INTRODUCTION

In Pseudomonas putida, the phenylacetic acid (PAA) catabolic gene clusters consist of 15 genes that are organized into five contiguous operons (del Peso-Santos et al., 2008; Di Gennaro et al., 2007; Jiménez et al., 2002; Luengo et al., 2001; Olivera et al., 1998). The PAA pathway (Fig. 1) is a major route of a complex functional unit that catalyses the transformation of structurally related compounds such as styrene, tropic acid, ethylbenzene and 2-phenylethylalanine into phenylacetyl-CoA (PA-CoA), a common intermediate (Luengo et al., 2001). It has been reported that PA-CoA, and not PAA, is the true inducer of the PAA catabolic pathway and that the PaaX repressor controls PAA-catabolic gene expression (Ferrández et al., 2000; García et al., 2000). Binding of PaaX to its cognate DNA binding sites is abolished when the inducer, PA-CoA, interacts with the PaaX protein (Bartolomé-Martín et al., 2004; del Peso-Santos et al., 2006; Ferrández et al., 2000; García et al., 2000). Interestingly, along with the StyR/StyS system, PaaX also regulates the styrene degradation pathway by binding to the promoter region of the styrene upper pathway in Pseudomonas sp. strain Y2 (del Peso-Santos et al., 2006). PaaX is also involved in the transcriptional regulation of the pac gene, which encodes penicillin G acylase in Escherichia coli W (Galán et al., 2004; Kim et al., 2004). PaaX is involved in the common central pathway of several aromatic biodegradation reactions (Luengo et al., 2001).

In glucose-amended minimal medium or Luria–Bertani (LB) medium, paaA expression in P. putida is repressed even in the presence of PAA (Kim et al., 2007; Luengo et al., 2001). This carbon catabolite repression (CCR) mechanism of PAA metabolism in P. putida is not yet fully understood. An RNA-binding global regulator in P. putida, Crc (Moreno & Rojo, 2008), appears to be involved in CCR...
of the degradation pathways for several aromatic compounds, such as benzoate and 4-hydroxybenzoate, but not PAA (Morales et al., 2004). In E. coli, the cAMP receptor protein Crp is involved in CCR of PAA degradation (Ferrández et al., 2000; Kimata et al., 1997; Notley-McRobb et al., 1997). However, the level of cAMP in P. putida remains constant regardless of the carbon source and cAMP is unlikely to be involved in CCR in this species (Basu et al., 2007; Siegel et al., 1977). CCR of PAA metabolism appears to be unaffected by organic acids, such as pyruvate or succinate (Kim et al., 2007), suggesting that CCR is generated by glucose intermediates (Fig. 1).

Glucose catabolically represses the toluene degradation system (del Castillo & Ramos, 2007; Holtet et al., 1994; Velázquez et al., 2004). CCR of toluene metabolism by glucose is mediated by 2-keto-3-deoxy-6-phosphogluconate (KDPG), an intermediate in the Entner–Doudoroff (ED) pathway of glucose metabolism in P. putida (del Castillo & Ramos, 2007; Velázquez et al., 2004). Interestingly, the regulation of glucose metabolism is controlled by the HexR repressor (Hager et al., 2000; Petruschka et al., 2002; Temple et al., 1994). We have shown that HexR binding to its cognate operator sites is inhibited by KDPG (Kim et al., 2008). It seems likely that KDPG is an important signal generated during glucose metabolism that may control the preferential utilization of glucose over other aromatic carbon sources in P. putida. Our hypothesis is that KDPG may be the intracellular CCR signal of PAA metabolism in P. putida KT2440, and our data indicate that KDPG is indeed required for CCR of PAA metabolism in this strain.

**METHODS**

**Bacterial strains, culture conditions and DNA manipulation.** Bacteria (Table 1) were grown at 37 °C (E. coli) or 30 °C (P. putida) in LB medium (Sambrook et al., 1989) or mineral salts medium (M9) (Sambrook et al., 1989) containing different carbon sources (sucinate, glucose, gluconate, fructose and pyruvate), each at a concentration of 10 mM. Plasmid isolation, gel electrophoresis, transformation and PCR were performed using standard procedures (Ausubel et al., 1999). P. putida KT2440R was selected from spontaneous mutations of P. putida KT2440 (laboratory stock) on LB medium containing rifampicin (200 µg ml⁻¹) after prolonged incubation and sequential transfers. Tetracycline (15 µg ml⁻¹) and kanamycin (50 µg ml⁻¹) were added to bacterial cultures when necessary. The edd and eda mutants were purchased from Bio-Iliberis R&D (Spain).

**Cloning procedures and mutant construction.** The broad-host-range promoter-probe vector pRK415gfp (Yin et al., 2003) was used to construct the reporter plasmid pRKPpaaA::gfp. Briefly, a 354 bp fragment from the paaA promoter was amplified using the primers kt paaA pro1 (5′-CGCCGATTCGCCGCCGGCCATGTAAGCA-3′) and kt paaA pro2 (5′-CGCCGATCCGCCGGCCGTCGGGATGTAATG-3′). The amplicon was inserted into the EcoRI/BamHI cloning site of the pRK415gfp vector to generate pRKpaaA::gfp. The constructed plasmid was introduced into E. coli Top10 by electroporation. Then, the pRKpaaA::gfp plasmid was introduced by triparental conjugation into P. putida KT2440R to create P. putida KT2440R(pRKpaaA::gfp). Conjugation was performed by filter mating using E. coli Top10(pRKpaaA::gfp), E. coli HB101(pRK2013) and P. putida KT2440R(pRKpaaA::gfp).
Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype or relevant characteristics*</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td><strong>P. putida KT2440</strong></td>
<td>Wild-type</td>
<td>Lab stock</td>
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<tr>
<td>KT2440R</td>
<td>Wild-type; Rf&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Lab stock</td>
</tr>
<tr>
<td>KT2440R (pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;)</td>
<td>Strain KT2440 harbouring pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;; Tc&lt;sup&gt;R&lt;/sup&gt; Rf&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>ΔpaaX</td>
<td>Derivation of strain KT2440, insertion of pVIK-PaaX; Rf&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
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<tr>
<td>ΔpaaX (pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;)</td>
<td>Strain ΔPaaX harbouring pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;; Rf&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
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<tr>
<td>ΔEdd</td>
<td>edd mini-Tn5-Km; Rf&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Duque et al. (2007)</td>
</tr>
<tr>
<td>ΔEdd (pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;)</td>
<td>Strain ΔEdd harbouring pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;; Rf&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt; Tc&lt;sup&gt;R&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>ΔEda</td>
<td>eda mini-Tn5-Km; Rf&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Duque et al. (2007)</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
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<tr>
<td>Top10</td>
<td>mcrA Δ(mrr-hsdRMS-mcrBC), φ80lacZ ΔM15</td>
<td>Promega</td>
</tr>
<tr>
<td>HB101</td>
<td>Strain harbouring prRK2013</td>
<td>Fredrickson et al. (1988)</td>
</tr>
<tr>
<td>S17-1/pir</td>
<td>Tp&lt;sup&gt;R&lt;/sup&gt; Sm&lt;sup&gt;R&lt;/sup&gt; recA thi pro hsdM&lt;sup&gt;T+&lt;/sup&gt; RP4-2-Tc::Mu:: Km Tn7; hsdR mutant</td>
<td>Simon et al. (1983)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
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<tr>
<td>pRK415gfp</td>
<td>Broad-host-range vector; Tc&lt;sup&gt;R&lt;/sup&gt; Mob +</td>
<td>Yin et al. (2003)</td>
</tr>
<tr>
<td>pRKP&lt;sub&gt;paaA::gfp&lt;/sub&gt;</td>
<td>pRK415gfp carrying the KT2440 paaA full promoter region</td>
<td>This study</td>
</tr>
<tr>
<td>pVK112</td>
<td>R6KoriI, suicide vector, LacZ translation fusion vector; Km&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Kalogeraki &amp; Winans (1997)</td>
</tr>
<tr>
<td>pVK-PaaX</td>
<td>Partial region of paaX gene cloned into pVK112</td>
<td>This study</td>
</tr>
<tr>
<td>pRK2013</td>
<td>tra&lt;sup&gt;R&lt;/sup&gt; Km&lt;sup&gt;R&lt;/sup&gt; derivative of RK2 containing ColE1 replicon</td>
<td>Fredrickson et al. (1988)</td>
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</table>

*Km<sup>R</sup>, Rf<sup>R</sup>, Sm<sup>R</sup>, Tc<sup>R</sup>,Tp<sup>R</sup>, Resistance to kanamycin, rifampicin, streptomycin, tetracycline or trimethoprim, respectively.

KT2440R as donor, helper and recipient, respectively (Park et al., 2003). Using the KT paaX KO1 (5'-CGCAGAATTCGAAAAGGTGGG-CGCCAGCAATCGGTTGTTGAGTTA-3') and KT paaX KO2 (5'-CGCAGAATTCGACGGCAAGTTA-3') primers, we deleted the amplified 538 bp fragment of the paaX internal region by cloning it into the EcoRI cloning site of the pVIK112 vector (Kalogeraki & Winans, 1997) to generate pVK-PaaX. The constructed plasmid was introduced into E. coli S17-1/pipir by electroporation. Conjugation was performed by filter mating with E. coli S17-1/pipir(pVK-PaaX) and P. putida KT2440R (Lee et al., 2006) as the donor and recipient, respectively. The transconjugant was selected on LB medium containing kanamycin and rifampicin at 30 °C. PCR with the paaX KO1/MCS-R primer pairs was performed to verify the transconjugant. The MCS-R primer was designed based on the pVK112 plasmid sequence (Park et al., 2003). P. putida KT2440R(ΔPaaX/pRK<sub>paaA::gfp</sub>) was constructed as described above (Table 1).

Quantification of GFP fluorescence. Overnight cultures of the reporter strains in LB medium were collected using a microcentrifuge (~15 800 g) and washed twice with M9 medium with no carbon source. The strains (1:250) were regrown to the exponential phase (~15 800 OD<sub>600</sub>, ~0.2) in each of the carbon sources. PAA (5 mM) was added during the early exponential phase. After a 3 h incubation, the samples were collected using a microcentrifuge (~15 800 g) and washed twice with PBS (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4). The OD<sub>600</sub> and GFP fluorescence intensity of the resuspended cultures were quantified using a microtiter plate reader (Victor3; Bio-Rad). The reporter strain expresses a stable GFP variant that absorbs light at 488 nm. One fluorescence unit was defined as [fluorescence intensity of cells–fluorescence intensity of PBS buffer]/OD<sub>600</sub> of cells.

Northern blot analysis. Total RNA was isolated from 5 or 10 ml cells from each growth phase using an RNeasy kit (Qiagen), according to the manufacturer’s instructions. Total RNA (5 μg) concentration was determined by measuring the absorbance at 260 nm. The fractionated RNA was transferred to a nylon membrane (Schleicher & Schuell) using a Turboblotter (Schleicher & Schuell), and the amount of paaA mRNA was determined by hybridizing the membrane with an [α-<sup>32</sup>P]-labelled probe specific for each gene (Takara Bio). The paaA-Pp1 (5'-CGCGCACGAGGTTGAGAGTTGTTGAGTTA-3') and paaA-Pp2 (5'-GGCCACGACCCGAAATCAA-3') primers were used as the specific probe. The band intensity of Northern blot data was measured using a densitometry instrument (800 × 1600 d.p.i.; Umax UTA 2100XL).

Determination of glucose, gluconate and PAA concentrations. To analyse residual substrates, the supernatants in each growth phase were collected using a microcentrifuge (15 800 g for 5 min). Residual glucose concentration was measured by the glucose oxidase enzymatic method (Keston, 1956). Residual gluconate concentration was measured using a gluconic acid assay kit (Megazyme), according to the manufacturer’s instructions. Residual PAA concentration was analysed by HPLC (Ferra´ndez et al., 1998; Martínez-Blanco et al., 1990). We used a Cosmosil 5C<sub>18</sub>-AR-II column (4.6 × 250 mm) with a Younglin model SP930D pump at room temperature. The mobile phase was 50% methanol/H<sub>2</sub>O at a flow rate of 1.0 ml min<sup>−1</sup>. Elution peaks corresponding to the retention time of an authentic PAA standard (2.6 min) were monitored at 220 nm.

**PA-CoA ligase assay.** PA-CoA ligase was assayed by measuring the rate of generation of phenylacetylglycine in the presence of ATP, CoA, PAA and neutral hydroxylamine (Martinez-Blanco et al., 1990). The assay mixture contained 12.5 μl 0.2 M MgCl<sub>2</sub>, 50 μl 0.1 M ATP, 15 μl 20 mM CoA, 30 μl 0.2 M PAA and 50 μl hydroxylamine solution (1 ml 5 M hydroxylamine hydrochloride, 250 μl distilled water and 1.25 ml 4 M KOH). In standard tests PAA, ATP, MgCl<sub>2</sub> or CoA were omitted. Reactions were started by adding 100 μl enzyme solution and the incubations were carried out for 1 h at 30 °C. Reactions were stopped by adding 450 μl ferric chloride reagent (0.37 M ferric chloride, 20 mM trichloroacetic acid and 0.66 M hydrochloric acid) and immediately incubated on ice for 30 min. The reactions were centrifuged for 2 min and monitored using an Optimed 2120 UV/Vis spectrophotometer (Mecasys) at 540 nm. The extinction
coefficient of phenylacetylhydroxamate under these conditions was 0.9 mM$^{-1}$ cm$^{-1}$. Protein concentration was measured by the method of Bradford (Ausubel et al., 1999).

**RESULTS AND DISCUSSION**

**PAA metabolism is repressed by the presence of glucose and gluconate**

Consistent with other observations (Morales et al., 2004; Schleissner et al., 1994), we have previously shown that the PAA catabolic genes (paaA, paaG and paaL) are repressed in glucose+PAA-amended minimal medium (Kim et al., 2007). We measured the paaA expression level using a GFP-based reporter strain, *P. putida* KT2440R(pRPK$_{paaA}$::gfp). The results indicated that the paaA promoter activity in PAA-amended minimal medium was repressed by the presence of glucose or gluconate, whereas fructose, pyruvate or succinate did not affect paaA-promoter expression, suggesting that there is CCR of PAA metabolism by glucose or gluconate (Fig. 2). These results suggested that an intermediate of these carbon sources may be a key signal for CCR. A similar CCR by glucose has been reported in the metabolism of toluene, which indicated that KDPG is the signal for toluene CCR (del Castillo & Ramos, 2007; Vela´zquez et al., 2004). Previously, we have shown that HexR, a repressor of glucose metabolism, binds to the zwf-1 promoter region, and that HexR binding is inhibited by KDPG. Taken together, we hypothesized that KDPG may be a crucial signal for CCR of PAA metabolism by glucose.

![Fig. 2](http://mic.sgmjournals.org)

**Fig. 2.** Quantification of GFP expression in wild-type reporter strains grown in the presence of various carbon sources with or without PAA. Measurement of GFP was performed as described in Methods. The following carbon sources and concentrations were used: glucose, 10 mM; gluconate, 10 mM; fructose, 10 mM; pyruvate, 10 mM; succinate, 10 mM; PAA, 5 mM.

**KDPG is the signal for the PAA catabolite repression**

*edd* (encoding a 6-phosphogluconate dehydratase) or *eda* (encoding a KDPG aldolase) gene-deleted strains were used to test our hypothesis. The *edd* mutant does not make KDPG, whereas the *eda* mutant accumulates KDPG (Fig. 1). Because the degradation of 6-phosphogluconate (6PG) or KDPG into central metabolites is blocked in the ED pathway, these mutants did not grow on glucose or gluconate (del Castillo et al., 2007; Vela´zquez et al., 2004; Table 2). However, the strains grew on other substrates or in LB medium (Table 2). Interestingly, the growth patterns of the mutant strains indicated that the *eda* mutant (KDPG overproducer) did not grow on minimal medium supplemented with glucose+PAA, whereas the wild-type and *edd* mutant strains did grow under such conditions; however, the *eda* mutant grew in minimal medium containing only PAA (Fig. 3a, Table 2). These growth patterns of wild-type and mutants were also observed under gluconate-amended conditions (Table 2). *eda* mutant cells also grew in the presence of glucose plus other aromatic carbon sources, including benzoate or 4-hydroxybenzoate, indicating that their utilization is not under CCR by glucose (Morales et al., 2004; Nichols & Harwood, 1995; Putrinsˇ et al., 2007).

Previously, we have shown that the expression of paaL, which encodes a PAA permease, is repressed by glucose in PAA-amended minimal medium (Kim et al., 2007). Therefore, we can rule out the possibility that the KDPG accumulated during glucose metabolism directly inhibits the PAA transport system in *P. putida* KT2440. KDPG may be involved in a repression system in which the PAA metabolic genes, including the genes involved in the transport of PAA, are not expressed. When the *eda* mutant cells were grown to exponential phase in LB medium and transferred to a minimal medium containing glucose+PAA, the glucose was completely metabolized after 16 h incubation, whereas the concentration of PAA remained the same (Fig. 3b). The uptake and/or the metabolism of glucose+PAA was repressed by glucose.

**Table 2. Growth of *P. putida* KT2440 and the *edd* and *eda* mutant strains in minimal medium with various substrates**

<table>
<thead>
<tr>
<th>Substrate type</th>
<th>Glu</th>
<th>Glu + PAA</th>
<th>Glc</th>
<th>Glc + PAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AEDD</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>AEDA</td>
<td>-</td>
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</tbody>
</table>
PAA appeared to be inhibited in the eda mutant in the presence of glucose (Fig. 3b). There was no decrease in viability of the eda mutant cells when cells cultured in glucose plus PAA medium were serially diluted and spotted onto LB agar (Fig. 3c).

**PAA catabolite repression is abolished in the edd mutant**

We observed a triauxic growth pattern in the wild-type cells when they were grown in the presence of glucose + PAA (Fig. 4a). The reason for the second lag period is not clear but may reflect the accumulation of PAA metabolites. The residual PAA concentration was not reduced while glucose remained in the culture medium (Fig. 4a). The consumption of PAA was initiated once the glucose was consumed, which demonstrated glucose as a repressor of PAA metabolism. Northern blot analysis revealed that paaA was strongly induced after the complete exhaustion of glucose (Fig. 4a). The growth rate of the edd mutant strain was diminished in minimal medium with glucose + PAA probably due to a blockage of glucose metabolism through the ED pathway (Fig. 4b). This observation is consistent with other reports in which the accumulation of phosphorylated intermediates of glucose metabolism was a suspected cause of slow growth under these conditions (Sawyer et al., 1977; Velázquez et al., 2004; Vicente & Canovas, 1973). Despite the poor growth of the edd mutant in the presence of glucose + PAA, the edd mutant simultaneously consumed glucose and PAA (Fig. 4b). Coincidently, paaA expression was highly induced in the early exponential phase under the same conditions (Fig. 4b). Our finding has also shown that this CCR of PAA occurs in the presence of gluconate and is abolished in the edd mutant (Fig. 4c, d). Interestingly, in the presence of gluconate + PAA, the final OD$_{600}$ of the edd mutant strain reached ~1 (Fig. 4d); with glucose + PAA it reached up to ~2 (Fig. 4b). We speculated that this discrepancy is due to the activity of glucose-6-phosphate dehydrogenase and availability of its precursor substrate (glucose). The edd mutant could obtain byproducts such as NADPH produced by glucose-6-phosphate dehydrogenase activity under conditions where glucose + PAA are present, but not in the presence of gluconate + PAA. This phenomenon might explain why all glucoses are not converted to gluconate, and glucose uptake via the direct glucose uptake route may be preferred in P. putida KT2440. Furthermore, the GFP reporter assay of edd mutant paaA promoter
activity in the presence of glucose + PAA or gluconate + PAA indicated that KDPG is involved in CCR of PAA under glucose- or gluconate-amended conditions (Fig. 4e). Our studies with these mutants demonstrated that the CCR of PAA metabolism by glucose or gluconate is mediated by KDPG, not by 6PG, in *P. putida*.

To confirm the results presented above, we also measured the activity of the first enzyme of PAA biodegradation, PA-CoA ligase, with glucose + PAA (Martínez-Blanco et al., 1990). In the presence of PAA as sole carbon source, PA-CoA ligase activity remained very high during exponential growth phase (OD$_{600}$ = 0.3–0.7) in both wild-type and the *edd* mutant (Fig. 5). However, no PA-CoA ligase activity was observed in the presence of PAA + glucose in wild-type cells (Fig. 5a). In contrast, the specific activity of PA-CoA ligase remained very high during exponential phase of the *edd* mutant (OD$_{600}$ = 0.5–0.7) even in the presence of glucose + PAA (Fig. 5b). From this, it is apparent that PAA and glucose are simultaneously consumed in the *edd* mutant.

**The PaaX protein is not responsible for CCR of PAA**

We inserted the *paaA::gfp* reporter into a strain in which PaaX is inactivated by Campbell-type homologous recombination to verify whether a repressor of a PAA-catabolic genes, PaaX, is involved in this CCR event. Targeted disruption of PaaX showed CCR of PAA metabolism in the presence of glucose and gluconate, but not fructose, pyruvate or succinate (Fig. 6). The absence of the PaaX repressor may have allowed constitutively high expression of *paaA* in the absence of PAA, leading to the
transcriptional initiation of paaA by RNA polymerase (Fig. 6). The data suggested that PaaX is not involved in CCR of PAA metabolism by glucose and that another repression mechanism might be preventing RNA polymerase from initiating transcription when repressive carbon sources are present. Because KDPG and blockage of RNA polymerase are required for CCR of PAA metabolism, it is plausible to suggest that there is a link between the KDPG signal and another repression system.

PAA catabolite repression occurred in the presence of either glucose or gluconate, but not with fructose, pyruvate or succinate (Fig. 2). These repressive carbon sources are metabolized via the ED pathway in P. putida. It was recently reported that P. putida KT2440 can simultaneously use all three peripheral pathways (glucokinase pathway, 2-ketogluconate loop, gluconokinase pathway) in glucose uptake (del Castillo et al., 2007). Despite the preference for the glucose uptake pathway, both 6PG and KDPG are key metabolites in the ED pathway of Pseudomonas strains (Kim et al., 2008).

Although PAA catabolic genes are intact in the eda mutant, the most intriguing finding of this study was that the eda deletion mutant did not grow on minimal medium in the presence of glucose + PAA (Table 2, Fig. 3a). We have provided the first evidence that KDPG is the signal for CCR of PAA metabolism by glucose, and that CCR occurs at the transcriptional level. Interestingly, we found that zwf-1, which encodes glucose-6-phosphate dehydrogenase, was highly induced by glucose and gluconate, whereas zwf-1 promoter activity was not induced by fructose, pyruvate or succinate, even though the metabolic pathway of fructose produces 6PG and KDPG (Kim et al., 2008). Our finding might be due to the fact that P. putida possesses another fructose metabolic pathway which does not produce KDPG (Vela´zquez et al., 2004). This would explain why fructose does not induce CCR of PAA catabolic genes; perhaps fructose generates KDPG levels that are not high enough to trigger catabolite repression.

It has been shown that KDPG inhibits the binding of HexR to its cognate promoter regions and thus enables expression genes involved in the ED pathway (Kim et al., 2008). KDPG is involved in the regulation of both paaA, the first gene of PAA metabolism, and zwf-1, the first gene of glucose metabolism. These results strongly suggest that the generation of KDPG in the ED pathway may signal CCR of PAA metabolism. Additional molecular studies of the role of KDPG in CCR of PAA metabolism of P. putida KT2440 are warranted.

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