JOURNAL OF BACTERIOLOGY, Dec. 1988, p. 5588–5593
0021-9193/88/125588-06$02.00/0

Vol. 170, No. 12

Regulation of the Galactose-Inducible *lac* Operon and the Histidine Utilization Operons in *pts* Mutants of *Klebsiella aerogenes*

SANDRA L. BALDAUF, MARY ANN CARDANI, AND ROBERT A. BENDER*
Department of Biology, University of Michigan, Ann Arbor, Michigan 48109

Received 5 May 1988/Accepted 31 August 1988

Galactose appears to be the physiological inducer of the chromosomal *lac* operon in *Klebsiella aerogenes*. Both lactose and galactose are poor inducers in strains having a functional galactose catabolism (*gal*) operon, but both are excellent inducers in *gal* mutants. Thus the slow growth of *K. aerogenes* on lactose reflects the rapid degradation of the inducer. Several *pts* mutations were characterized and shown to affect both inducer exclusion and permanent catabolite repression. The β-galactosidase of *pts* mutants cannot be induced at all by lactose, and *pts* mutants appear to have a permanent and constitutive inducer exclusion phenotype. In addition, *pts* mutants show a reduced rate of glucose metabolism, leading to slower growth on glucose and a reduced degree of glucose-mediated permanent catabolite repression. The *crr*-type pseudorevertants of *pts* mutations relieve the constitutive inducer exclusion for *lac* but do not restore the full level of glucose-mediated permanent catabolite repression and only slightly weaken the glucose-mediated inducer exclusion. Except for weakening the glucose-mediated permanent catabolite repression, *pts* and *crr* mutations have no effect on expression of the histidine utilization (*hut*) operons.

Many wild-type strains of *Klebsiella aerogenes* (currently *K. pneumoniae* [13]), including strain W70, have at least two *lac* operons (4, 9, 19): a plasmid-encoded *lac* operon that appears very similar to the *lac* operon of *Escherichia coli* and a chromosomally encoded *lac* operon that is less similar. Although the *K. aerogenes* chromosomal *lac* operon is known to resemble the *E. coli lac* operon in being inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) and requiring the products of the *cya* and *crp* genes for expression (18), several questions remain unanswered. Perhaps the most fundamental of these is why *K. aerogenes*, with a fully functional chromosomal *lac* operon, scores only weakly lactose positive in vivo. This weak growth could reflect either an enzymological or a regulatory defect. We have analyzed three types of regulation: the operon-specific repressor control, the permanent catabolite repression mediated by the catabolite gene activator protein (CAP) and cyclic AMP (cAMP), and the inducer exclusion mediated by the phosphoenolpyruvate:sugar phosphotransferase system (PTS). Our results suggest that the defect involves an unusual pattern of *lac* operon induction in *K. aerogenes*.

The first type of regulation, operon-specific repressor control, involves the interactions of the *lac* repressor (*lacI* gene product) with inducers and with the operator (*lacO*) locus. The deduced amino acid sequence of all or most of the *Klebsiella lacI* gene is known (5), and six amino acids thought to be critical for the interaction of the *E. coli lac* repressor with inducer are not conserved in the *K. aerogenes lac* repressor, suggesting that interaction of the repressor with inducers may be different (5). In fact, melibiose, an inducer of *E. coli lac*, both represses and prevents induction of *lac* in *K. aerogenes* (20). Interestingly, although the *E. coli* and *K. aerogenes lac* repressors are surprisingly divergent (only 40% amino acid identity), those homologies that do remain lie precisely in the regions where the two *lac* repressors show similarity with the *galR* product, the repressor of the *gal* operon (5).

The second type of regulation, permanent catabolite repression mediated by glucose, is stronger in *K. aerogenes* than in *E. coli* (7), probably reflecting the fact that *K. aerogenes* metabolizes glucose not only via the Embden-Meyerhof pathway but also via a very active glucose dehydrogenase (11, 12), resulting in rapid breakdown of glucose. In both organisms, *lac* operon expression is strongly dependent on CAP-cAMP-mediated activation; mutants lacking CAP or adenylate cyclase exhibit drastically reduced *lac* operon expression (18).

The third type of regulation, PTS-mediated inducer exclusion in *Klebsiella* species, has been only briefly studied to date (23). The *Klebsiella* system appears to resemble the analogous system in *E. coli*, where induction of the *lac* operon is prevented by direct interaction with and inhibition of the lactose permease by the *crr* gene product in its unphosphorylated form. (For a review of the PTS, see reference 17.)

MATERIALS AND METHODS

Strains and growth conditions. Bacterial strains used in these studies are listed in Table 1. All strains except KG2003 and KG2004 are derived from KC1043, which is strain MK9000 (22) cured of the *lac* plasmid and the drug resistance plasmid present in the wild strains (4) and which is sensitive to bacteriophage P1 (6). Strains KG2003 and KG2004 have been described elsewhere (8). Minimal medium was W salts (3) supplemented with 0.2% (wt/vol) ammonium sulfate and organic compounds as listed in the tables. Biotin (0.3 μM) and sodium succinate (0.5%) were added where necessary. For transductions involving the *cysA* marker (see Table 4), the chlorides of magnesium and ammonium replaced the sulfates, and either sodium sulfate or sodium sulfite was provided at 0.8 mM. Cultures were grown at 30°C with shaking, and cells were harvested for assays in the log phase (50 Klett units with a green filter).

Enzyme assays. β-Galactosidase was assayed essentially as described previously (18), except that the reaction was carried out at 30°C and the cells were removed by centrifugation before the absorbance was read. Histidase was assayed as described previously (18). Cell protein was esti-

* Corresponding author.

TABLE 1. *K. aerogenes* strains used[a]

Strain	Genotype	Origin or reference
KC1043	*hutC515*	MK9000[b] (22)
KC1047	Δ(*gal-hut-bio-chl*)*401*	2DG[r] Chl[r] derivative of KC1043[c]
KC1297	Wild type	P1 transduction of MK1 × KC1047[d]
KC1108	*pts-3011 hutC515*	EMS[e] mutagenesis of KC1043
KC1382	*pts-3021* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1383	*pts-3031* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1384	*pts-3032* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1385	*pts-3041* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1386	*pts-3042* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1387	*pts-3043* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1388	*pts-3044* Δ(*gal-hut-bio-chl*)*401*	EMS mutagenesis of KC1047
KC1426	*pts-3021*	P1 transduction of KC1297 × KC1382
KC1427	*pts-3031*	P1 transduction of KC1297 × KC1383
KC1428	*pts-3032*	P1 transduction of KC1297 × KC1384
KC1429	*pts-3042*	P1 transduction of KC1297 × KC1386
KC1448	*pts-3011 crr-11 hutC515*	Mal[+] derivative of KC1108[c]
KC1449	*pts-3021 crr-21* Δ(*gal-hut-bio-chl*)*401*	Mal[+] derivative of KC1382
KC1509	*pts-3011 crr-12 hutC515*	Mal[+] derivative of KC1108
KC1510	*pts-3021 crr-22* Δ(*gal-hut-bio-chl*)*401*	Mal[+] derivative of KC1382
KC1511	*pts-3031 crr-31* Δ(*gal-hut-bio-chl*)*401*	Mal[+] derivative of KC1383
KC1513	*pts-3041 crr-51* Δ(*gal-hut-bio-chl*)*401*	Mal[+] derivative of KC1385
KC1515	*pts-3043 crr-71* Δ(*gal-hut-bio-chl*)*401*	Mal[+] derivative of KC1387
KC1516	*pts-3044 crr-81* Δ(*gal-hut-bio-chl*)*401*	Mal[+] derivative of KC1388
KC1599	*cysA401 hutC515*	This work
KG2003	*hutH518 hutC516*	8
KG2004	*hutH518 hutC516 gal-1*	8

[a] All strains are derived from *K. aerogenes* MK1 (18).
[b] KC1043 is MK9000 cured of its *lac* plasmid and drug resistance plasmid (4).
[c] A spontaneous mutation. 2DG[r] Chl[r] is simultaneous resistance to 2-deoxygalactose and chlorate anaerobically. Mal[+] is the ability to use maltose as the sole carbon source.
[d] Bacteriophage P1 grown on strain MK1 was used to transduce strain KC1047 to Hut[+] (ability to use histidine as the sole carbon or nitrogen source).
[e] Mutagenesis with EMS as described previously (22).

mated by the method of Lowry et al. (15) with whole cells (untreated) and bovine serum albumin as a standard. Enzyme activities are reported as nanomoles of substrate cleaved or product formed per minute per milligram of cell protein.

Genetic procedures. Mutagenesis with ethyl methanesulfonate (EMS) (18), P1-mediated generalized transduction (6), and transformation of competent cells with plasmid DNA (14) have been described elsewhere. The *cysA* mutant (lacking sulfate permease) was isolated as a spontaneous mutant able to grow in the presence of 0.1 mM potassium chromate, similar to the method used for *E. coli* (1). This mutant, KC1599, grew as well as the wild-type KC1043 when 0.8 mM sodium sulfite was the sole source of sulfur in the medium but did not grow when 0.8 mM sodium sulfate was used. In a crude sulfate accumulation assay, sulfite-grown cells were washed with sulfur-free medium and suspended in medium containing chloramphenicol, $H_2{}^{35}SO_4$, and nonradioactive sulfate (0.5 mM). After 2 min the cells were filtered, and the radioactivity retained by the cells was measured. Wild-type cells accumulated 13 pmol of sulfate, whereas an equal amount of KC1599 cells accumulated no sulfate (<0.1 pmol). Strain KC1599 reverted spontaneously to growth on sulfate with a frequency of 10^{-9} to 10^{-10}. Taken together, these data indicate that strain KC1599 carries a simple mutation in *cysA*.

RESULTS

Galactose as inducer of the *lac* operon. The original intention of this work was to investigate the role of the PTS in the carbon regulation of the histidine utilization (*hut*) operons. As a result, many of the original mutations were isolated in strains carrying a deletion of the *gal-hut-bio-chl* region of the chromosome to facilitate later genetic manipulations with the *hut* operons. However, control studies showed a striking difference in the regulation of *lac* expression between strains with and without this deletion (Table 2). In particular, it was apparent that although lactose was almost as good an inducer of the *lac* operon as IPTG in the *gal-chl* deletion mutant KC1047, lactose was a very poor inducer of the *lac* operon in the wild-type KC1043, whether lactose was present along with another poor carbon source such as citrate or whether lactose was the only carbon source provided (Table 2). Furthermore, the wild-type strain grew slowly when lactose was the only carbon source (doubling time, 2.5 to 3 h). The failure of *lac* induction in the presence of lactose was not due to an inhibitory effect of lactose on induction, since IPTG was fully effective in the presence or

TABLE 2. Induction of β-galactosidase formation

Medium	β-Galactosidase sp act[a] of:			
	KC1043 *gal*[+]	KC1047 Δ(*gal-chl*)	KG2003 *gal*[+]	KG2004 *gal-2*
Lactose	100	1,669	NT[b]	NT
Lactose-IPTG	2,343	2,219	NT	NT
Citrate	10	3	NT	NT
Citrate-lactose	185	1,373	70	1,068
Citrate-galactose	141	1,333	51	1,343
Citrate-IPTG	1,909	1,948	1,870	1,989

[a] Specific activity in nanomoles of σ-nitrophenyl-β-D-galactopyranoside degraded per minute per milligram of cell protein.
[b] NT, Not tested.

TABLE 3. Growth response of PTS mutants to carbon sources

Carbon source	Growth of:			
	KC1043 *pts*$^+$	KC1426 *ptsI*	KC1108 *ptsH*	KC1448 *pts crr*
Fructose	+	−[a]	+	−
Glucose	+	±	±	±
Inositol	+	−	−	−
Mannitol	+	−	−	−
Mannose	+	±	±	±
myo-inositol	+	−	−	−
α-Methylglucoside	+	−	−	−
Ribose	+	−	−	−
Sorbitol	+	−	−	−
Sorbose	+	−	−	−
Sucrose	+	−	−	−
Trehalose	+	−[a]	−	−
Arabinose	+	±	±	+
Glycerol	+	±	±	+
Lactose	+	−	−	+
Maltose	+	−	−	+
Melibiose	+	±	±	+
Raffinose	+	±	±	+
Galactose	+	+	+	+
Gluconate	+	+	+	+

[a] When strain KC1382 (identical to KC1426 except that is also carries a *gal-chl* deletion) was tested, it grew quite well, although slower than the wild type, on both fructose and trehalose.

TABLE 4. Linkage of *pts* and *cysA* mutations

KC1599 (*cysA*) as donor		*pts* mutant tested[a]	KC1599 (*cysA*) as recipient	
cysA/*pts*$^+$[b]	% Linkage		*pts*/*cysA*$^+$[c]	% Linkage
97/139	70	KC1108	87/187	46
69/90	77	KC1382	78/179	44
66/87	76	KC1383	52/101	51
97/118	82	KC1384	81/184	44
81/108	75	KC1385	82/195	42
67/114	59[d]	KC1386	79/180	44
92/126	73	KC1387	55/170	32
69/84	82	KC1388	58/182	32

[a] For the data in the left columns, phage P1 grown on strain KC1559 (*cysA pts*$^+$) was used to transduce each of the PTS mutants to growth on sorbitol-minimal medium with sodium sulfite as the sulfur source (nonselective for *cysA*). The *cysA* marker was scored by picking the PTS$^+$ colonies to plates with sodium sulfate as the sulfur source (selective for *cysA*$^+$). For the data in the right columns, phage grown on each of the PTS mutants (*pts cysA*$^+$) was used to transduce strain KC1599 (*pts cysA*) to growth on gluconate minimal medium (nonselective for *pts*) with sodium sulfate as sulfur source. The *pts* marker was scored by picking the *cysA*$^+$ colonies to plates with sorbitol as carbon source (selective for *pts*$^+$).

[b] Number of *cysA* mutants/number of *pts*$^+$ transductants.

[c] Number of *pts* mutants/number of *cysA* transductants.

[d] Strain KC1386 reverts to the wild type at much higher frequency than the other PTS mutants. Control plates of untransduced cells gave about one-third as many colonies as the transduction. Thus 59% represents a lower limit of cotransduction, and the correct value is probably not different from that seen with the other PTS mutants.

the absence of lactose (Table 2). Similarly, IPTG allowed faster growth on lactose (doubling time, about 1.5 h). Thus, something encoded by the wild-type *gal-chl* region was responsible for reducing the ability of lactose to induce the *lac* operon of wild-type *K. aerogenes*. A similar isogenic pair of strains differing only by a mutation in the *gal* operon was analyzed; the same result was found. The *lac* operon of the galactose-negative mutant (KG2004) was readily induced by lactose, whereas the *lac* operon of the isogenic galactose-positive strain (KG2003) was only weakly induced by lactose (Table 2). This suggested that galactose metabolism may be involved in the effect, so the ability of galactose to induce the *lac* operon was tested directly. Galactose was found to induce the *lac* operon in exactly the same pattern as lactose: the galactose-negative mutants KC1047 and KG2004 were readily induced, whereas the strains capable of degrading the galactose, KC1043 and KG2003, were only weakly induced by galactose, suggesting that the metabolite of lactose responsible for inducing the *K. aerogenes lac* operon is galactose.

Isolation of PTS mutants. After mutagenesis with EMS, eight mutants showing a sugar-negative phenotype on MacConkey indicator agar supplemented with a mixture of maltose and sorbitol were isolated. Two of these strains (KC1384 and KC1386) were leaky on all carbon sources tested and were not studied in detail. One of the mutant strains, KC1108, derived from strain KC1043, differed from the other strains (derived from strain KC1047) in that it was capable of rapid growth on fructose, whereas the other seven were not, even when the *gal-chl* region was restored by transduction. Since growth on fructose is characteristic of *ptsH* but not *ptsI* mutants (24), strain KC1108 was tentatively classified as *ptsH* (see below). A number of sugars were tested; the Pts$^-$ Gal$^+$ strains failed to utilize a number of sugars (Table 3). The mutants also grew more slowly than the wild type with xylose or citrate as a carbon source but just as rapidly as the wild type with gluconate or galactose.

Six of the mutants were plated on sorbitol or maltose medium, and revertants able to grow on these sugars were isolated. With sorbitol, revertants were isolated at frequencies of 10^{-8} to 10^{-10}, and all of these revertants had the same pattern of sugar utilization as the wild type. With maltose, revertants were about 100-fold more frequent, and these fell into three classes. The most common class was able to use a subset of the sugars tested, including lactose, maltose, melibiose, and raffinose, but was unable to use mannose, mannitol, sorbose, sorbitol, or sucrose (Table 3). About 1% of the maltose-positive revertants were able to use all the sugars tested and resembled the sorbitol-selected revertants in every respect. A third class of mutants, representing about 6% of the total maltose-positive revertants, recovered only maltose-utilizing ability. The sorbitol-positive revertants appear to be revertants to the PTS-positive phenotype, the common class of maltose-selected revertants resemble the *crr* mutants of *E. coli*, and the maltose-specific revertants resemble the "PTS-resistant maltose permease" mutants of *E. coli* which can be derived from PTS mutants (21).

On the basis of the phenotypes of the mutants and the revertants, we assumed that the original mutants contained *pts* mutations. We tested this assumption by analyzing the linkage of the mutations to the *cysA* (sulfate permease) gene, which lies near the *pts* operon in *E. coli* (1), and by complementation of the mutations with cloned *pts* genes from *E. coli*. A *cysA* mutant of *K. aerogenes* was isolated as described in Materials and Methods. P1-mediated transduction was used to determine the linkage of each of the mutations to *cysA* and, conversely, of *cysA* to each of the putative PTS mutations. All of the mutations showed approximately the same degree of linkage (about 45%) to *cysA*, and *cysA* was about 75% linked to each of the putative PTS mutations (Table 4).

When plasmid pDS22, carrying the *ptsHI* operon of *E. coli* (generously provided by D. Saffen and S. Roseman), was introduced into the mutants by transformation, all of the ampicillin-resistant transformants of the mutants recovered the ability to utilize both sorbitol and maltose, thus con-

TABLE 5. Effect of carbon source on β-galactosidase induction

Medium	β-Galactosidase sp act[a] of:			
	KC1047 *pts*$^+$	KC1382 *pts*	KC1449 *pts crr*	KC1510 *pts crr*
Citrate	3	<2	NT	NT
Citrate-lactose	1,374	<2	109	825
Citrate-IPTG	1,948	1,703	962	770
Citrate-lactose-cAMP	1,122	<2	1,122	569
Glucose	<0.2	<2	NT	<2
Glucose-lactose	<2	<2	3	5
Glucose-IPTG	308	723	772	760
Glucose-lactose-cAMP	NT	<2	3	5

[a] See footnotes *a* and *b* of Table 2.

TABLE 6. Regulation of histidase expression in a *pts* mutant

Enzyme tested and medium	Sp act[a] of:	
	KC1043 *hutC pts*$^+$	KC1426 *hutC*$^+$ *pts*
Histidase		
Citrate-histidine	271	204
Glucose-histidine	66	275
β-Galactosidase		
Citrate	10	3
Citrate-lactose	185	3
Citrate-IPTG	1,909	1,642

[a] See footnote *a* of Table 2.

firming the identity of the mutations as *pts* mutations. In addition, plasmid pDS10, which carries only the *ptsH* gene of *E. coli*, was able to complement the mutation in strain KC1108, confirming that *pts-3011* is indeed a *ptsH* mutation.

Regulation of *lac* operon expression in PTS mutants. The *lac* operon expression of enteric bacteria responds to glucose addition at several levels (16). Two of these are shown for *K. aerogenes* in Table 5. The presence of glucose in the medium completely prevents the ability of lactose to induce β-galactosidase by a phenomenon known as (inter alia) inducer exclusion (16, 17) (Table 5). In addition, even when IPTG was used as inducer, glucose reduced *lac* expression via CAP-cAMP-mediated permanent catabolite repression (Table 5). The *pts* strain KC1382 showed no induction of *lac* by lactose even when glucose was replaced by a poor carbon source such as citrate, even in the presence of cAMP (Table 5). Furthermore, the failure of KC1382 to grow with lactose as sole carbon source was consistent with the idea that lactose was incapable of inducing *lac* expression in this strain. KC1382 differed from the wild type further in that the permanent catabolite repression caused by glucose was less severe in the mutant (about twofold) than in the wild-type (about sixfold) (Table 5).

The *crr*-type revertants of the *pts* mutants fell into two distinct classes; KC1449 and KC1510 (both derived from the same parental *pts* strain) are representative of the two types. In strain KC1449, lactose was weakly able to induce *lac* expression in the presence of a poor carbon source such as citrate, and almost full induction (i.e., equal to IPTG induction) was restored in the presence of cAMP (Table 5). In the presence of glucose, the permanent catabolite repression remained weak (Table 5). Surprisingly, the glucose-mediated inducer exclusion was only slightly weakened (Table 5). Strain KC1510 was similar, except that lactose was capable of inducing *lac* expression to high levels in citrate medium, even in the absence of cAMP. Again both the permanent catabolite repression and the inducer exclusion mediated by glucose were weaker than in the wild type. Five other *pts* mutants were examined and showed essentially the same regulatory pattern as KC1382. In addition, each of the five gave rise to *crr* revertants, and these revertants resembled either KC1449 or KC1510.

Regulation of *hut* operon expression in PTS mutants. For the experiments described in Table 5, strains carrying the *gal-hut-bio-chl* deletion were used to eliminate any confusion about lactose induction (see above). The *gal-hut-bio-chl* region of KC1382 was restored with *hutC*$^+$ DNA (carrying an active *hut*-specific repressor) to allow us to test for the inducer exclusion phenomenon. The resulting strain, KC1426, was analyzed for effects of the *pts* mutation on *hut* operon expression, monitored as expression of histidase from the *hutH* gene. The inducibility of *hut* by histidine was unaffected by the *pts* mutation (Table 6), but glucose was unable to mediate permanent catabolite repression in KC1426. The constitutive inducer exclusion of lactose in the *pts* strain was also visible in a *gal*$^+$ background, where lactose was a relatively weak inducer even in a *pts*$^+$ background (Table 6).

DISCUSSION

The data presented here argue that, in *K. aerogenes*, the physiologically relevant inducer of the *lac* operon is galactose. In *E. coli*, allolactose (generated by the action of basal levels of β-galactosidase on lactose) serves as a physiological inducer, allowing full induction of *lac* even in *gal*$^+$ strains. The failure of lactose to effect full induction of *K. aerogenes lac* via allolactose may reflect the differences in repressor structure in the inducer-binding site and recalls the fact that melibiose, an inducer of *lac* in E. coli, prevents induction of *lac* in *K. aerogenes*. However, we cannot exclude the possibility that galactosides such as allolactose or lactose might be capable of binding to and inactivating the *K. aerogenes lac* repressor but are degraded too rapidly by β-galactosidase to accumulate to inducing concentrations. In the absence of a *lacZ* mutant (which we have been unable to isolate in this strain), we can be sure only that lactose fails as a physiologically effective inducer. The argument that a lower basal level of β-galactosidase in *K. aerogenes* might be insufficient to generate sufficient quantities of allolactose is unlikely, since lactose does cause a partial induction, thus raising the β-galactosidase level high enough to trigger full induction if the *E. coli* model were applicable.

The notion that galactose is the inducer for *K. aerogenes lac* is hardly startling when one considers the following: (i) galactose, like allolactose, is a product of the β-galactosidase reaction; (ii) galactose, like allolactose, is further degraded; (iii) galactose also acts as an inducer for *E. coli lac*, although less efficiently than allolactose (2); and (iv) both the *E. coli* and *K. aerogenes lac* repressors show considerable homology to the *gal* repressor protein (5). The physiological consequence of using galactose as an inducer of *lac* appears to be that the active galactose catabolism of *K. aerogenes* degrades the inducer so fast that it never accumulates to levels high enough to allow significant induction, and the strain is thus functionally almost lactose negative. This may explain why so many of the wild-type isolates of *K. aerogenes* carry a separate *lac* operon with *E. coli*-like induction properties on a plasmid.

The *pts* mutants described here affect *lac* regulation at two

levels. (i) At the level of inducer exclusion, induction of β-galactosidase by lactose (requiring lactose permease activity) is completely inhibited, whereas induction by IPTG (not requiring lactose permease activity) is normal. (ii) At the level of permanent catabolite repression, the *pts* mutants grow more slowly than the wild type on glucose, thus reducing the effectiveness of the glucose-mediated catabolite repression. The *pts* mutants described here have a phenotype that is similar but not identical to those of mutants isolated by Tanaka and Lin (23). They are similar in that both show no growth on mannitol, sorbitol, or (for *ptsI*) fructose; slow growth on glycerol, glucose, arabinose, and mannose; and wild-type growth on galactose. They differ, however, in that our *ptsI K. aerogenes* mutants show no growth with maltose, ribose, inositol, or lactose, whereas theirs showed weak growth with maltose, wild-type growth with ribose and inositol, and, surprisingly, wild-type growth with lactose. Although the weak growth on maltose may reflect differences in reagents, leakiness of the mutation, or the different isolation procedures (our mutants were isolated as maltose nonfermenters, theirs as mannitol nonfermenters), the wild-type growth of their *ptsI* mutant on lactose remains unexplained, even if we assume that their strain retained the plasmid with an *E. coli*-like *lac*.

Curiously, *ptsI* strains carrying the *gal-chl* deletion grow quite well, although still slower than the wild type, with fructose or trehalose as the carbon source. With all the other carbon sources listed in Table 3, no difference was seen between the gal^+ strains and *gal* mutants. The connection between fructose and trehalose probably has nothing to do with the *ptsH* product, since the *ptsH* mutant remains fully negative on trehalose despite its wild-type growth on fructose. The effect of the *gal-chl* deletion on the PTS-dependent metabolism of these two sugars remains unexplained.

One clear difference between *pts* mutants of *K. aerogenes* and those of *E. coli* is that the former grow relatively well on glucose, presumably via their active glucose dehydrogenase and perhaps hexokinase. This is further supported by the observation that, under conditions of glucose excess, a substantial fraction of the glucose transport in *K. aerogenes* is independent of the PTS system, even in the wild type (10). Although the growth of *K. aerogenes pts* strains is much better than that of *E. coli pts* strains, it is still slower than that of wild-type *K. aerogenes*. This is further reflected in the fact that *K. aerogenes pts* strains show (at best) a weak glucose-mediated permanent catabolite repression of both PTS-responsive systems (*lac*) and PTS-nonresponsive systems (*hut*). When catabolite-repressible operons such as *hut* are compared in *E. coli* and *K. aerogenes* backgrounds, the glucose-mediated permanent catabolite repression is found to be stronger in *K. aerogenes* (7), presumably because both the Embden-Meyerhof pathway (beginning with the PTS-dependent phosphorylation of glucose) and the glucose dehydrogenase pathway (independent of PTS) contribute to a very rapid glucose catabolism in *K. aerogenes* (11), whereas *E. coli* uses only the former system.

Finally, except for the fact that glucose-mediated permanent catabolite repression is weakened in *pts* mutants, the regulation of the histidine utilization (*hut*) operons of *K. aerogenes* is independent of the PTS, in that there is no inducer exclusion of histidine and the CAP-cAMP-mediated activation of *hut* is unimpaired in *pts* mutants.

ACKNOWLEDGMENTS

We are grateful to D. Saffen and S. Roseman for generously providing plasmids pDS10 and pDS22.

This work was supported by Public Health Service grant AI 15822 from the National Institutes of Health.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli*, edition 7. Microbiol. Rev. **47**:180–230.
2. **Barkley, M. D., and S. Bourgeois.** 1978. Repressor recognition of operator and effectors, p. 177–220. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Bender, R. A., K. A. Janssen, A. D. Resnick, M. Blumenberg, F. Foor, and B. Magasanik.** 1977. Biochemical parameters of glutamine synthetase from *Klebsiella aerogenes*. J. Bacteriol. **129**:1001–1009.
4. **Brenchley, J. E., and B. Magasanik.** 1972. *Klebsiella aerogenes* strain carrying drug-resistance determinants and a *lac* plasmid. J. Bacteriol. **112**:200–205.
5. **Buvinger, W. E., and M. Riley.** 1985. Nucleotide sequence of *Klebsiella pneumoniae lac* genes. J. Bacteriol. **163**:850–857.
6. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. J. Bacteriol. **118**:810–814.
7. **Goldberg, R. B., F. R. Bloom, and B. Magasanik.** 1976. Regulation of histidase synthesis in intergeneric hybrids of enteric bacteria. J. Bacteriol. **127**:114–119.
8. **Goldberg, R. B., and B. Magasanik.** 1975. Gene order of the histidine utilization (*hut*) operons in *Klebsiella aerogenes*. J. Bacteriol. **122**:1025–1031.
9. **Hall, B. G., and E. C. R. Reeve.** 1977. A third β-galactosidase in a strain of *Klebsiella* that possesses two *lac* genes. J. Bacteriol. **132**:219–223.
10. **Heijssel, O. M., G. P. M. A. Hardy, J. C. Lansbergen, D. W. Tempest, and R. W. O'Brien.** 1980. Influence of growth environment on the phosphoenolpyruvate:glucose phosphotransferase activities of *Escherichia coli* and *Klebsiella aerogenes*: a comparative study. Arch. Microbiol. **125**:175–179.
11. **Neidhardt, F. C.** 1960. Mutant of *Aerobacter aerogenes* lacking glucose repression. J. Bacteriol. **80**:536–543.
12. **Neijssel, O. M., D. W. Tempest, P. W. Postma, J. A. Duine, and J. Frank, Jr.** 1983. Glucose metabolism by K^+-limited *Klebsiella aerogenes*: evidence for the involvement of a quinoprotein glucose dehydrogenase. FEMS Microbiol. Lett. **20**:35–39.
13. **Orskov, I.** 1984. Genus VI. *Klebsiella* Trevisan 1885, p. 321–324. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
14. **Lederberg, E. M., and S. N. Cohen.** 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. **119**:1072–1074.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Magasanik, B.** 1970. Glucose effects: inducer exclusion and repression, p. 189–219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Postma, P. W., and J. W. Lengeler.** 1985. Phosphoenolpyruvate: carbohydrate phosphotransferase system of bacteria. Microbiol. Rev. **49**:232–269.
18. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen-limited growth of *Klebsiella aerogenes*. J. Biol. Chem. **246**: 6288–6296.
19. **Reeve, E. C. R., and J. A. Braithwaite.** 1973. Lac^+ plasmids are responsible for the strong lactose-positive phenotype found in many strains of *Klebsiella* species. Genet. Res. **22**:329–333.
20. **Reeve, E. C. R., and J. A. Braithwaite.** 1974. The lactose system in *Klebsiella aerogenes* V9A. IV. A comparison of the *lac* operons of *Klebsiella* and *Escherichia coli*. Genet. Res. **24**:323–331.
21. **Saier, M. H., H. Straud, L. S. Massman, J. J. Judice, M. J. Newman, and B. U. Feucht.** 1978. Pemease-specific mutations in *Salmonella typhimurium* and *Escherichia coli* that release the

glycerol, maltose, melibiose, and lactose transport systems from regulation by the phosphoenolpyruvate:sugar phosphotransferase system. J. Bacteriol. **133**:1358–1367.

22. **Streicher, S. L., R. A. Bender, and B. Magasanik.** 1975. Genetic control of glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **121**:320–331.
23. **Tanaka, S., and E. C. C. Lin.** 1967. Two classes of pleiotropic mutants of *Aerobacter aerogenes* lacking components of a phosphoenolpyruvate-dependent phosphotransferase system. Proc. Natl. Acad. Sci. USA **57**:913–919.
24. **Walter, R. W., and R. L. Anderson.** 1973. Evidence that the inducible phosphoenolpyruvate:D-fructose 1-phosphotransferase system of *Aerobacter aerogenes* does not require HPr. Biochem. Biophys. Res. Commun. **52**:93–97.