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Crc Is Involved in Catabolite Repression Control of the *bkd* Operons of *Pseudomonas putida* and *Pseudomonas aeruginosa*

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Crc (catabolite repression control) protein of *Pseudomonas aeruginosa* has shown to be involved in carbon regulation of several pathways. In this study, the role of Crc in catabolite repression control has been studied in *Pseudomonas putida*. The *bkd* operons of *P. putida* and *P. aeruginosa* encode the inducible multienzyme complex branched-chain keto acid dehydrogenase, which is regulated in both species by catabolite repression. We report here that this effect is mediated in both species by Crc. A 13-kb cloned DNA fragment containing the *P. putida crc* gene region was sequenced. Crc regulates the expression of branched-chain keto acid dehydrogenase, glucose-6-phosphate dehydrogenase, and amidase in both species but not urocanase, although the carbon sources responsible for catabolite repression in the two species differ. Transposon mutants affected in their expression of BkdR, the transcriptional activator of the *bkd* operon, were isolated and identified as *crc* and *vacB* (*rnr*) mutants. These mutants suggested that catabolite repression in pseudomonads might, in part, involve control of BkdR levels.

Pseudomonads play an important role in nature because of their ability to metabolize natural and manufactured organic chemicals. Many of these compounds are environmental pollutants, such as benzene, toluene, xylene, ethylbenzene, styrene, and chlorobenzoates (18), and their removal has been named bioremediation. Although the enzymic pathways responsible for degradation of these pollutants may be effective when the target compound is the sole growth-supporting substrate, in nature these compounds are present as mixtures, and some substrates may be degraded preferentially. Catabolite repression control refers to the ability of an organism to preferentially metabolize one carbon source over another when both are present in the growth medium. Because of the importance of pseudomonads to bioremediation efforts, understanding the control of catabolite repression is important so that more efficient, genetically modified organisms can be utilized in the removal of these environmental pollutants.

The molecular mechanisms of catabolite repression control have been extensively characterized in enteric bacteria, where glucose is the preferred carbon source. In these organisms, enzymes of the phosphoenolpyruvate-dependent phosphotransferase system mediate catabolite repression control by regulation of cyclic AMP (cAMP) concentration via adenylate cyclase activity (22). The strongest repressing substrates in *Pseudomonas* spp. are acetate, tricarboxylic acid cycle intermediates, and glucose (4, 10, 26). Unlike *Escherichia coli*, in *Pseudomonas* species adenylate cyclase activity, cAMP phosphodiesterase activity, and cAMP pools do not fluctuate with carbon source, nor does the addition of cAMP relieve repression of catabolite responsive pathways (21, 25). In addition, only one phosphotransferase system (fructose) has been iden-

tified in *Pseudomonas* (5), suggesting that PTS components are not involved in catabolite repression control in pseudomonads. The only protein thus far shown to be involved in catabolite repression in *Pseudomonas* is Crc of *P. aeruginosa*, but a function has not been identified for this protein (11). Crc has some sequence similarity (25 to 32% identity) to DNA repair enzymes of both prokaryotes and eukaryotes. However, Crc does not appear to have endonuclease activity or to bind DNA, suggesting that it has some other function.

Expression of branched-chain keto acid dehydrogenase (BCKAD) of *P. putida* is regulated by carbon and nitrogen sources (29). The *bkd* operon, which encodes BCKAD, and its regulation by BkdR, a positive transcriptional regulator, has been well characterized in *P. putida* (13–16, 29); therefore, *P. putida* BCKAD is a good model system for studying catabolite repression control of catabolic pathways in pseudomonads.

The objective of the present research was to determine if Crc played a role in catabolite repression control in *P. putida*, as well as in *P. aeruginosa*, by using the *bkd* operon as a model system. The phenotypes of the *P. aeruginosa* and *P. putida crc* mutants were compared, and although the carbon sources responsible for catabolite repression in the two species differ, the pathways regulated by Crc are identical in the two species.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *P. putida* was grown with aeration at 30°C, while *P. aeruginosa* and *E. coli* were grown with aeration at 37°C. Starter cultures were grown in 2 ml of 2xYT medium (23). For BCKAD assays, *P. putida* and *P. aeruginosa* were grown in nitrogen-free valine-isoleucine medium (17) which contained 0.3% valine and 0.1% isoleucine (wt/vol) as the sole carbon and nitrogen sources (17).

Both amino acids are supplied since growth on valine alone is toxic to the cell. For other assays, *P. putida* was grown in basal salts medium (BSM) (17) plus an inducing carbon source. For urocanase assays the carbon source was 10 mM histidine, for amidase assays the carbon source was 40 mM lactamide, and for glucose-6-phosphate dehydrogenase (G6PDH) the carbon source was 20 mM mannitol. The concentrations of catabolite repressors added to the medium were

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
P. putida		
JS386	Φ(*bkdR-lacZ*)*132*(Hyb)	14
JS391	Φ(*bkdR-lacZ*)*132*(Hyb) *crc*::Mini-Tn*5*	This study
JS393	Φ(*bkdR-lacZ*)*132*(Hyb) *rnr*::Mini-Tn*5*	This study
JS394	*crc*::Km^r	This study
PpG2	Wild type	I. C. Gunsalus
P. aeruginosa		
PAO1	Wild type	P. V. Phibbs, Jr.
PAO8020	Δ*crc*::Tc^r	11
E. coli		
DH5α	F$^-$ φ80d *lacZ*ΔM15 Δ(*lacZYA-argF*) *endA1 recA13 supE44 hsdR17*	Gibco/BRL
HB101	F$^-$ *hsdS20 supE44 recA13 ara14 proA2ara14 proA2 rpsL20*(Sm^r) *xyl5 mlt-1*λ$^-$ *galK2 lacY1*	Gibco/BRL
JM109	*recA1 endA1 gyrA96 hsdR17 supE44 relA1* Δ(*lac-proAB*) (F′ *traD36 proAB lacI*^{q}*Z*ΔM15)	
Plasmids		
pJRS191	40-kb insert of *P. putida* DNA in pLAFR3; includes *crc* and *rnr*	This study
pJRS194	*crc*$^+$, 1.45-kb PCR insert in pUCPM19	This study
pJRS196	*crc*$^+$, 2-kb insert in pUCPM19	This study
pJRS197	*Eco*RI/*Kpn*I *crc* with Km^r at *Bsa*AI in pUCPM19 minus stabilization fragment	This study
pLAFR1	Broad-host-range cosmid cloning vector	6
pLAFR3	Broad-host-range cosmid cloning vector	28
pPZ352	PAO1 *crc*$^+$	11
pRK2013	Helper plasmid; ColE1 *mob*$^+$ *tra*$^+$ (RK2) Km^r (8)	8
pUC19	Cloning vector	31
pUCP19	*Escherichia-Pseudomonas* shuttle vector derived from pUC19	24
pUCPM18/19	pUCP19 with *mob* fragment	This study
pUTKm	Tn*5*-based delivery plasmid in *E. coli* CC118	9

as follows: 20 mM glucose, 40 mM lactate, 40 mM succinate, 30 mM glutamate, and 20 mM gluconate. The concentrations of antibiotics used to inhibit growth of *P. putida* were as follows: carbenicillin, 2 mg/ml; kanamycin, 90 μg/ml; and tetracycline, 100 μg/ml. The concentrations of antibiotics used to inhibit growth of *P. aeruginosa* were as follows: carbenicillin, 1 mg/ml; kanamycin, 500 μg/ml; and tetracycline, 100 μg/ml. The concentrations of antibiotics used to inhibit growth of *E. coli* were as follows: ampicillin, 200 μg/ml; kanamycin, 90 μg/ml; and tetracycline, 50 μg/ml.

Enzyme assays. Cultures for enzyme assays were grown in 100 ml of medium to an A_{660} of 0.6 to 0.8, harvested, and then treated as described previously for enzyme assays (17). The following enzyme assays were performed as described previously: BCKAD (27), amidase (26), urocanase (7), and G6PDH (10). One unit of BCKAD is 1 μmol of NADH formed/min/mg of protein, and one unit of G6PDH is 1 μmol of NADPH formed/min/mg of protein. Amidase assays were done on whole-cell suspensions, and the specific activity was measured as micromoles of lactamide per A_{660}. The data in Fig. 5 are expressed as the percent activity relative to the lactamide plus lactate culture.

Construction and screening of *P. putida* genomic cosmid library. A complete *Bam*HI digest of PpG2 chromosomal DNA was sized over a sucrose gradient, and fragments larger than 6 kb were ligated with *Bam*HI-digested pLAFR3 (28) with T4 DNA ligase at a chromosome-to-vector ratio of 5:1. In vitro packaging and transformation of *E. coli* JM109 was performed according to the manufacturer's recommendations (Gigapack III Gold Packaging Extract; Stratagene). The library was screened with the 2.0 kb *Sst*I fragment of pPZ352, which contains the PAO1 *crc* gene (11), and was labeled by using the RadPrime DNA labeling system (Life Technologies). The library was plated on 2xYT agar containing tetracycline, colonies were lifted by using Colony/Plaque Screen (Dupont/NEN Research Products), and hybridization and washing conditions were performed as suggested by the manufacturer.

Nucleic acid preparations and manipulations. Chromosomal and cosmid DNAs used for library construction were purified by using cesium chloride gradients. Plasmid DNA was purified by using QIAprep Spin Miniprep kit (Qiagen). Chromosomal DNA was purified by using the Qiagen Midi kit or the Gentra Systems Puregene kit. Basic DNA manipulations were performed as described earlier (23). Transfer of plasmids from *E. coli* to *P. putida* was done by triparental mating with pRK2013 (8).

Oligonucleotide synthesis was performed by the University of Oklahoma Health Sciences Center Molecular Biology Resource Facility and Life Technologies.

Plasmid constructions. pUCP18/19 are pUC-derived plasmids which contain a fragment from RP1 (19) which permits stable maintenance of these plasmids in

Pseudomonas species (24). These plasmids were further modified in this study so that they could be used to transform *Pseudomonas* species by triparental mating. In order to accomplish this, the *Xma*III fragment from pLAFRI, (6), which contains the mobilization (*mob*) site, was blunted and cloned into the *Sma*I site of pUC18. The 750-bp *mob* fragment was isolated after digestion with *Kpn*I and *Bam*HI and blunted. Next, pUCP18/19 was digested with *Ssp*I and blunted, and the 750-bp blunted *mob* fragment was cloned into the blunted vector, providing pUCPM18 and pUCPM19.

pJRS191 was obtained from the pLAFR3 cosmid library and contains a fragment of *P. putida* DNA, of approximately 40 kb, inserted into the *Bam*HI. pJRS196 contains a 2.0-kb fragment from pJRS191 that includes the entire structural genes of *crc* and *pyrE*, cloned into the *Kpn*I site of pUCPM19.

Mutant construction. The coding region of *crc* was amplified from pJRS191 by using primers S163 (GAACAGGCCGGCATTGAAGAAATA) and S165 (GC GCTGGACATGAGCAAGCTGGGCG). The PCR product was digested with *Eco*RI and *Kpn*I and ligated with pUCPM19 digested with the same restriction enzymes; this plasmid was designated pJRS194. The stabilization fragment was removed from plasmids which were to be used for homologous recombination. To remove the stabilization fragment of pUCPM19, pJRS194 was digested with *Nde*I and *Eco*RI, and the 4.6-kb fragment was religated. Transposon pUTKm (9) was digested with *Alw*NI, and the 1.25-kb fragment containing the Km^r gene was blunt ended and ligated with the construct described above digested with *Bsa*AI. The resulting plasmid, pJRS197, contained the Km^r gene cloned in the opposite orientation to *crc* 447 bp downstream of the ATG of *crc*. Conjugal transfer of this suicide plasmid into PpG2 was accomplished by triparental mating with pRK2013 (8). Mutants were isolated by using *Pseudomonas* isolation agar containing kanamycin. Because all mutants isolated were also Cb^r and therefore were single crossovers, restriction digests and Southern blotting were used to identify recombination events that occurred upstream of the Km^r gene insertion site. Double crossovers were detected by the loss of carbenicillin resistance, and this recombination event was confirmed by restriction digests and Southern blotting with the *Nde*I/*Alw*NI fragment of pUC19 containing *bla* as a probe. The mutant with *crc* inactivated by insertion of a Km^r cassette was named *P. putida* JS394.

Transposon mutagenesis and screening. To isolate transposon mutants affected in *bkdR* expression, triparental matings of *P. putida* JS386, *E. coli* CC118(pUTKm), and *E. coli* HB101(pRK2013) were plated on Difco *Pseudomonas* Isolation Agar plus kanamycin plus 0.004% X-Gal (wt/vol; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Approximately 10,000 colonies were screened on the basis of color, since JS386 is light blue on this medium. Both white and dark blue colonies were collected, and phenotypes were confirmed by
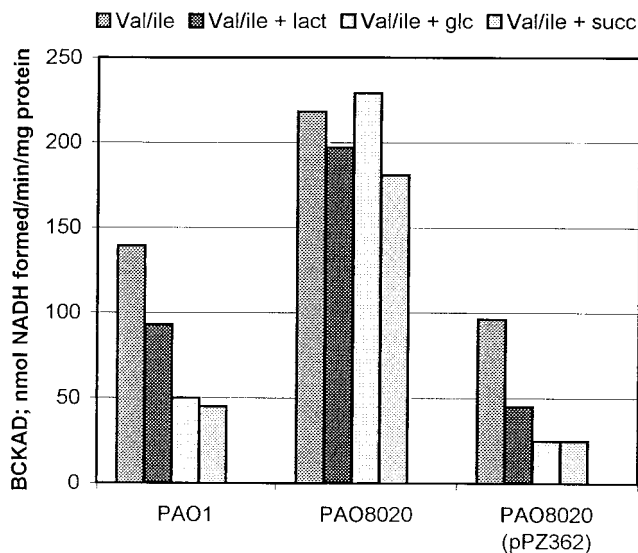
FIG. 1. Effect of mutation in *crc* on catabolite repression of BCKAD in *P. aeruginosa*. *P. aeruginosa* PAO1, PAO8020, and PAO8020(pPZ352) were grown in valine-isoleucine medium either alone or supplemented with lactate, glucose, or succinate (columns in each group from left to right, respectively) as described in Materials and Methods. Data are the averages of three separate experiments.

β-galactosidase assays. Southern blots were used to identify mutants with insertions in the *bkdR-bkdA1* intergenic region or in *lacZ*, and these mutants were discarded. Four white colonies and three dark blue colonies were selected for further study. Genomic DNA from each mutant was digested with several restriction enzymes and Southern blotted with the 3.0-kb *Eco*RI-Km^r fragment to identify fragments of a desired size for cloning. The transposon insertion sites for three of the white colonies and all of the dark blue colonies were identified by cloning into pUC19 and sequencing the DNA insert by the Oklahoma University Health Sciences Center DNA Sequencing Facility.

DNA sequencing and analysis of nucleotide sequences. DNA Sequencing was performed by Bruce A. Roe's lab at the University of Oklahoma Advanced Center for Genomic Technology (Norman, Okla.). Cosmid DNA was purified by means of a cleared-lysate diatomaceous earth method (20). Sequencing was undertaken by using the double-stranded, shotgun-based approach (2). The resulting sequences were screened to eliminate vector, assembled into contiguous fragments, and proofread by using the Phred/Phrap/Consed system developed by P. Green (http://chimera.biotech.washington.edu/uwgc). Contigs larger than 2 kb were deposited before publication in the "unfinished" division of the high-throughput genome sequencing GenBank database and were given accession number AC004396.

RESULTS

Involvement of Crc in catabolite repression of the *bkd* operon of *P. aeruginosa* PAO1. Each strain of *P. aeruginosa* was grown on valine-isoleucine medium alone or supplemented with the additional carbon sources lactate, glucose, or succinate. Addition of glucose or succinate to the inducing (valine-isoleucine) medium caused a 65 to 70% repression of BCKAD activity in the wild-type strain PAO1, and even lactate caused

some repression (Fig. 1). Repression was completely relieved in the *crc* mutant PAO8020, where similar BCKAD activities were seen in inducing medium with or without glucose or succinate. Repression was restored when PAO8020 was complemented with *crc*$^+$ from *P. aeruginosa* on a plasmid (pPZ352). These results indicate that *crc* is responsible for a significant fraction of repression of the *P. aeruginosa bkd* operon by glucose and succinate synthetic media. The levels of BCKAD activity in the *crc* mutant were elevated compared to the wild type under all growth conditions, suggesting that constitutive levels of Crc in *P. aeruginosa* PAO1 cause some repression of *bkd* operon expression in the absence of repressing carbon source.

Isolation of *P. putida crc*. *P. putida crc* was isolated in order to determine if Crc was involved in catabolite repression of the *bkd* operon of *P. putida*. A PpG2 chromosomal DNA library was constructed in pLAFR3 and screened with a 2.0-kb *Sst*I fragment of pPZ352 containing the *P. aeruginosa crc* gene. The positive clone with a ~40-kb insert of *P. putida*, pJRS191, was partly sequenced, and the consensus sequence of a 13-kb contig containing the *crc* gene was obtained (Fig. 2).

P. putida crc is 780 bp long, and the encoded protein is 86% identical and 93% similar to PAO1 Crc. The 3.1-kb PAO1 *crc* region includes three genes: *pyrE* upstream of, and *rph* downstream of, *crc* (11). This organization is conserved in the PpG2 *crc* region, with the exception of the *crc-rph* intergenic region, which is 479 bp in PAO1 but only 66 bp in PpG2 (Fig. 2).

A BLAST (1) search of GenBank was undertaken to identify open reading frames in the remaining 10 kb of the contig, and the annotation based on this similarity search is shown in Fig. 2. In addition to genes described in the legend to Fig. 2, a LysR family regulator appears to be encoded upstream of the open reading frame with similarity to glutamine synthetase, but the 3′ end of the open reading frame could not be identified. The 2-kb sequence between the open reading frames with similarity to glutamine synthetase and GMP kinase appears to contain two open reading frames based on sequence similarity to open reading frames of unknown function in the DNA databases. However, the 5′ and 3′ ends of each could not be defined due to low similarity and incomplete open reading frames obtained in the search.

Effect of Crc on catabolite repression of the *bkd* operon of *P. putida*. *P. putida* JS394, which possesses a chromosomal *crc* inactivated by insertion of a kanamycin cassette, was created by homologous recombination. For complementation studies, *P. putida crc* was cloned into pUCPM19. This construct was numbered pJRS196 and was used to transform *P. putida* JS394. *P. putida* PpG2, JS394, and JS394(pJRS196) were grown in valine-isoleucine medium alone and with lactate, glucose, or succinate (Fig. 3). BCKAD activity obtained in the presence of lactate and glucose was about one-half to one-fourth the activity obtained in cells grown in valine-isoleucine medium. BCKAD activity obtained in the presence of succinate was
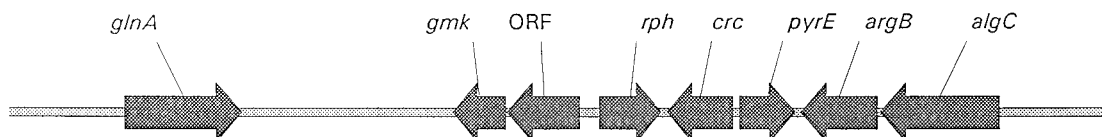

FIG. 2. Annotation of contig 270 from pJRS191. Open reading frames were identified by similarity searching by using the BLAST program (1). Similarities were found to the following proteins (identities at the protein level are given in parentheses): *glnA*, glutamine synthetase (61% to *E. coli*); *gmk*, GMP kinase (61% to *E. coli*); *rph*, RNase PH (84% to *P. aeruginosa*); *crc*, Crc (86% to *P. aeruginosa*); *pyrE*, orotate phosphoribosyltransferase (80% to *P. aeruginosa*); *argB*, acetylglutamate kinase (46% to *Methanococcus jannaschii*); and *algC*, phosphomannomutase (75% to *P. aeruginosa*). The only similarity found to the open reading frame (ORF) was to other reading frames of unknown function identified by genomic sequencing.
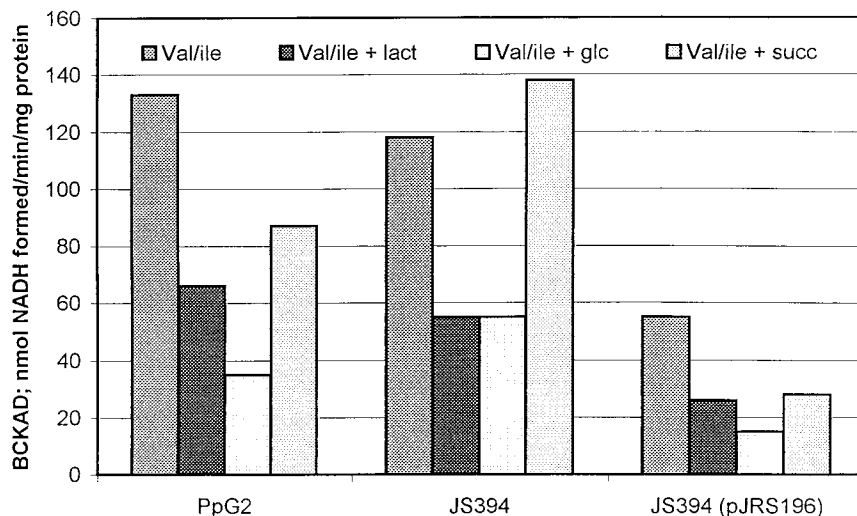
FIG. 3. Effect of mutation in *crc* on catabolite repression of BCKAD in *P. putida*. *P. putida* strains PpG2, JS394, and JS394(pJRS196) were grown in valine-isoleucine medium either alone or supplemented with lactate, glucose, or succinate (columns in each group from left to right, respectively). Kanamycin was always added to medium in which JS394 was grown, and carbenicillin was added to medium in which strains containing pJRS196 were grown. Data are the averages of two separate experiments.

about three-fourths of the control value. There was some relief of catabolite repression in *P. putida* JS394 when succinate was the repressor (Fig. 3). However, when *P. putida* JS394 was complemented with *crc in trans*, there was distinct evidence of catabolite repression by lactate, glucose, and succinate, probably due the effect of multiple copies of *crc* (Fig. 3).

While it can be concluded that Crc plays a role in catabolite repression control of the *bkd* operon of both *P. aeruginosa* and *P. putida*, there are some differences. Crc does not have as much control over expression of the *bkd* operon of *P. putida* as it does in *P. aeruginosa*, and succinate is a much better repressor in *P. aeruginosa*. Another difference is that the elevated levels of BCKAD activity seen under all growth conditions in the *P. aeruginosa crc* mutant (Fig. 1) were not observed in the *P. putida crc* mutant.

Effect of *P. putida* Crc on catabolite repression of G6PDH and amidase. Crc also controls expression of G6PDH and amidase in *P. aeruginosa* (4), and the effect of Crc on expression of these enzymes was also studied in *P. putida*. G6PDH activities were studied in extracts of *P. putida* PpG2, JS394, and JS394(pJRS196) grown in BSM containing mannitol plus lactate or succinate. In this case, the control medium is mannitol plus lactate because growth in mannitol alone is very slow. As seen in Fig. 4, G6PDH activity in *P. putida* PpG2 is subject to strong catabolite repression by succinate; G6PDH activity in mannitol plus succinate was only about one-third that obtained in mannitol plus lactate medium. However, G6PDH activities in JS394 cultures grown in the presence of mannitol plus lactate and mannitol plus succinate were nearly identical, demonstrating relief of catabolite repression in the *crc* mutant. When JS394 was complemented with pJRS196, the repression by succinate was restored, demonstrating that *P. putida* Crc is involved in catabolite repression of G6PDH by succinate.

The effect of Crc on catabolite repression control of amidase in *P. putida* PpG2 is shown in Fig. 5. The control medium contained lactamide plus lactate, and the results are expressed as the percent activity obtained with *P. putida* grown in this medium. Succinate is a nonrepressing carbon source, which is in contrast to the situation in *P. aeruginosa*, where succinate is a strong repressor of amidase activity (12). Amidase activity

obtained when gluconate was the repressor was less than 20% of the activity obtained in the control medium. Amidase activity obtained from cells grown in the presence of glutamate and glucose was 30 to 50% of the activity obtained in the control medium. Repression by glutamate, glucose, and gluconate was restored in the complemented mutant, *P. putida* JS394 (pJRS196). Therefore, Crc plays a major role in catabolite repression control of amidase by glutamate, glucose, and gluconate in *P. putida*, as well as in *P. aeruginosa*, but again the carbon sources responsible for catabolite repression differ.

***P. putida* Crc is not involved in catabolite repression of urocanase activity.** Repression of urocanase and histidase activities by succinate was not relieved in *P. aeruginosa crc* mu-
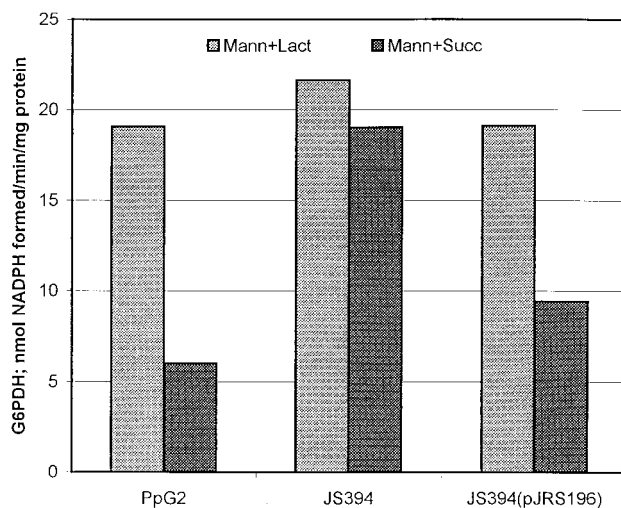

FIG. 4. Effect of mutation in *crc* on catabolite repression of G6PDH of *P. putida*. *P. putida* PpG2, JS394, and JS394(pJRS196) were grown in BSM containing mannitol plus lactate or containing mannitol plus succinate (left and right columns in each group, respectively). Data are the averages of two separate experiments.
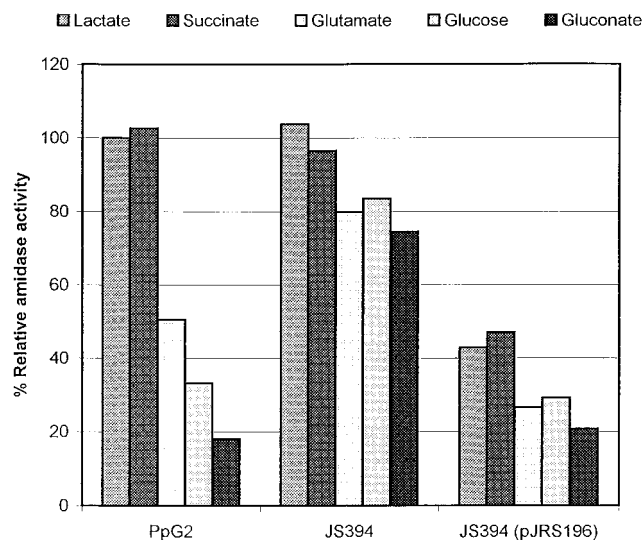
FIG. 5. Effect of mutation in *crc* in catabolite repression of amidase in *P. putida*. *P. putida* strains PpG2, JS394, and JS394(pJRS196) were grown in BSM containing lactamide plus lactate, succinate, glutamate, glucose, or gluconate (columns in each group from left to right, respectively). Amidase activity is expressed as a percentage of the activity seen in *P. putida* PpG2 grown in lactamide plus lactate, which was included with each experiment.

tants (P. Phibbs and C. MacGregor, unpublished data); therefore, Crc does not play a role in catabolite repression control of the *hut* operon. For comparison, *P. putida* PpG2 and JS394 were grown on BSM containing histidine plus lactate, succinate, or glucose and then assayed for urocanase activity. The specific activities of urocanase in PpG2 and JS394 grown on histidine plus glucose were the same for both strains and were about two-thirds that of the activity obtained when they were grown in histidine plus lactate. Thus, the results also suggest that Crc is not involved in catabolite repression control of the *hut* operon in *P. putida*, although repression was not very strong.

Crc and RNase R affect expression of *bkdR*. Crc could act directly on transcription of the *bkd* operon or indirectly on

expression of *bkdR*. To test the latter possibility, transposon mutagenesis of the *bkdR-lacZ* fusion of strain JS386 was undertaken to isolate and identify mutants affected in *bkdR* expression (see Materials and Methods). The strategy was to isolate dark blue colonies, which should be derepressed for expression of *bkdR*. Three dark blue colonies were isolated, two of which had insertions in *crc*. The other dark blue colony had an insertion in *vacB*, which encodes a protein involved in posttranscriptional regulation of virulence genes in *Shigella flexneri* (30). VacB has recently been identified as RNase R in *E. coli* (3), and this terminology will be used here. The structural gene for RNase R is *rnr* and was found on the same contig which contains *crc* (Table 1). The amino acid sequence of RNase R from *P. putida* is 50% identical to RNase R from *E. coli* and is 48% identical to RNase R from *S. flexneri*.

To examine the effects of Crc and RNase R on *bkdR* expression, β-galactosidase assays were performed on *P. putida* JS386, JS391 (*crc*), and JS393 (*rnr*) grown in valine-isoleucine plus lactate, succinate, or glucose (Fig. 6). There was no repression of β-galactosidase activity by glucose and succinate in *P. putida* JS386 which contains the *bkdR-lacZ* translational fusion. *P. putida* JS391 and *P. putida* JS393 had three- to fourfold-higher levels of β-galactosidase activity than JS386 under all growth conditions, indicating that Crc and RNase R had negative effects on the expression of *bkdR*. One interesting difference was that catabolite repression of amidase was not relieved in the *rnr* mutant.

DISCUSSION

Crc is involved in the control of catabolite repression of BCKAD (Fig. 1), G6PDH, and amidase in *P. aeruginosa* (4), but not of urocanase (Phibbs and MacGregor, unpublished). The same can be concluded for Crc of *P. putida* (Fig. 3 to 5), although the effect on catabolite repression of BCKAD is not as strong, and in *P. aeruginosa* it is weak (Fig. 1 and 3). While the same catabolic enzymes are affected in both species, the catabolite repressors have different degrees of effectiveness. Succinate is a good repressor in *P. aeruginosa* (Fig. 1) (4) but is a weak repressor in *P. putida* (Fig. 3 and 5). The high degree of sequence similarity between Crc from *P. aeruginosa* and *P. putida* and the results of the catabolite repression control stud-
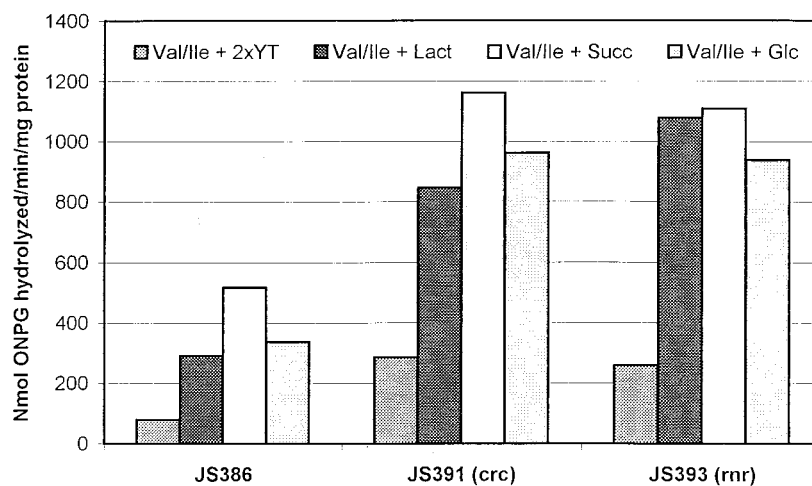

FIG. 6. Effect of mutations in *crc* and *rnr* on expression of *bkdR* in *P. putida* strains with a *bkdR-lacZ* translational fusion. *P. putida* strains JS386, JS391, and JS393 were grown in synthetic medium with valine-isoleucine plus 2xYT, lactate, succinate, or glucose (columns in each group from left to right, respectively). Data are the averages of at least three separate experiments.

ies (Fig. 3 to 5) support the conclusion that Crc carries out the same functions in both species. There are two differences which bear examination, namely, the small intergenic region between *crc* and *rph* in *P. putida* (69 bp) compared to that in *P. aeruginosa* (479 bp) and the increased expression of BCKAD relative to the wild type seen in *P. aeruginosa* (Fig. 1) but not *P. putida* (Fig. 3).

The fact that catabolite repression in pseudomonads does not involve cAMP makes the role of Crc in catabolite repression all the more interesting. Crc does not appear to be a DNA-binding protein and is therefore not likely to interfere with transcription. Other possibilities are that Crc acts posttranscriptionally by interfering with the expression of the mRNA or posttranslationally by modifying BkdR. However, the sequence similarity of Crc to endonucleases (11) makes the latter possibility remote. It has been pointed out that it was not possible to demonstrate the binding of Crc from *P. aeruginosa* DNA (11) and that has been our experience with *P. putida* DNA as well (K. L. Hester and J. R. Sokatch, unpublished data). The transposon mutants (Fig. 6) were isolated to test the possibility that Crc had an effect on expression of *bkdR* and, indeed, of the three colonies isolated with increased expression of LacZ from the *crc-lacZ* fusion, two were *crc* mutants. The other mutant with a similar phenotype was RNase R, which is interesting because Crc has some similarity to exonucleases (11). The accompanying study (9a) presents evidence that Crc acts posttranscriptionally.

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