Less is more: reduced catechol production permits *Pseudomonas putida* F1 to grow on styrene

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*Pseudomonas putida* F1 is unable to grow on styrene due to the accumulation of 3-vinylcatechol, a toxic metabolite that is produced through the toluene degradation (*tod*) pathway and causes catechol-2,3-dioxygenase (C23O) inactivation. In this study, we characterized a spontaneous F1 mutant, designated SF1, which acquired the ability to grow on styrene and did not accumulate 3-vinylcatechol. Whereas adaptation to new aromatic substrates has typically been shown to involve increased C23O activity or the acquisition of resistance to C23O inactivation, SF1 retained wild-type C23O activity. Surprisingly, SF1 grew more slowly on toluene, its native substrate, and exhibited reduced toluene dioxygenase (TDO) activity (approximately 50% of that of F1), the enzyme responsible for ring hydroxylation and subsequent production of 3-vinylcatechol. DNA sequence analysis of the *tod* operon of SF1 revealed a single base pair mutation in *todA* (C479T), a gene encoding the reductase component of TDO. Replacement of the wild-type *todA* allele in F1 with *todA*C479T reduced TDO activity to SF1 levels, obviated vinylcatechol accumulation, and conferred the ability to grow on styrene. This novel ‘less is more’ strategy – reduced catechol production as a means to expand growth substrate range – sheds light on an alternative approach for managing catechol toxicity during the metabolism of aromatic compounds.

**INTRODUCTION**

Although the biodegradation of aromatic compounds has been studied extensively for over 60 years (Stanier, 1948; Evans *et al.*, 1949; Gibson *et al.*, 1968; van der Meer *et al.*, 1992; Díaz, 2004; Cao *et al.*, 2009), there is still much to learn about bacterial adaptations which augment catabolic capacity or expand growth substrate range. Despite the continued discovery of novel strategies for growth on aromatics (George & Hay, 2011), the actual mechanism of aromatic degradation is highly conserved. In general, pathways converge towards intermediates such as protocatechuate, gentisate, hydroquinone and catechol prior to ring cleavage by a dioxygenase (Gibson & Subramanian, 1984). Of these potential intermediates, catechols, produced during the biodegradation of numerous aromatic pollutants such as benzene and toluene, are among the most common (Vaillancourt *et al.*, 2006). Catechol intermediates are enzymically cleaved by either extradiol or intradiol dioxygenases, which cleave the ring at the 2,3-(meta) or 1,2- (ortho) position of the catechol, respectively (Harayama & Rekik, 1989). Compared with intradiol dioxygenases, extradiol dioxygenases (catechol-2,3-dioxygenases; C23Os) cleave a larger array of substrates and also occur in a wider range of biodegradation pathways (Vaillancourt *et al.*, 2006). Consequently, C23Os are generally recognized as more versatile ring-cleavage enzymes.

C23Os may be substrate-inhibited through a variety of potential mechanisms, including iron chelation, iron oxidation and suicide inactivation (reviewed by Vaillancourt *et al.*, 2006). C23O inhibition often leads to catechol accumulation and limits the range of substrates available to a microorganism for growth (Klecka & Gibson, 1981; Bartels *et al.*, 1984; Ramos *et al.*, 1987; Rojo *et al.*, 1987; Cerdan *et al.*, 1994, 1995; Vaillancourt *et al.*, 2002; Ward *et al.*, 2004). Several studies have highlighted the deleterious effects of catechol accumulation during aromatic hydrocarbon metabolism (Pérez-Pantoja *et al.*, 2003; Park *et al.*, 2004; Park & Madsen, 2004). In addition to suicide inhibition, catechols can initiate toxicity through a diverse range of molecular mechanisms, ranging from the production of reactive oxygen species to direct protein damage (Schweigert *et al.*, 2001a, b). Given this toxicity, bacterial cells have developed multiple strategies for preventing catechol accumulation. Previously described adaptations include expression of C23O-reactivating [2Fe–2S] ferredoxins (Polissi & Harayama, 1993; Cerdan *et al.*, 1994; Hugo *et al.*, 1998, 2000; Park *et al.*, 2002), recruitment of inactivation-resistant C23Os (Ramos *et al.*, 2004), and adaptation of C23O activity (Harayama & Rekik, 1989).

Abbreviations: C23O, catechol-2,3-dioxygenase; TDO, toluene dioxygenase; 6-vinyl HODA, 2-hydroxy-6-vinylhexa-2,4-dienoate.

Two supplementary figures are available with the online version of this paper.
Table 1. Strains and plasmids used in this study

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<th>Strain or plasmid</th>
<th>Characteristics</th>
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<tr>
<td><em>P. putida</em> F1</td>
<td>CmR, AmpR; model organism containing the <em>tod</em> operon</td>
<td>Gibson et al. (1968)</td>
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<td><em>P. putida</em> SF1</td>
<td>Styrene-adapted F1 mutant containing <em>todA</em>&lt;sub&gt;C479T&lt;/sub&gt; allele</td>
<td>This study</td>
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<tr>
<td>F1* todA*&lt;sub&gt;C479T&lt;/sub&gt;</td>
<td>F1 double recombinant containing <em>todA</em>&lt;sub&gt;C479T&lt;/sub&gt; allele (naive with respect to styrene)</td>
<td>This study</td>
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<td><em>E. coli</em> S17-1 <em>Δpir</em></td>
<td>*Δpir; hsdR pro recA RP4 2-Tc::Mu-Km::Tn7; pro, res&lt;sup&gt;+&lt;/sup&gt;, mod&lt;sup&gt;+&lt;/sup&gt;, Str&lt;sup&gt;R&lt;/sup&gt;, Trm&lt;sup&gt;R&lt;/sup&gt;</td>
<td>de Lorenzo &amp; Timmis (1994)</td>
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<td><em>E. coli</em> BW21037</td>
<td>DE3(lac)&lt;sub&gt;24&lt;/sub&gt; uaaD(A mutant)::pir-116 Tn5lac4Cm&lt;sup&gt;R&lt;/sup&gt; lacI&lt;sub&gt;Q&lt;/sub&gt; ΔlacZM15 recA1aphnC1ΩDEFGHIJKLMNOP33–30 ΔphoA532</td>
<td>Metcalf et al. (1996)</td>
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**Plasmids**

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<td>pKNG101</td>
<td>Suicide vector, <em>pir</em>&lt;sup&gt;+&lt;/sup&gt; oriR6K mobRR2 sacB Str&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Kaniga et al. (1991)</td>
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<tr>
<td>pBBR1MCS-5</td>
<td>Gm&lt;sup&gt;R&lt;/sup&gt;, broad-host-range expression vector</td>
<td>Kovach et al. (1995)</td>
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<td>pPROBE-NT</td>
<td>Km&lt;sup&gt;R&lt;/sup&gt;, Used to generate GFP transcriptional fusions</td>
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<td>pKNGkm <strong>todA</strong>&lt;sub&gt;C479T&lt;/sub&gt;</td>
<td>Km&lt;sup&gt;R&lt;/sup&gt; derivative of pKNG101 containing a 212 bp fragment of <em>todA</em>&lt;sub&gt;SF1&lt;/sub&gt;; used to generate F1 <strong>todA</strong>&lt;sub&gt;C479T&lt;/sub&gt;</td>
<td>This study</td>
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<td>pTodA</td>
<td>Derivative of pBBR1MCS-5 containing <em>todA</em> from F1; TodA expression vector</td>
<td>This study</td>
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<td>pPROBE:&lt;b&gt;P&lt;/b&gt;&lt;sub&gt;<em>todX</em>&lt;/sub&gt;</td>
<td>GFP transcriptional fusion containing P&lt;sub&gt;<em>todX</em>&lt;/sub&gt;</td>
<td>This study</td>
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1987; Rojo et al., 1987; Cerdan et al., 1994) and selection for chromosomal gene duplications which increase catechol consumption (van der Meer et al., 1998; Müller et al., 2003; Pérez-Pantoja et al., 2003). In the case of chlorocatechols, it has been demonstrated that cells must develop a delicate balance between catechol-producing and catechol-consuming reactions to avoid toxicity and permit growth on 3-chlorobenzoic acid (Pérez-Pantoja et al., 2003).

Recently, we found that C23O inactivation is the crucial factor which prevents *Pseudomonas putida* F1 from growing on styrene (George et al., 2011). Inactivation of TodE, the native C23O of the toluene degradation (*tod*) pathway, resulted in 3-vinylcatechol accumulation, toxicity and cell death. Increasing the expression of TodE was sufficient to avoid catechol toxicity and permitted F1 to grow on styrene. During that work we also obtained spontaneous F1 mutants capable of growing on styrene.

In this study, we describe a novel strategy used by a *P. putida* F1 mutant to overcome the catechol toxicity associated with styrene metabolism. Through a combination of genetic assays and genetic analyses, we demonstrate that a single mutation leading to decreased toluene dioxygenase (TDO) activity is sufficient to reduce production of 3-vinylcatechol and its associated deleterious effects, thereby permitting growth on styrene.

**METHODS**

**Bacterial strains, plasmids and growth conditions.** *P. putida* F1, *P. putida* SF1, F1 **todA**<sub>C479T</sub> and derivative plasmid-carrying strains were grown with shaking (100 r.p.m.) at room temperature in minimal salts medium (MSM) (Focht, 1994) containing suitable growth substrates. Toluene or styrene was provided as vapour for growth on agar plates or at a concentration of 5 mM in liquid media. When specified, acetate was supplied at a concentration of 5 mM in MSM liquid media. *Escherichia coli* S17-1 *Δpir* (de Lorenzo & Timmis, 1994) strains were grown at 37 °C in LB containing 50 μg streptomycin ml<sup>−1</sup>. Strains containing pBBR1MCS-5 and pKNGkm **todA**<sub>C479T</sub> were selected on 25 mg gentamicin ml<sup>−1</sup> or 50 μg kanamycin ml<sup>−1</sup>, respectively. A list of strains and plasmids used in this study is presented in Table 1.

**Cloning and DNA manipulation.** Primers for genes in the *tod* operon were designed based on the published genomic sequence of *P. putida* F1 (GenBank accession no. NC_009512). PCR was performed using HotStart Master Mix from Qiagen and a PTC-200 DNA Engine thermocycler from MJ Research. PCR fragments were digested with appropriate enzymes and ligated into pBBR1MCS-5 (Gm<sup>R</sup>) or pKNG101 (Str<sup>R</sup>) using T4 DNA ligase (Promega). The resultant plasmids were transformed into appropriate hosts by electroporation. Transformants were selected on LB plates containing antibiotics and screened by PCR. All DNA sequencing was performed by the Bioresource Center at Cornell University on an ABI3700 DNA analyser.

**Isolation of styrene-adapted F1 (SF1).** *P. putida* F1 was grown overnight at room temperature in MSM acetate, washed twice in an equal volume of 30 mM KPO<sub>4</sub> buffer (pH 7.0), and serially diluted to 10<sup>−7</sup> in PB. Volumes (5 μl) of each dilution were spotted onto MSM agar and exposed to styrene vapours. After approximately 10 days of exposure, mutant colonies capable of growing on styrene appeared at a frequency of 10<sup>−6</sup>. One colony capable of robust growth was designated styrene-adapted F1 (SF1) and selected for further study.

**todA**<sub>c</sub> allele replacement in F1. A 212 bp fragment of *todA* (nucleotides 3377–3588 of GenBank accession no. J04996) was PCR-amplified from SF1 and cloned between the BamHI and Apal sites of pKNG101 (Kaniga et al., 1991). DNA sequencing was used to confirm the presence of the C479T transition following transformation into *E. coli* BW21037 (Metcalf et al., 1996) by electroporation. Since F1 exhibits background resistance to streptomycin, a kanamycin (Km)-resistant derivative of the plasmid was generated (pKNGkm **todA**<sub>C479T</sub>) in *E. coli* S17-1 *Δpir* using the EZ-Tn5 <KAN-2> insertion kit (Epicentre). Biparental mating of S17-1 *Δpir* pKNGkm **todA**<sub>C479T</sub> with F1 was used to generate Km<sup>R</sup> F1 recombinants with pKNGkm **todA**<sub>C479T</sub> integrated into the chromosome. PCR with an
upstream chromosomal primer and a pKNG101-specific primer yielded amplicons in all tested colonies, confirming integration into todA as expected. Since plasmid integration knocked out the ability of the F1 recombinants to grow on toluene, double-crossover mutants were selected on MSM agar exposed to toluene vapour in the absence of Km. Colonies capable of growth on toluene appeared at a frequency of approximately 10−5 and were Km−, which confirmed loss of pKNGKm todA<sub>C23O</sub>. To screen double recombinants, PCR was used to amplify the 212 bp todA region from putative double-crossover mutants. These fragments were then digested with the restriction endonuclease Hpy99I, which only cuts the wild-type allele. DNA sequencing was then employed to confirm the presence of the C479T mutation. A single confirmed double-crossover mutant that had never been exposed to styrene, designated F1 tod<sub>C479T</sub>A, was used for further study.

**Resting cell assays.** For resting cell enzymic assays, cells were grown to late exponential phase (OD<sub>600</sub> 0.8) at room temperature in MSM acetate and inoculated at 1:100 into fresh MSM medium containing acetate and toluene. Upon reaching late exponential phase, cells were harvested by centrifugation (10 000 g for 10 min at 4 °C), washed twice in 30 mM PB, and resuspended in an equal volume of sterile buffer.

**Measurement of C23O activity.** C23O activity was measured as the rate of increase in A<sub>460</sub>, following the addition of 2 mM catechol, as described elsewhere (Sala-Trepat & Evans, 1971). Briefly, 200 μl volumes of resting cells in PB were assayed in triplicate using a Bio-Tek Synergy HT-I microplate reader. Following the addition of catechol, initial rates of meta-cleavage were calculated and normalized to their OD<sub>600</sub>. Concentrations of catechol meta-fission product were calculated using a reported molar absorption coefficient of 40 μM<sup>−1</sup> cm<sup>−1</sup> (Seah et al., 1998).

**C23O inactivation assays.** To quantify C23O inactivation following substrate cleavage, cultures were exposed to 2 mM of the various substrates. For 3-vinylcatechol exposures, cultures were resuspended in filtered PB containing 50 μM substrate. 3-Vinylcatechol was synthesized biologically as previously described (George et al., 2011). At set time points, 1 ml aliquots were taken, centrifuged at 16 000 g for 1 min and resuspended in an equal volume of PB. C23O activity was measured as described above.

**Measurement of TDO activity.** TDO activity was quantified using the indoxyl fluorescence method (Woo et al., 2000). In resting whole-cell cultures, TDO activity was measured as the rate of indoxyl formation following the addition of indole in N,N-dimethylformamide to a final concentration of 250 μM. Assays were performed in triplicate using excitation and emission wavelengths of 360 and 460 nm, respectively, with a Bio-Tek Synergy HT-I microplate reader.

**RESULTS**

**SF1 resists C23O inactivation following styrene exposure, but is equally susceptible to inactivation by 3-vinylcatechol**

Previously we found that F1 accumulates toxic levels of 3-vinylcatechol and loses C23O activity when exposed to styrene (George et al., 2011). Intriguingly, we noticed that SF1, an F1 mutant capable of robust growth on styrene, did not accumulate 3-vinylcatechol and retained C23O activity when exposed to styrene (Fig. 1). Based on our previous work and that of others describing growth on compounds such as chlorinated biphenyls and chlorobenzenes (Vaillancourt et al., 2002, 2005; Pérez-Pantoja et al., 2003), we initially hypothesized that the lack of 3-vinylcatechol accumulation in SF1 was due to an increase in C23O activity or the acquisition of 3-vinylcatechol-resistant C23O activity. Surprisingly, however, the level of C23O activity of SF1 was the same as that of F1 and was just as susceptible to inactivation by 3-vinylcatechol (Fig. 1). DNA sequencing also revealed that SF1 todE, encoding the native C23O of the tod operon, was 100% identical to that of F1 (accession no. NC_009512).

**TDO activity is reduced in SF1, but C23O activity is identical**

These preliminary observations suggested that SF1 might be employing a different strategy to overcome 3-vinylcatechol toxicity. The reduced growth rate (μ) of SF1 on toluene (0.24 h<sup>−1</sup> versus 0.47 h<sup>−1</sup> in the F1 wild-type), but not on acetate, provided the first hint as to what this might be (Fig. 2a). This discrepancy suggested that changes in the tod pathway that affected toluene degradation were potentially responsible for the newly acquired ability of SF1 to grow on styrene.

The similarity in C23O activity between F1 and SF1, despite clear differences in growth on toluene, suggested the potential involvement of an enzyme upstream of the C23O. In the tod pathway, transformation of styrene into 3-vinylcatechol is catalysed by the multi-component TDO,
encoded by todC1C2BA, and by cis-toluene dihydrodiol dehydrogenase, encoded by todD (Zylstra & Gibson, 1989). These enzymes catalyse the hydroxylation and reoaromatization of the ring, respectively. A simple fluorescence assay (Woo et al., 2000) was used to determine whether there was a difference in TDO activity between F1 and SF1 following induction with toluene. As Fig. 2(b) demonstrates, there was nearly a twofold decrease in TDO activity in SF1.

To ensure that this variation was not due to differential induction of tod pathway enzymes, C23O activity, which is transcribed from the same promoter as TDO activity, was measured and found to be identical in both strains (Fig. 2b). Transcriptional regulation of the tod operon in SF1 was also investigated using a P_{todX}–GFP reporter construct and found to be indistinguishable from regulation in F1 (see Supplementary Fig. S1 available with the online version of this paper). DNA sequencing of key regulatory genes and promoters in SF1, including todS, todT and todX, showed them also to be 100% identical to those of F1 (accession no. NC_009512).

todA from SF1 contains a single nucleotide polymorphism

The reduced TDO activity in SF1 suggested the existence of one or more mutations in todC1C2BA, the genes encoding the components of TDO. Sequencing of todC1, todC2 and todB revealed no nucleotide changes compared with F1. Sequencing of todA, which encodes the reductase component of TDO, however, revealed the existence of a single C to T transition at position 479 from the todA start codon in accession number J04996 (Zylstra & Gibson, 1989).

Introduction of todA_{C479T} into F1 confers reduced TDO activity, decreased C23O inactivation after styrene exposure, and the ability to grow on styrene

To determine whether the todA_{C479T} allele permitted SF1 to grow on styrene and caused its reduced TDO activity phenotype, the mutant allele was introduced into the chromosome of styrene-naive F1 through homologous recombination, generating F1 todA_{C479T}.

TDO activity in F1 todA_{C479T} was nearly identical to that in SF1 (Fig. 3a), as was the observed growth on toluene (Fig. 3b). We also tested the corollary of this hypothesis by introducing a wild-type copy of todA into SF1 (SF1pTodA). The expression of wild-type TodA in SF1pTodA increased TDO activity, although it did not completely restore it to F1 levels. The reduced growth rate on toluene, however, was eliminated by expression of wild-type TodA in SF1pTodA (Fig. 3b).

The presence of the todA_{C479T} allele in F1 also affected the level of C23O activity following styrene exposure: after a 1 h exposure to styrene, C23O activity in F1 todA_{C479T} was still more than 90% of that of an unexposed control (Fig. 3c). Compared with F1, this represented a sevenfold increase in C23O activity. SF1pTodA, which expressed both mutant and wild-type todA alleles, exhibited a 2.5-fold reduction in C23O activity compared with the parent strain. Despite the differences in activity following styrene exposure, the C23Os of each strain remained equally susceptible to 3-vinylcatechol (Fig. 3c).

As expected given its C23O inactivation behaviour, F1 todA_{C479T} was capable of growth on styrene as a sole...
carbon source (Fig. 3d), while growth of SF1pTodA on styrene was impeded, although not entirely eliminated despite the accumulation of polymerized 3-vinylcatechol (Fig. 4). The intermediate phenotype of SF1 pTodA, also observed in TDO and C23O activity measurements, was likely the result of chromosomal expression of todA<sub>C479T</sub> in addition to plasmid-encoded wild-type todA.

**DISCUSSION**

In this study, we characterized a novel mutant of *P. putida* F1 with reduced TDO activity that was capable of growing on styrene. This prevented C23O inactivation and more generalized catechol-related toxicity (Fig. 4).

TDO activity in SF1 was reduced as a consequence of the C479T transition in todA, which resulted in the replacement of Thr160 with Met160 (TodAT<sub>160M</sub>). This reduction in activity was likely caused by subtle structural changes in regions associated with electron transfer and docking of the ferredoxin component of the TDO holoenzyme. The ferredoxin (encoded by todB) shuttles electrons from the reductase (todA) to the terminal dioxygenase (todC1C2), with the latter ultimately catalysing the addition of dioxygen to the aromatic ring to form a dihydrodiol (Gibson et al., 1970). TodA is actually comprised of three domains: an FAD-binding domain (residues 1–108 and 239–317), an NADH-binding domain (residues 109–238), and a C-terminal domain (residues 318–410) (Friemann et al., 2009). Lys48 and Glu157 have been suggested to play conserved roles in electron transfer from NADH to FAD, while Trp320 of the C-terminal domain has been suggested to mediate electron shuttling from FAD to the Reiske centre of TodB, the ferredoxin (Friemann et al., 2009).

**Fig. 3.** Phenotypic effects of the todA<sub>C479T</sub> allele. (a) TDO activity. In F1 todA<sub>C479T</sub>, the mutant allele reduced TDO activity to SF1 levels. Conversely, SF1 with the wild-type todA allele (SF1 pTodA) had higher TDO activity than SF1 (P=0.001), although it was not as high as that of F1 (P=0.003). TDO activity was measured in resting cultures as described in Methods. Error bars, 1sd (n=3). (b) Growth on toluene. The reduced growth rate on toluene correlated with reduced TDO activity. F1 todA<sub>C479T</sub> mimicked the behaviour of SF1, while SF1pTodA nearly matched the growth characteristics of F1. Error bars, 1sd (n=3). (c) C23O inactivation behaviour. As expected, resting cultures of F1 todA<sub>C479T</sub> maintained wild-type C23O activity following exposure to styrene but not 3-vinylcatechol. In SF1pTodA, C23O inactivation was increased following styrene exposure relative to SF1. Error bars, 1sd (n=3). (d) Growth on styrene. F1 todA<sub>C479T</sub> was capable of growth on styrene as a sole carbon source, showing transient accumulation of yellow-coloured 2-hydroxy-6-vinylhexa-2,4-dienoate (6-vinyl HODA). Growth of SF1 (pTodA) was inhibited. Strains were grown for 48 h on MSM agar with styrene vapour.
TodAT160M, replacement of Thr160 with Met160 would be expected to prevent the formation of two hydrogen bonds from the threonine side chain hydroxyl to the main chain oxygen of Cys156 and nitrogen of Ala324, respectively (Fig. S2). Cys156 is located within the α-helix containing the putative catalytic residue Glu157, while Ala324 resides adjacent to the β-sheet containing Trp320. Although Thr160 does not interact with Glu157 or Trp320 directly, the dissolution of nearby hydrogen bonds following substitution to Met160 likely alters the position of these key catalytic residues.

The results of this study – namely, the ‘less is more’ approach of SF1 to mitigating catechol toxicity – contrast markedly with those observed during adaptation to other aromatic hydrocarbons. Although previous studies have also underscored the need for efficient catechol turnover in aromatic degradation pathways, particularly in the case of chlorinated catechol intermediates (van der Meer et al., 1998; Müller et al., 2003; Pérez-Pantoja et al., 2003), the organisms studied achieved this in a manner substantially different from SF1.

For example, while working with Ralstonia eutropha JMP134(pJP4), Pérez-Pantoja et al. (2003) found that multiple copies of tfdC, encoding chlorocatechol 1,2-dioxygenase, increased catechol turnover and were necessary for growth on 3-chlorobenzoate. Mutants lacking multiple copies of tfdC were unable to grow on 3-chlorobenzoate and were subject to toxicity due to the accumulation of chlorocatechols. Importantly, the authors noted that increasing the rate of chlorocatechol production through the heterologous expression of a broad-substrate toluate dioxygenase (xylS–xylXYZL) had a negative effect on growth. Their work made it clear that efficient growth on 3-chlorobenzoate requires the proper gene dosage and a delicate balance between chlorocatechol-producing and chlorocatechol-consumming reactions. This need for proper gene dosage has likely played a role in the genetic organization and evolution of catabolic pathways for chloroaromatic degradation and led to the separation of so-called ‘upper pathways’ that hydroxylate the ring and ‘lower pathways’ which cleave it (van der Meer et al., 1998; Laemmli et al., 2000; Müller et al., 2003; Trefault et al., 2004).

With respect to transcription, Choi et al. (2003) demonstrated that adaptation of F1 to growth on biphenyl, n-propylbenzene and n-butylbenzene required mutations in todS as well as cymR, allowing for induction of the tod operon and recruitment of CmtE as an alternative catabolic enzyme, respectively. In the closely related strain P. putida KL47, mutations in todS which increased transcription of the tod pathway were again required for catabolic adaptation to substrates such as biphenyl (Lee et al., 2006). In the current study, however, it is perhaps not surprising that tod operon regulation was identical in SF1 and F1: since the entire catabolic todXFC1C2BADEGIH operon is transcribed from a single promoter (Lau et al., 1997; Mosqueda et al., 1999; Lacal et al., 2006, 2008 Busch et al., 2007), upregulation of beneficial C23O activity would also have increased deleterious TDO activity.

In the absence of differentially regulated upper and lower pathways, SF1 was forced to find another way to alter the ratio of TDO to C23O activity. It accomplished this by taking advantage of the less active TDO encoded by the C479T allele of todA. While the ‘less is more’ strategy described in this work was only demonstrated in response to styrene, it is likely that this strategy for alleviating C23O inactivation will permit adaptation to other aromatic substrates which produce inactivating catechols.
ACKNOWLEDGEMENTS

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REFERENCES


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degradation of chloro- and methylaromatics.


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