt	Ap^r, 2.5-kb *Xba*I-*Sal*I fragment of pROR501 cloned into pUC18	This study
pRC50Pt	Tc^r Cm^r, 2.5-kb *Eco*RI-*Hin*dIII fragment of pUC18Pt cloned into pRC50; *phcT*::*lacZ* transcriptional fusion	This study

[a] Abbreviations: Phl$^+$, growth on phenol; Phl$^-$, no growth on phenol; Ap^r, ampicillin resistant; Cb^r, carbenicillin resistant; Ap^r/Cb^r, resistant to both ampicillin and carbenicillin; Tc^r, tetracycline resistant; Cm^r, chloramphenicol resistant.

both the *phcT* and *phcS* genes. The *phcT* gene on pSK1 was disrupted by inserting a 1.7-kb *Eco*RV fragment of pMT5056, which carried a tetracycline resistance (Tc^r) gene, into the blunted *Sac*II-*Sal*I site of pSK1 (pSK01T). A *Not*I fragment containing the mobilization cassette of pMT5071 was subsequently inserted into pSK01T. The plasmid thus constructed, pSK02T, was mobilized (7) from *E. coli* S17-1 to strain R5, and Tc^r selection was done on an M9 agar plate containing 200 mg of phenol per liter, 5% (wt/vol) of sucrose, and tetracycline. The transconjugants (*phcT* knockouts: strain R5T) were chosen for their sensitivity to carbenicillin, and their chromosomal DNAs were analyzed by the PCR to confirm that gene replacement had indeed occurred (data not shown).

The *phcR* gene on pBS2 was disrupted by inserting a 1.7-kb *Pvu*II fragment of pMT5056, which carried a Tc^r gene, into the *Pvu*II-*Pvu*II site of pBS2 (pBS01R). The *Not*I fragment of pMT5071 was subsequently inserted into pBS01R. The plasmid thus constructed, pBS02R, was mobilized from *E. coli* S17-1 to strain R5, and Tc^r selection was done on an M9 agar plate containing 600 mg of sodium acetate per liter, 5% (wt/vol) sucrose, and tetracycline. The transconjugants (*phcR* knockouts: strain R5R) were chosen and analyzed as just described above.

The *phcT* and *phcS* genes on pSK1 were disrupted by inserting the 1.7-kb *Pvu*II fragment of pMT5056 into the blunted *Sac*II-*Apa*I site of pSK1 (pSK01TS). The *Not*I fragment of pMT5071 was subsequently inserted into pSK01TS. The plasmid thus constructed, pSK02TS, was mobilized from *E. coli* S17-1 to strain R5, and Tc^r selection was done on an M9 agar plate containing 200 mg of phenol per liter, 5% (wt/vol) sucrose, and tetracycline. The transconjugants (knockouts defective in both the *phcT* and *phcS* genes: strain R5TS) were chosen and analyzed as described above.

Assay for phenol-oxygenating activity. The phenol-oxygenating activity (oxygen uptake rate) was measured at 25°C with a Clark-type oxygen electrode (5/6 Oxygraph; Gilson) as described previously (37). The activity, which was measured in the presence of 10 mM potassium cyanide following the addition of phenol (final concentration, 10 μM), represents the amount of oxygen consumed equally by phenol hydroxylase (PH) and catechol 2,3-dioxygenase (C23DOase). A previous study had indicated that the phenol-oxygenating activity was double the PH activity, showing that the activity of C23DOase is higher than that of PH (42). The cell weight (dry weight) was determined as described previously (37). Cells from a continuous culture were sampled immediately before the activity was measured.

Induction experiment. The expression of mPH under batch culture conditions was examined as described below. *C. testosteroni* strains were grown in LB medium to the stationary phase, harvested, washed with MP medium, resuspended in the original culture volume of MP medium, and finally exposed to 2 mM phenol at 30°C for the indicated periods of time during shaking at 100 rpm. Before the phenol-oxygenating activity was measured, the culture was washed with MP medium and then resuspended in the same medium. *C. testosteroni* strains transformed with a pRC50 derivative were grown to the stationary phase in LB medium containing Cm and then washed and resuspended in MP medium containing phenol as just described above. Before the activity of β-galactosidase, the *lacZ* gene product, was measured (see below), the culture was washed with MP medium and then resuspended in the same medium. After each experiment, maintenance of the plasmid was checked by using nonselective and selective plates (supplemented with Cm).

Other methods. The conditions used for continuous culture and sampling from the culture were as described previously (38). The C23DOase activity was measured by the method described previously (38). The protein concentration was determined by the method of Bradford (5) with a protein assay kit (Bio-Rad), using bovine serum albumin as the standard. The activity of β-galactosidase was determined by the protocol described by Miller (17).

Nucleotide sequence accession number. The nucleotide sequence of the 2.5-kb *Xba*I-*Sal*I region has been deposited in the DDBJ/EMBL/GenBank database under accession no. AB061422.

RESULTS AND DISCUSSION

Identification of the *phcT* gene. We analyzed the DNA region downstream of *phcS* cloned in pROR501 (38). Sequencing of the 2.5-kb *Xba*I-*Sal*I region identified a 771-bp open

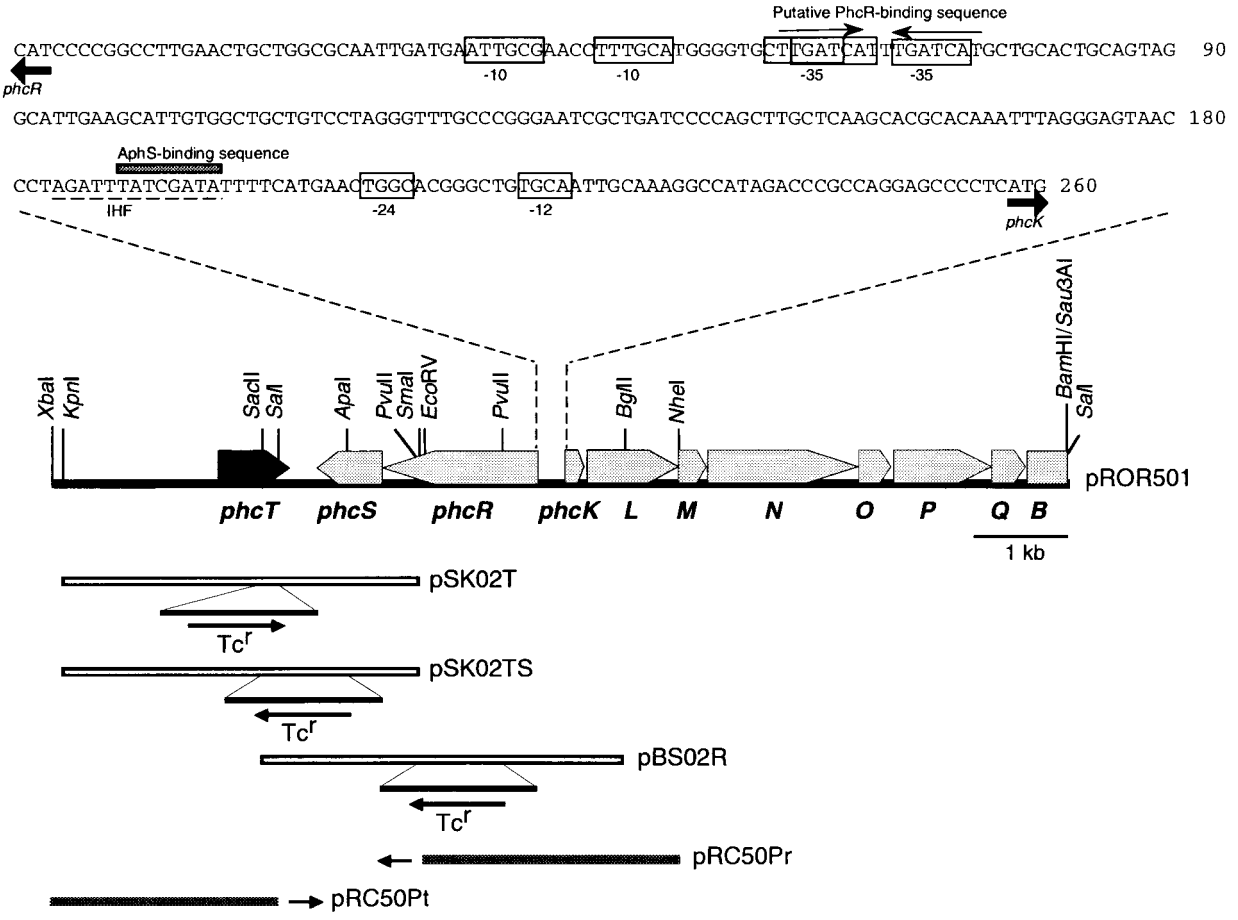

FIG. 1. Genetic organization of the regulatory and structural genes for mPH in *C. testosteroni* R5. *phcT* (in black) was identified in this study. The other genes have been identified in our previous studies (37, 38). pSK02T carries the *phcT* gene which was disrupted by inserting a Tc^r cassette, pSK02TS carries the *phcT* and *phcS* genes which were disrupted by inserting a Tc^r cassette, and pBS02R carries *phcR* which was disrupted by inserting a Tc^r cassette. The arrows indicate the direction of Tc^r transcription. pRC50Pr and pRC50Pt are derivatives of pRC50, pRC50Pr carrying an *Eco*RV-*Nhe*I fragment which contains the *phcR* promoter region and pRC50Pt carrying an *Xba*I-*Sal*I fragment which contains the *phcT* promoter region. The two small arrows indicate *lacZ* fused to these promoters on plasmids pRC50Pr and pRC50Pt. The nucleotide sequence of the *phcR-phcK* promoter region is also shown. Putative −35 and −10 sequences of the *phcR* promoter and putative −24 and −12 sequences of the *phcK* promoter are boxed (37). A putative IHF recognition sequence is indicated by the dashed underline (37). A putative PhcR-binding sequence, which is similar to the recognition sites of DmpR and XylR (30), is indicated by the pair of arrows. The shaded box indicates an AphS-binding sequence (1).

reading frame, named *phcT*, preceded by a putative Shine-Dalgarno sequence (27) (Fig. 1). This *phcT* gene encodes a protein of 257 amino acid residues with a predicted molecular mass of 28 kDa. The deduced product has 19% identity and 45% similarity to XylS, a transcriptional activator of the *meta*-cleavage pathway genes on *Pseudomonas putida* TOL plasmid pWW0 which are involved in the degradation of benzoate and substituted benzoates (13, 16, 35). The residues conserved in the AraC/XylS family of transcriptional regulators shown by Gallegos et al. (10) were well conserved in the PhcT sequence (Fig. 2). One characteristic of the AraC/XylS family is to show two putative helix-turn-helix (HTH) motifs within this conserved region that are likely to be involved in DNA binding (10). One HTH motif was indicated to be located between amino acid residues 175 and 196 in PhcT with the method described by Dodd and Egan (8). The other HTH motif conserved in the AraC/XylS family was predicted to be located between amino acid residues 211 and 252 in PhcT by a protein sequence motif search program (http://motif.genome.ad.jp/MOTIF.html). Therefore, PhcT was indicated to be a member

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Consensus: A-----S---L---F----G----------R---A---L---------(I/V)--(I/V)A---G(F/Y)-----F---F(R/K)---G--P
XylS(234): A     S   L   F    G          K   I   L           I     I  A   G  F       F   Y  R     G  P
PhcT(181): A     G   L   F    G          R   A   I           V     V  A   G  Y       F   F  R     G  P
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FIG. 2. Comparison of the PhcT amino acid sequence with the consensus sequence for the AraC/XylS family (10). Hyphens indicate nonconserved amino acid residues. The position of the first amino acid residue in each of the XylS and PhcT proteins is indicated by the numbers in parentheses.

TABLE 2. Effect of disrupting *phcT* on the expression of the mPH operon in *C. testosteroni* R5 grown on phenol in a continuous culture

Strain	Phenol-oxygenating activity (μmol min^{-1} [g of dry cells]$^{-1}$)	C23DOase activity (μmol min^{-1} [mg of protein]$^{-1}$)
R5[a]	213 ± 5 (100)	2.27 ± 0.14 (100)
R5T	117 ± 7 (55)	1.58 ± 0.11 (70)

[a] Data are from our previous study (38). Each value is the mean ± standard error from two or three independent cultures. Values in parentheses show the percentage of the value from strain R5.

of the AraC/XylS family of transcriptional regulators. A gene corresponding to *phcT* has not previously been found in the mPH gene clusters of other bacteria.

Enhancement of the mPH operon expression by PhcT. We constructed a *phcT* knockout of *C. testosteroni* strain R5 (R5T) to examine the physiological role of *phcT*. Strain R5T was cultured in a chemostat with phenol as the sole carbon source, and the phenol-oxygenating activity and C23DOase activity of the culture of strain R5T were compared with those of parental strain R5 (Table 2). A C23DOase gene was found downstream of the *phc* mPH genes (*phcB* in Fig. 1) (37) and was thought to be transcribed in the same unit as mPH genes (28). Therefore, C23DOase activity was measured to monitor the transcriptional level of the mPH genes. The phenol-oxygenating activity of strain R5T was 55% of that of strain R5, and the level of C23DOase activity was positively correlated with that of the phenol-oxygenating activity (Table 2). These results suggest that PhcT promoted the expression of the mPH operon at the transcriptional level. The protein profiles in strains R5T and R5, which had been grown in a chemostat with phenol, were also analyzed by SDS-PAGE using mPH subunit proteins (PhcKLMNOP) overexpressed in *E. coli* as molecular markers. The quantities of the mPH subunits determined by SDS-PAGE showed good correlation with the phenol-oxygenating activity (data not shown).

The expression of the mPH operon in response to phenol in strains R5T and R5 was also examined in batch cultures (Fig. 3). It was reduced upon introduction of the *phcT* disruption. The patterns of mPH expression matched the transcriptional patterns of the mPH operon monitored by *phcKL*::*lacZ* transcriptional fusion (Fig. 4A and B), suggesting that PhcT promoted mPH expression at the transcriptional level. Neither the transcriptional level of *phcR*, a transcriptional activator gene for the mPH operon, nor that of *phcT* was affected by phenol (Fig. 4). Interestingly, the transcription level of *phcR* was much higher in strain R5T than in strain R5 (Fig. 4A and B). In spite of the higher level of expression of *phcR* in strain R5T, the phenol-oxygenating activity was lower in this strain than in the wild-type strain. These results suggest that PhcT exerted positive control on the mPH operon and negative control on *phcR* and that PhcR was not rate limiting for the expression of the mPH operon. Similar regulation has been reported for the genes involved in the toluene/xylene degradation pathway encoded on the *P. putida* TOL plasmid. XylR activated the σ^{54}-dependent promoter of *xylS* (*Ps*1) while repressing its own σ^{70} promoters (*Pr*1 and *Pr*2). Such regulation was observed because the σ^{70}-RNA polymerase binding sites of *Pr*1 and *Pr*2 overlap the upstream activating sequences of the divergently organized σ^{54} promoter *Ps*1 (4, 15).

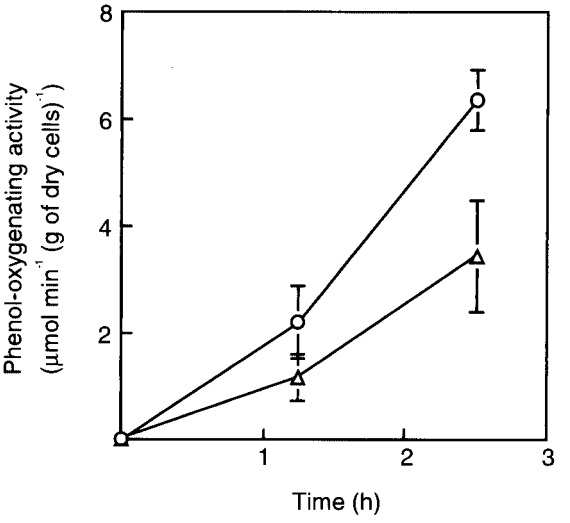

FIG. 3. mPH expression in *C. testosteron*i strains R5 and R5T in response to phenol. Stationary-phase cultures of strains R5 (circles) and R5T (triangles) grown on LB medium were exposed to MP medium supplemented with 2 mM phenol (at time 0). Each value is the mean ± standard error from four independent experiments.

PhcT as an auxiliary factor for the PhcR-dependent transcriptional activation of the mPH operon. We constructed a *phcR* knockout of *C. testosteroni* R5 (R5R) to examine whether PhcT alone would be sufficient to cause expression of the mPH operon. Strain R5R transformed with pRC50Pt showed higher β-galactosidase activity than strain R5R transformed with pRC50 (Fig. 4C), indicating that PhcT was expressed in the R5R strain. Strain R5R was unable to grow on phenol as the sole carbon source (data not shown). These results strongly suggest that PhcT alone was insufficient to induce expression of the Phc mPH operon.

Role of PhcT in the gratuitous expression of the mPH operon. We have reported that a *phcS* knockout of *C. testosteroni* R5 (R5S) expressed the mPH operon even in the absence of the genuine substrate for mPH (38). To test the involvement of PhcT in this gratuitous expression, we constructed *C. testosteroni* R5 defective in both the *phcS* and *phcT* genes (R5TS). Strain R5TS was continuously cultured in a chemostat with acetate as the sole carbon source, and the phenol-oxygenating activity of the culture was measured. The activity of strain R5TS grown on acetate was at the same level as that of strain R5S grown on acetate (data not shown), indicating that PhcT was not involved in the gratuitous expression of the mPH operon.

Proposed model for the control of the phenol-oxidizing operon in *Comamonas*. Together with our previous results (37, 38), a model for the novel transcriptional regulation of the mPH operon is proposed (Fig. 5). In the absence of the genuine substrate for mPH, PhcR-dependent gratuitous expression of the mPH operon was blocked by PhcS (38). As an AphS (PhcS-like protein)-binding site overlapped a putative integration host factor (IHF) recognition sequence (Fig. 1), bending of the promoter region for the mPH operon by the IHF for contact between PhcR and the σ^{54}-RNA polymerase holoenzyme (23) might have been hampered by the binding of PhcS on the IHF recognition sequence. The hypothesis that the

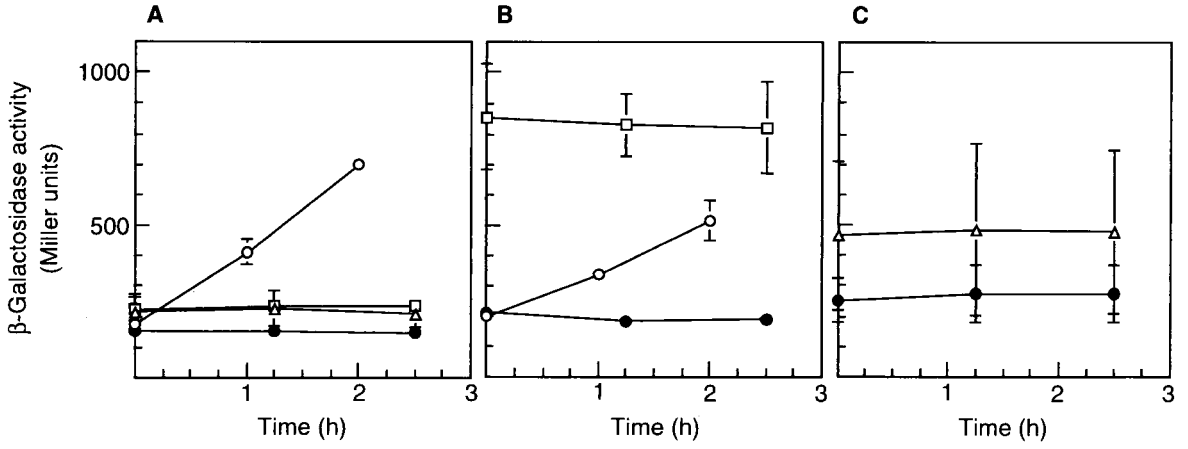

FIG. 4. Transcriptional activities of the *phcK*, *phcR*, and *phcT* promoters in *C. testosteroni* R5 (A), of the *phcK* and *phcR* promoters in *C. testosteroni* R5T (B), and of the *phcT* promoter in *C. testosteroni* R5R (C) in response to phenol. The strains were transformed with pRC50Pk, which carries the transcriptional fusion of *phcKL*::*lacZ* (circles), pRC50Pr, which carries the transcriptional fusion of *phcR*::*lacZ* (squares), pRC50Pt, which carries the transcriptional fusion of *phcT*::*lacZ* (triangles), or pRC50 (control vector [solid circles]). The first and second genes of the mPH operon are *phcK* and *phcL*, respectively (Fig. 1). At time zero, each stationary-phase culture was transferred to MP medium supplemented with 2 mM phenol. Each value is the mean ± standard error from three or four independent experiments.

binding of IHF to the IHF recognition sequence is inhibited in a competitive manner by PhcS has not been experimentally tested. PhcT was not involved in the gratuitous expression (Fig. 5B). In the presence of the genuine substrate for mPH, the action of PhcS was prevented by an as yet uncharacterized factor, X, and PhcR could interact with the σ^{54}-RNA polymerase holoenzyme. Phenol itself could not be the factor X, since PhcS was suggested to cause transcriptional repression of the Phc mPH genes in the presence of phenol in a heterologous *Pseudomonas aeruginosa* host (38). PhcT reduced the transcription of *phcR* but enhanced the PhcR-mediated transcriptional activation of the mPH promoter (Fig. 5A). We speculate that PhcR in strain R5 was enough to fully activate the mPH promoter and that excess PhcR in strain R5T was not used for this transcriptional activation. The mode of action of PhcT is not yet clear, but one possibility is that PhcT bound to the *phcR* promoter region decreased the transcription of *phcR* but interacted with PhcR to enhance the transcription of the mPH operon (Fig. 1 and 5A).

We have thus demonstrated in this study that acquisition of PhcT was largely responsible for the high phenol-oxygenating activity of strain R5. Acquiring PhcT may also be advantageous for limiting the excess expression of PhcR. These features must have been beneficial for strain R5 to predominate over other

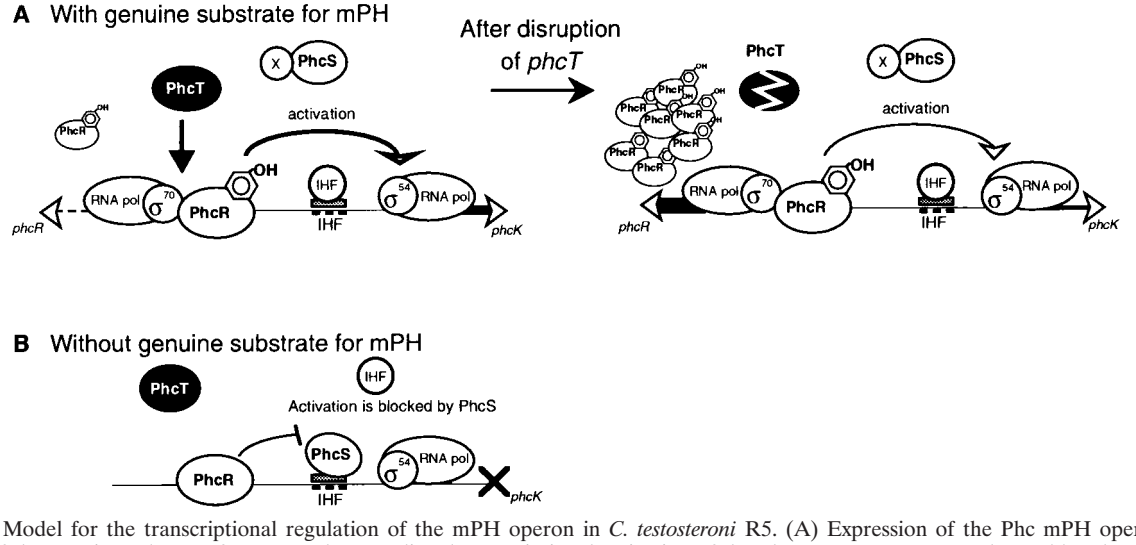

FIG. 5. Model for the transcriptional regulation of the mPH operon in *C. testosteroni* R5. (A) Expression of the Phc mPH operon in the presence of the genuine substrate for mPH. PhcR-mediated transcriptional activation of the Phc mPH operon was enhanced by PhcT-mediated regulation, while transcription of *phcR* was largely inhibited by this regulation. The action of PhcS was prevented by factor X. In the absence of PhcT, PhcR-mediated transcriptional activation was not enhanced, and the inhibition of *phcR* transcription was relieved. (B) Expression of the Phc mPH operon in the absence of the genuine substrate for mPH. PhcR-mediated transcriptional activation of the Phc mPH operon was repressed by PhcS (38). PhcT was not involved in the gratuitous expression which occurred in strain R5S in the absence of the genuine substrate for mPH.

members of the microbial community and survive in the natural environment.

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