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MINIREVIEW

Regulation of a Bacterial Aromatic Monooxygenase Pathway in Response to Solvent Exposure

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Research in our laboratory focuses on the determinants of biodegradability of xenobiotic compounds. The model system that is currently being investigated in detail is a study of mechanisms governing regulation of metabolic diversity for degradation of benzene, toluene, ethylbenzene, and xylenes (collectively designated BTEX), as well as the chlorinated solvents perchloroethylene (PCE) and trichloroethylene (TCE). These compounds have been chosen as model substrates because of their ubiquity as groundwater contaminants in the Unites States, and because they are a primary target of federal and state regulations aimed at aquifer restoration owing to their potential negative impact on human health.

The results of the past several years of research have led us to the conclusion that bacteria such as *Ralstonia pickettii* PKO1 are representative of a group of bacterial strains which, from our work to date, appear to be largely indistinguishable from closely related species by previous criteria, but which have evolved a suite of adaptive traits that allow for growth and oxygen metabolism in oxygen-limited environments. These adaptive traits include the ability to carry out significant nitrate-dependent degradation of aromatic hydrocarbons under conditions of oxygen limitation; the presence of key catabolic enzymes with kinetic characteristics that allow for effective turnover of limiting substrates; and transcriptional enhancement of promoters of key catabolic operons linked to the onset of denitrification. Such results have led us to advance the hypothesis that uniquely adapted bacteria, typified by strain PKO1 (Kukor and Olsen, 1990a; Mikesell *et al.*, 1993; Olsen *et al.*, 1994b, 1995), are capable of degrading aromatic hydrocarbons in hypoxic aquifer environments by a physiological strategy of "oxygen sparing", in which aerobic nitrate respiration allows for utilization of low residual levels of oxygen for critical substrate oxygenase reactions.

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The genes encoding enzymes for the toluene/benzene/TCE catabolic pathway have been cloned from strain PKO1 (Olsen *et al.*, 1994a) and have been shown to be organized into three operons: the *tbuA1UBVA2C* and *tbuT* operon encoding the initial toluene-3-monooxygenase and the transcriptional activator TbuT (Byrne and Olsen, 1996), the *tbuD* operon encoding phenol/cresol hydroxylase (Kukor and Olsen, 1990b,1992), and the *tbuWEFGKIHJ* operon encoding enzymes of the *meta*-cleavage pathway for conversion of catechol and methylcatechols to tricarboxylic acid cycle intermediates (Kukor and Olsen, 1991) (Fig. 1).

Transcriptional fusion assays as well as physiological analyses have shown that TbuT, a σ^{54}-dependent NtrC-like activator, controls transcription of each of these operons in response to aromatic effector compounds.

The two major promoters that control transcription of the *tbu* catabolic pathway genes are *PtbuA1* and *PtbuD*. These promoters are divergently arranged. Sequence and functional analyses have shown that both of these promoters are dependent on the alternative sigma factor, σ^{54}, and that activation of the promoters is dependent on binding to DNA upstream activation sequences (UAS) located 100 to 200 bp upstream of the promoters that they regulate. The sequence motif 5- TTGANCAAATC-3, which is highly homologous to the palindromic region upstream of

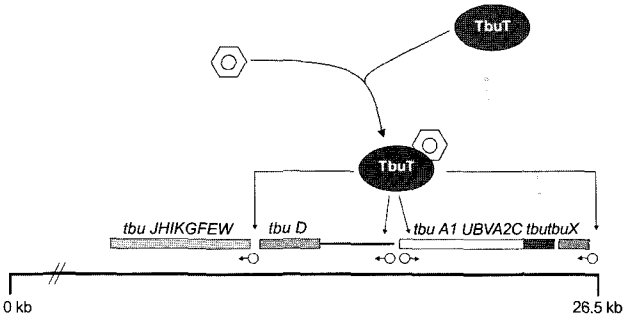

Fig. 1. Arrangement of the *tbu* operons.

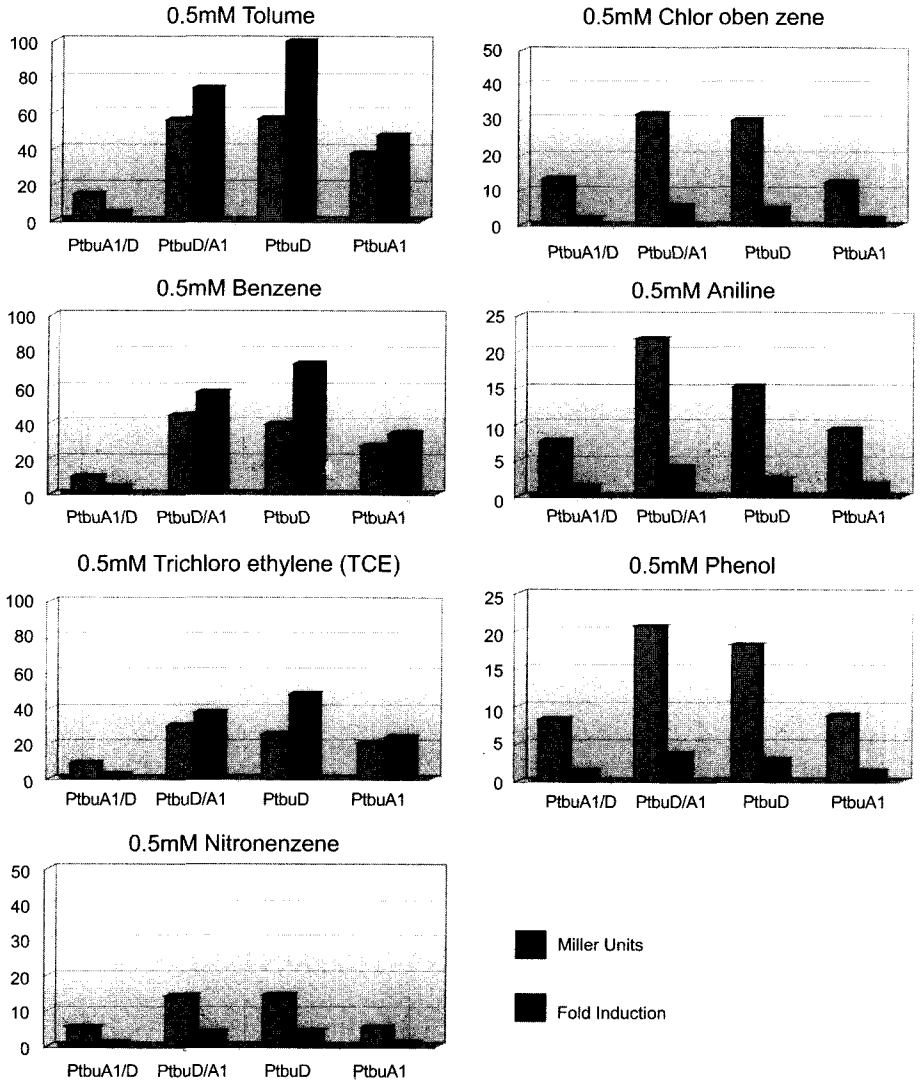

Fig. 2. Comparative promoter response.

Pu, the upper pathway promoter from the TOL plasmid to which the transcriptional activator XylR has been shown to bind (de Lorenzo *et al.*, 1991; Inouye *et al.*, 1990), is present in the UAS region upstream of both *PtbuA1* and *PtbuD*, however it is of interest to note that the spacing between the conserved inverted repeats differs between *PtbuA1* and *PtbuD*. This difference in UAS palindrome architecture is correlated with promoter activity, i.e., greater promoter activity is observed when *tbuT* is in *trans* with promoter/UAS *lacZ* fusions of *PtbuD*, than with fusions of *PtbuA1*(Fig. 2). This shows that promoter function can be affected by the structure of the site to which the transcriptional activator binds.

In addition to promoter strength being controlled directly by the nature of TbuT binding to the UAS palindrome, we have also demonstrated that the chemical structure of the activator can exert an effect on the strength of the promoter response. Compounds with methyl or halogen substituents are better effectors than those containing nitro-, hydroxyl, or amino groups.

The *tbu* regulon appears to be unique among bacterial regulatory systems in that the binding of a single transcriptional activator, TbuT, to multiple promoter-upstream regions of different architecture appears to allow for differential promoter response.

In addition to the transcriptional activator, *tbuT*, *tbuX* has also been found to play a role in the ability of strain PKO1 to utilize hydrocarbons. TbuX is a putative outer membrane protein, and its deletion affects the overall physiology of PKO1 cells vis-à-vis toluene utilization. At very low toluene concentrations, the TbuX knockout appears to have a growth advantage over the wild-type, whereas at higher toluene concentrations the wild-type grows faster than the knockout. These results suggest that TbuX may play a role in hydrocarbon utilization that is more complex than that of a simple porin.

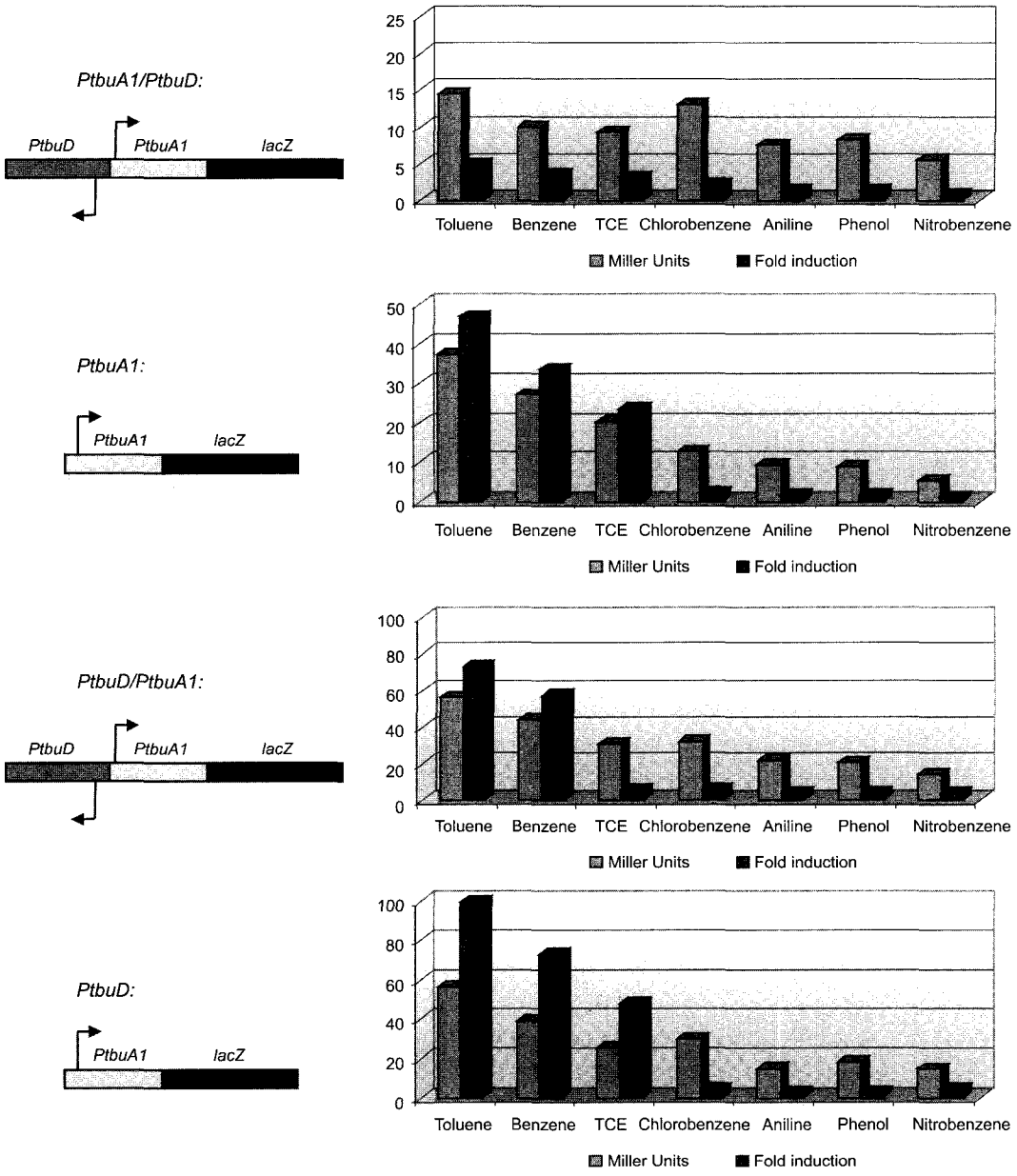

Fig. 3. Strength of promoter response.

Our previous work on strain PKO1 has demonstrated that this organism is capable of oxidative attack on hydrocarbons in low-oxygen environments when nitrate is available as an alternate respiratory electron acceptor. In part this is due to transcriptional enhancement of promoters of key catabolic operons linked to the onset of denitrification. This appears to be manifest as an elevated "basal level" of transcription of *PtbuA1* or *PtbuD* under conditions where nitrate is present or when the oxygen concentration has been reduced. Identification of sequences upstream of the major *tbu* promoters that are homologous to the binding site for ANR, the anaerobic regulator of arginine deiminase and nitrate reduction of *Pseudomonas aeruginosa* PAO (Gamper *et al.*, 1991), suggests that expression of the *tbu* pathway operons is influenced by oxygen-limited, denitrifying conditions. Moreover, this elevated basal level of transcription under low oxygen conditions in the presence of nitrate suggests the possibility that transcription of the *tbu* promoters may be controlled by elements other than TbuT (Kukor and Olsen, 1996).

The immediate goal of our research on the PKO1 model system is to further elucidate an adaptive strategy of microbial utilization of mixed electron acceptors (viz., oxygen and nitrate) from the perspective of "global regulation" of catabolic operons encoding critical aromatic oxygenases. A longer range goal is to assess the func-

wt = Wildtype

C8 = PKO1*tbuX* Knockout

Absorbance at 600 nm	
- = <	
+ =	- 0.4
++ = >	- 0.6
+++ = >	- 0.8
++++ = >	- 1.0
+++++ = >	

		10 hr	13.5 hr	15.5 hr	22.5 hr	37 hr	47 hr
1mM	wt	-	-	+	+	+	+
	C8	-	+	+	+	+	+
5 mM	wt	-	+	++	+++	+++	+++
	C8	-	+	++	+++	+++	+++
10 mM	wt	-	+	+++	++++	++++	++++
	C8	-	++	+++	++++	++++	++++
15 mM	wt	-	-	-	++++	+++++	+++++
	C8	-	-	-	++	+++++	+++++
20 mM	wt	-	-	-	-	-	-
	C8	-	-	-	-	-	-

Fig. 4. Growth rates of PKO1 wildtype vs. PKO1 *tbuX* knockout at different concentrations of toluene.

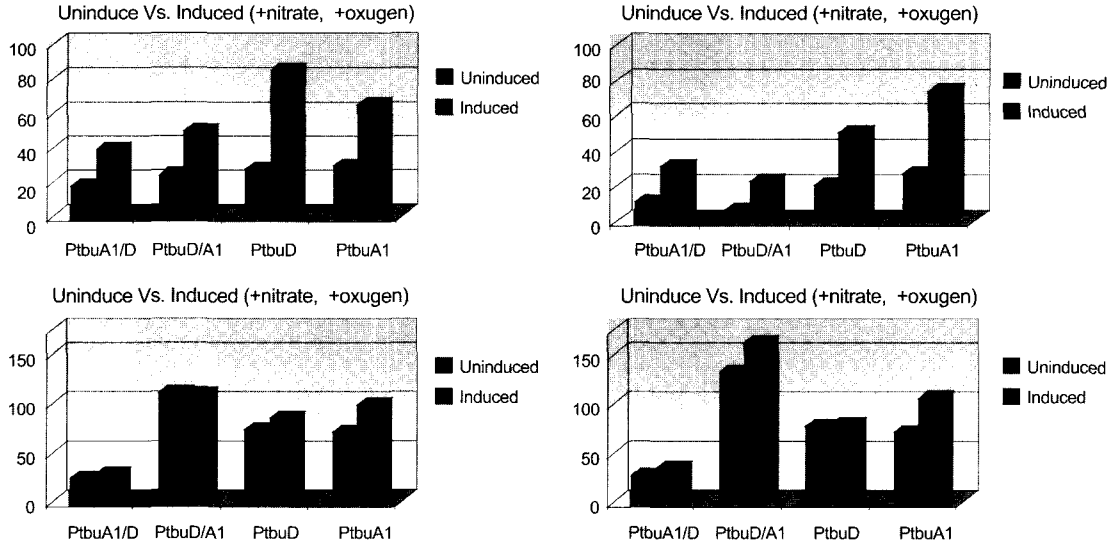

Fig. 5. Oxygen and nitrate effects on p*tbu* promoror activity.

tional role of such bacteria as components of complex microbial communities, with particular emphasis on their adaptation towards functionality in oligotrophic, oxygen-variable environments.

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