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Genetics of the lac-PTS system of *Klebsiella*

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SUMMARY

The isolation of a temperature sensitive *pts I* mutant which fails to utilize lactose provides strong evidence that *Klebsiella* strain CT-1 utilizes lactose via a phosphoenolpyruvate dependent lactose-phosphotransferase system (PTS-lac). We designate this lactose utilization system *elu* for *evolved lactose utilization*. Analysis of a series of Lac^- mutants identifies two genes, *eluA* and *eluB*, whose function is required for lactose utilization by this pathway. The functions specified by these genes are not known, but neither locus specifies the hydrolytic enzyme phospho-β-galactosidase. A mutant of CT-1, strain RPD-2, exhibits a half-maximal growth rate at a lactose concentration 40 fold lower than that of strain CT-1; and it has a K_m for lactose uptake that is 40 fold lower than that of strain CT-1. That mutation defines the locus *eluC*, which is assumed to specify the enzyme II(lac) of the PTS-lactose system. From the observations that (i) cellobiose induces the phospho-β-galactosidase enzyme, (ii) pregrowth in cellobiose dramatically reduces the growth lag when cells are shifted into lactose minimal medium, (iii) *eluB* mutants exhibit a growth lag when shifted into cellobiose minimal medium, and (iv) lactose induces a phospho-β-glucosidase enzyme; we speculate that the phospho-β-glucosidase enzyme is the same enzyme as the phospho-β-glucosidase that normally functions in cellobiose metabolism. We conclude that the original mutation that allowed CT-1 to utilize lactose was a regulatory mutation that permitted inducible expression of the *eluC* gene.

1. INTRODUCTION

Klebsiella strain RE1755, a lactose-negative mutant of *Klebsiella* strain RE1544, spontaneously lost both the chromosome specified β-galactosidase (BGase-II), and the plasmid specified β-galactosidase (BGase-I) of its parent strain (Reeve, 1976). Strain RE1755 does synthesize an inducible β-galactosidase designated β-galactosidase-III (BGase-III) (Hall & Reeve, 1977). Synthesis of β-galactosidase-III is inducible by lactose and by β-methyl-galactoside (Hall, 1979), but strain

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RE1755 is unable to utilize lactose because (i) BGase-III has very low activity toward lactose, and (ii) strain RE1755 possesses no β-galactoside permease (Hall, 1980). Strain RE1755A is a streptomycin resistant nalidixic acid resistant mutant of strain RE1755 (Hall, 1979). Strain CT-1 is a spontaneous Lac^+ mutant of strain RE1755A. CT-1 failed to induce β-galactosidase synthesis during growth on lactose, and the properties of BG ase-III were unchanged from those of the parent strain. Strain CT-1 did induce synthesis of a phospho-β-galactosidase during growth on lactose (Hall, 1979), suggesting that the phospho-β-galactosidase played a role in lactose utilization. This idea was supported by the observation that in many Lac^- mutants of strain CT-1 the phospho-β-galactosidase activity was uninducible. The parental strain, RE1755A, also synthesized phospho-β-galactosidase when grown in the presence of lactose, but even when fully induced it was unable to utilize lactose (Hall, 1979).

These observations led to the hypothesis that strain CT-1 transported lactose by a phosphoenolpyruvate dependent phosphotransferase system (PTS), that phosphorylated lactose during transport. This idea was supported by direct studies of lactose-dependent release of pyruvate from phosphoenolpyruvate (PEP), and PEP dependent hydrolysis of O-nitrophenyl-β-D-galactoside (ONPG) by permeabilized cells (Imai & Hall, 1981). High background activities in those assays prohibited drawing a firm conclusion about the role of a possible lac-PTS system in lactose utilization by strain CT-1. In this paper we report further evidence which allows us to conclude firmly that strain CT-1 metabolizes lactose via a lac-PTS system and a phospho-β-galactosidase.

It had been noted (Hall, 1979) that the growth rate of strain CT-1 depended strongly upon the external lactose concentration, no growth occurring below 1 mM lactose. Studies of lactose transport kinetics (Imai & Hall, 1981) showed a strong correlation ($r = 0{\cdot}997$) between growth rate and lactose transport rate, with the half-maximal growth rate occurring at a lactose concentration (5·4 mM) that was nearly identical with the K_m for lactose transport (6 mM lactose). It was concluded that in strain CT-1 the transport rate limits the growth rate on lactose, and it was predicted that mutants capable of growth at lactose concentrations under 1 mM would possess altered transport systems, rather than altered hydrolytic enzymes. In this report we verify that prediction.

Finally, we note some interactions between the system for cellobiose utilization and lactose utilization in these Lac^+ strains; and we speculate on the evolutionary basis for those interactions.

2. MATERIALS AND METHODS

(i) *Bacterial strains*

All strains are dervied from *Klebsiella* strain RE1755 (Reeve, 1976). Genotypes are given in Table 1.

(ii) *Media*

The phosphate buffered mineral salts minimal medium previously described (Hall, 1979) was employed except for those experiments involving growth at 30 °C and 42 °C. For those experiments the NaCl concentration was increased to 20 g per litre to prevent cell clumping at 30 °C.

Table 1. Klebsiella *strains*

Strain	Genotype*	
RE1755A	*eluR*^{0}, *nal*, *rpsL*	[Gal$^-$ Lac$^-$]†
CT-1	*eluR*$^+$ mutant of RE1755A	[Lac$^+$]
CT-3	*eluA1* mutant of CT-1	[Lac$^-$]
CT-4	*eluA2* mutant of CT-1	[Lac$^-$]
CT-5	*eluA3* mutant of CT-1	[Lac$^-$]
CT-6	*eluB1* mutant of CT-1	[Lac$^-$]
CT-7	*eluB2* mutant of CT-1	[Lac$^-$]
CT-8	*eluB3* mutant of CT-1	[Lac$^-$]
CT-9	*ptsH1* mutant of CT-1	[Lac$^-$]
CT-10	*ptsH2* mutant of CT-1	[Lac$^-$]
CT-11	*ptsH3* mutant of CT-1	[Lac$^-$]
CT-12	*eluB4* mutant of CT-1	[Lac$^-$]
CT-13	*eluB5* mutant of CT-1	[Lac$^-$]
CT-14	*eluB6* mutant of CT-1	[Lac$^-$]
RPD-2	*eluC*$^+$(*AL*) mutant of CT-1	[Lac$^+$]
mtl-2	*ptsI* mutant of CT-1	[Lac$^-$]
BG-7	*bgtaI* mutant of RE1755A	[Lac$^-$]
BG71	*bgtA1*, *eluR*$^+$, transductant from CT-1 into BG-7	[Lac$^+$]
BG71-CR6	*bgtA1*, *eluR*$^+$, *ptsI*(*ts*) mutant of BG-71	

* Genetic nomenclature: *eluA*: Lactose negative, phospho-galactosidase uninducible by lactose (Hall, 1979). *eluB*: Lactose negative, phospho-galactosidase uninducible by lactose (Hall, 1979), lag in growth on cellobiose. *eluR*^{0}: Wild type regulatory allele. Does not permit expression of lactose transport system. Lactose negative. *eluR*$^+$: Mutant regulatory allele. Permits expression of lactose transport system. Lactose positive. *eluC*$^+$(*AL*): Mutant allele of structural gene for enzyme II(Lac). Mutant gene product has increased affinity for lactose. *bgtA*: Structural gene for β-galactosidase-III.

† [ ] enclose relevant phenotype.

(iii) *Growth rates*

Growth rates were usually determined by monitoring the increase in turbidity at 600 or 625 nm in a Gilford spectrophotometer. At low carbon source concentrations (< 1 mM), however, the carbon source is rapidly exhausted at cell densities employed for monitoring turbidity. For the experiments measuring the growth rates of strain RPD-2 at low lactose concentrations cells were pregrown in 10 mM lactose, washed, and diluted to give about 1000 cells per ml in minimal medium containing 30–300 μM lactose. At various times the colony forming units (CFU) were determined by the pour plate method using LB agar (1 % tryptone, 0·5 % yeast extract, 1 % NaCl, 1·5 % agar).

Growth rates are reported as the first order growth rate constant in units of reciprocal hours, and were determined from the slope of the last squares fit of ln (CFU) or ln (A_{625}) *v.* time.

(iv) *Transductions, mutagenesis, and mutant selection*

Transductions were mediated by bacteriophage P1 *cam ts100*. Mutagenesis was by ethyl methane sulphonate. Mutants were isolated by counter selection with ampicillin. All of these procedures were previously described (Hall, 1979).

(v) *Lactose transport assays*

Lactose transport was measured from the rate of ^{14}C-lactose uptake as previously described (Imai & Hall, 1981).

(vi) *Enzyme assays*

Phospho-β-galactosidase activity in cell free extracts was assayed as previously described (Hall, 1979).

Phospho-β-galactosidase activity was also measured in cells permeabilized with toluene. Cells were incubated in 5 mM-O-nitrophenyl-β-D-galactoside-6-phosphate (PONPG) in buffer 68 (Hall, 1979) until sufficient yellow colour had appeared. The assay was terminated by the addition of 0·33 volumes of 1 M-Na_2CO_3, cells were removed by filtration, and the concentration of O-nitrophenol (ONP) was determined from the absorbance of the clear fluid at 420 nm.

O-nitrophenyl-β-D-galactoside (ONPG) transport activity was measured as the hydrolysis of 5 mM-ONPG by intact cells as previously described (Hall, 1979) except that buffer 68 was employed in the place of TM buffer.

Phospho-β-glucosidase activity was measured as the hydrolysis of O-nitrophenyl-β-D-glucoside (ONPGlu) by intact cells exactly as was done for the hydrolysis of ONPG.

For all assays one unit of activity is the hydrolysis of 1 nmol of substrate per minute.

3. RESULTS

(i) *Analysis of Lac$^-$ mutants*

A series of Lac$^-$ mutants isolated from strain CT-1 (Hall, 1979) was subjected to analysis by reciprocal bacteriophage P1 transductions (Table 2). From the observation that RE1755A can transduce all of these mutants to Lac$^+$, and that all of the mutants can likewise transduce RE1755A to Lac$^+$, it is apparent that none of these mutations is in the same gene as that which resulted in acquisition of the Lac$^+$ phenotype in strain CT-1. This genetic analysis confirms the earlier interpretation based upon phenotypic data (Hall, 1979).

The mutants in Table 2 can be placed into three classes such that mutants in the same class transduce each other to Lac$^+$ rarely, while those in different classes transduce each other to Lac$^+$ frequently. According to this analysis one class includes strains CT-3, -4, and -5; a second class includes strains CT-6, -7, -8, -12, -13, and -14; and a third class includes strains CT-9, -10, and -11.

Transport and concomitant phosphorylation of many sugars is mediated by the PEP dependent phosphotransferase system called PTS. Included in this system are the constitutively synthesized common proteins enyme I, which catalyzes the transfer of a high energy phosphate from PEP to the second common protein, HPr. Also included are a family of sugar specific membrane bound proteins, enzymes II; and proteins which may be either membrane bound or soluble and are designated factors III (for reviews of the properties of the PTS system (see Saier, 1977; and Postma & Roseman, 1976). Thus there is an enzyme II (man) for mannitol, and an enzyme II(glu) for glucose, etc. Sugars which are transported by this system are called PTS sugars. In the *Enterobacteriaceae* these include glucose, fructose, mannitol, sorbitol, cellobiose and other β-galactosides, mannose, and *N*-acetyl-glucosamine among others, but do not include galactose, melibiose, glycerol, or lactose (Saier, 1977; Postma & Roseman, 1976; Fox & Wilson, 1968). In some gram positive organisms, however, notably *Staphylococci* and *Streptococci*, lactose *is* a PTS sugar (Simoni & Roseman, 1973; McKay *et al.* 1970). Mutations in the *ptsI* gene for enzyme I, or in the *ptsH* gene for HPr protein result in a failure to utilize PTS sugars. Walter & Anderson (1973) have shown, however, that *ptsH* but not *ptsI* mutants of *Aerobacter aerogenes* PRL-R3 (now classified as *Klebsiella pneumoniae* variety *oxytoca* (Mortlock, 1981)) can utilize the PTS sugar fructose.

The mutants in Table 2 were screened for utilization of the PTS sugars mannitol, sorbitol, phenyl-β-glucoside, glucose, fructose, and mannose. The class which includes CT-9, -10, and -11 was unable to utilize any of the PTS sugars except fructose. These mutants are therefore designated *ptsH*. The class which includes CT-6, -7, -8, -12, -13, and -14 was unimpaired in the utilization of PTS sugars except that the appearance of colonies on cellobiose minimal medium was delayed. These mutants are designated *eluB*, for *evolved lactose utilization*. The class which includes CT-3, -4, and -5 is unimpaired in the utilization of any PTS sugars tested. These are designated *eluA*.

The finding that a class of Lac$^-$ mutants was *ptsH* supported the hypothesis that in strain CT-1 lactose was utilized by a PTS transport system. That hypothesis was strengthened by the finding that a *ptsI* mutant (isolated as a mannitol negative mutant and found to be negative on all PTS sugars tested, including fructose) was also lactose negative. A lysate of bacteriophage P1 prepared on that strain, strain mtl-2, transduced the *eluA* mutant CT-4 and the *eluB* mutant CT-7 to Lac$^+$ at high frequencies (13·2 and 11·3 transductants μl^{-1} of lysate respectively), but transduced the *ptsH* mutant CT-10 to Lac$^+$ at a low frequency (0·8 μl^{-1}). Based upon this analysis it seems likely that the *ptsH* and *ptsI* genes are closely linked in this species of *Klebsiella* just as they are in *Salmonella typhimurium* and *Escherichia coli* (Bachmann & Low, 1980; Sanderson & Hartman, 1978).

Table 2. *Reciprocal transductions**

Recipient strain	Donor strain											
	CT-3	CT-4	CT-5	CT-6	CT-7	CT-8	CT-9	CT-10	CT-11	CT-12	CT-13	CT-14
CT-3	0	0·34	0·53	15·3	13·4	6·5	11·1	15·1	17·7	18·3	16·2	22·8
CT-4	0·29	0	0·72	13·3	11·4	9·0	10·9	16·6	13·9	13·7	11·5	10·8
CT-5	0·25	0·45	0	21·2	15·7	11·11	12·2	16·3	19·0	20·7	13·6	17·2
CT-6	7·0	10·5	14·5	0	0·5	0·46	7·5	11·1	10·9	0·34	0·32	0·34
CT-7	9·8	9·1	12·3	0·5	0	0·1	7·5	12·5	10·4	0·19	0	0
CT-8	6·4	8·0	13·7	0·06	0·1	0	8·0	11·6	11·4	0·27	0	0·09
CT-9	10·7	10·9	17·8	12·1	16·4	6·5	0	0	0·32	15·4	9·1	14·3
CT-10	10·2	13·9	13·9	9·5	9·7	10·2	0	0	0·08	19·9	12·2	14·1
CT-11	11·1	16·2	14·2	11·5	14·6	9·9	0·01	0·32	0	13·8	11·4	11·5
CT-12	5·2	8·8	9·1	0	0	0	6·0	9·6	6·3	0	0·08	0
CT-13	6·0	10·7	10·6	0·14	0·14	0	6·8	8·6	9·7	0·05	0	0
CT-14	7·5	15·5	11·7	0·26	0·10	0·37	10·4	14·1	16·4	0·11	0·11	0

* Transductants per microlitre of lysate, corrected for revertants. Values are means of two experiments. All values were reproducible ±20% between the two experiments.

Although the above observations implicated the PTS system in lactose utilization, it was possible that the effects of the *ptsH* and *ptsI* mutations were indirect. Some *ptsH* and *ptsI* mutations are known to affect the utilization of non-PTS sugars such as lactose, melibiose, and galactose (Postma & Roseman, 1976). Those effects are attributed to inducer exclusion (Saier & Roseman, 1972; Postma & Roseman, 1976) and the possibility remained that inducer exclusion could account for the Lac$^-$ phenotype of the *ptsH* and *ptsI* mutants of strain CT-1.

(ii) *Isolation of a temperature sensitive* ptsI *mutant*

The basal level of BGase-III in strain CT-1 had made interpretation of some earlier experiments difficult (Imai & Hall, 1981), accordingly we began by isolating a BGase-III negative mutant of strain RE1755A.

BGase-III is inducible by β-methyl-galactoside in strain RE1755A (Hall, 1979), and it hydrolyzes phenyl-β-galactoside rapidly enough to permit growth (Hall, 1980). Strain RE1755A was mutagenized, and the mutagenized culture was grown in the presence of β-methyl-galactoside to induce BGase-III synthesis. To enrich for BGase-III negative mutants, the induced culture was subjected to ampicillin selection for failure to utilize phenyl-β-galactoside. Following two such rounds of selection the culture was plated onto non-selective medium, and individual colonies were scored for the presence of a β-methyl-galactoside inducible β-galactosidase.

One such mutant, strain BG-7, synthesized no detectable β-galactosidase activity. We designate the gene for BGase-III *bgtA*, and strain BG-7 is accordingly *bgtA1*.

The Lac$^+$ phenotype was transduced from strain CT-1 into strain BG-7, and the Lac$^+$ transductant was called strain BG-71. Strain BG-71 retains the *bgtA1* allele, and synthesizes no BGase-III activity.

Strain BG-71 was mutagenized and subjected to two rounds of ampicillin selection for failure to utilize PTS sugars at 42 °C. The first round selected against utilization of mannitol or sorbitol; and, to avoid selection of sugar specific enzyme II or factor III mutants, the second round was against cellobiose utilization. Following selection the stock was plated onto MacConkey-mannitol indicator agar and incubated at 42 °C. Mannitol negative colonies were identified, and the plates were incubated for a subsequent 24 hr at 30 °C. Those colonies which were mannitol negative at 42 °C, but positive at 30 °C were tested for utilization of the PTS sugars mannose, mannitol, sorbitol, glucose, fructose, and cellobiose at 30 °C and 42 °C. One isolate, strain BG71-CR6, was negative on all PTS sugars at 42 °C, and was positive on all PTS sugars at 30 °C. It was accordingly designated *ptsI*(*ts*).

Strain BG71-CR6 was Lac$^+$ at 30 °C and Lac$^-$ at 42 °C. To determine whether the temperature sensitive Lac$^-$ phenotype resulted from inducer exclusion or from a failure to transport lactose via a lac-PTS system, temperature shift experiments were performed. If the Lac$^-$ phenotype resulted from inducer exclusion, BG71-CR6

cells growing on lactose at 30 °C, and therefore induced for the lactose utilization system, should continue to grow on lactose at 42 °C until a rate limiting component of the lactose utilization system was diluted out by growth. If, on the other hand, the temperature sensitive phenotype resulted from thermolability of the enzyme I protein itself, and lactose was transported by a lac-PTS system, then growth on lactose should cease virtually immediately after the culture is shifted to 42 °C.

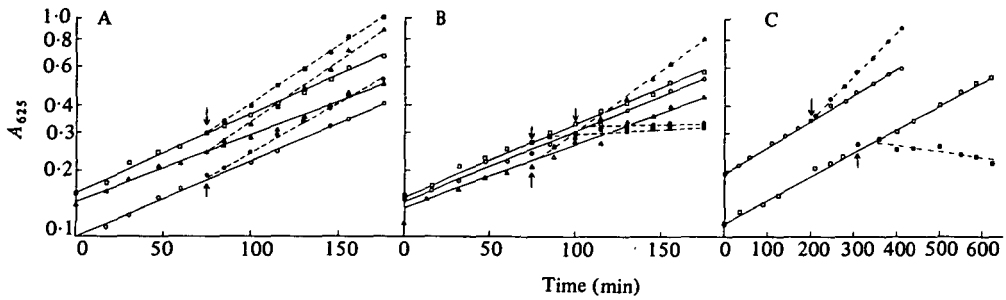

Fig. 1. Growth of strains BG-71 and BG71-CR6 at 30 °C and 42 °C. A portion of each culture growing at 30 °C was shifted to 42 °C at the time indicated by the arrow. Open symbols and solid line, growth at 30 °C; closed symbols and dashed lines, growth at 42 °C. *Panel A*: strain BG71. Circles = cellobiose, squares = fructose, triangle = galactose. *Panel B*: Strain BG71-CR6. Circles = cellobiose, squares = fructose, triangles = galactose. *Panel C*: Growth on lactose. Circles = strain BG-71, squares = strain BG71-CR6.

Figure 1*A* shows that the growth rate of the parental strain BG-71 increases upon being shifted to 42 °C, whether the carbon source is a non-PTS sugar (galactose) or a PTS sugar (fructose or cellobiose). Panel B shows that the growth of the mutant strain BG-71-CR6 ceases upon shift to 42 °C if the carbon source is a PTS sugar, but increases if the carbon source is the non-PTS sugar galactose. Growth of BG71-CR6 on mannitol and sorbitol also ceased within 10 min of being shifted to 42 °C (data not shown). Panel C shows that the temperature sensitive *ptsI* strain BG71-CR6 ceased growing on lactose within ten minutes of being shifted to 42 °C, while the parental strain BG71 exhibited an increased growth rate at the elevated temperature. Table 3 shows the growth rates of these strains on various sugars.

The rapid cessation of lactose utilization by strain BG71-CR6 at 42 °C is not consistent with inducer exclusion, but is entirely consistent with the utilization of lactose via a lac-PTS system.

To confirm that the *ptsI(ts)* mutation directly affected β-galactoside transport, we measured the effect of temperature on the transport of ONPG. (These strains do not transport thio-methyl-β-galactoside (TMG) at all, thus TMG uptake could not be used to assess the affect of temperature on transport). ONPG was previously shown to compete for the uptake of lactose by strain CT-1 (Imai & Hall, 1981), and is therefore presumed to enter by the lactose transport system. Since strains

BG71 and BG71-CR6 lack β-galactosidase activity, the hydrolysis of ONPG depends upon the activity of the phospho-β-galactosidase, an enzyme which is active only toward phosphorylated ONPG (Hall, 1979). Phosphorylation of ONPG requires an intact cell membrane (Hall, 1979; and Table 4), and thus the rate of ONPG hydrolysis by intact cells is a measure of the rate of transport associated ONPG phosphorylation. The activity of the phospho-β-galactosidase enzyme can be measured directly in toluene treated cells by using commercial ONPG-6-phosphate as a substrate. Table 4 shows that phospho-β-galactosidase activity is reduced about two-fold at 42 °C. in both strains, but *transport* is virtually eliminated at 42 °C only in the temperature sensitive *ptsI* strain BG71-CR6.

Table 3. *Growth Rates*

	30 °C		42 °C	
Substrate	BG71	BG71-CR6	BG71	BG71-CR6
Sorbitol	N.D.	0·389	N.D.	< 0·01
Mannitol	N.D.	0·474	N.D.	< 0·01
Fructose	0·493	0·441	0·743	< 0·01
Cellobiose	0·488	0·442	0·623	< 0·01
Galactose	0·424	0·437	0·778	0·772
Lactose	0·161	0·150	0·250	< 0·01

Values are first order growth rate constants in reciprocal hours.
N.D. = not determined.

Table 4. *Phospho-β-galactosidase activity and ONPG transport*

	BG-71	BG71-CR6
Phospho-β-galactosidase, 30 °C	5·69	5·66
Phospho-β-galactosidase, 42 °C	2·99	2·55
ONPG transport, 30 °C	8·54	5·96
ONPG transport, 42 °C	8·33	0·25

Values are units per 10^9 cells.

Taken together, the properties of strain BG71-CR6 confirm the hypothesis that lactose is transported and phosphorylated by a lac-PTS system in these strains of *Klebsiella*.

(iii) *A mutant that grows at low lactose concentrations*

Strain CT-1 does not grow on 1 mM lactose (Hall, 1979). A spontaneous mutant of CT-1, strain RPD-2, was isolated by selecting for growth on 1 mM lactose minimal agar. We have predicted that such a mutant would possess an improved lactose transport system (Imai & Hall, 1981).

The growth rate of strain RPD-2 on 1·0 mM lactose was $0{\cdot}345 \pm 0{\cdot}018\ h^{-1}$, not significantly different from its rate of $0{\cdot}367 \pm 0{\cdot}013\ h^{-1}$ on 10 mM lactose. The growth rate was measured over a range from 30–300 μM lactose (Fig. 2), and the

half-maximal growth rate occurred at 130 micromolar lactose, a concentration forty-fold lower than that required for the half-maximal growth rate of the parent strain CT-1 (Imai & Hall, 1981).

The kinetics of lactose uptake were examined using highly purified ^{14}C-lactose. The apparent K_m for lactose uptake was 150 μM lactose (Fig. 3), and the apparent V_{max} was 110 nmol/min./mg of cell protein. The apparent K_m is thus 40 fold lower than that of the parental strain CT-1 (Imai & Hall, 1981), while the apparent V_{max} for lactose is virtually unaltered. There is a strong correlation between transport rate and the growth rate of strain RPD-2 on lactose (correlation coefficient $r = 0{\cdot}97$), implying that transport still limits the rate of *in vivo* lactose hydrolysis by strain RPD-2.

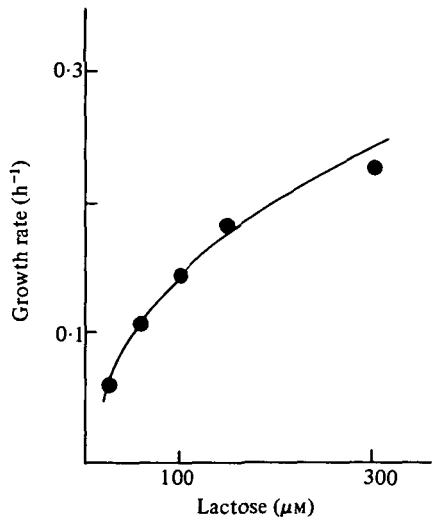

Fig. 2. Growth rate of strain RPD-2 as function of lactose concentration.

ONPG is a competitive inhibitor of lactose uptake by strain RPD-2 (Fig. 3), with an apparent K_i of 2 mM-ONPG. That apparent K_i is only four-fold lower than that observed in the parental strain CT-1 (Imai & Hall, 1981), indicating that the mutation affected the affinity of the system for lactose much more than it did the affinity for ONPG.

These data suggest that the mutation in strain RPD-2 is in the structural gene for the enzyme II (lac). To determine whether any of the previously identified *elu* genes was identical with or linked to the gene for enzyme II (lac), a lysate of phage P1 prepared on strain RPD-2 was used to transduce strains RE1755A, CT-4, CT-7, and CT-10. Lac$^+$ transductants were selected on minimal medium containing 10 mM lactose, and from each cross 100 transductants were scored for the ability to grow on 1 mM lactose. None of the Lac$^+$ transductants was able to grow on 1 mM lactose, and it is therefore concluded that the gene for enzyme II (lac) is not tightly

linked to any of the previously identified loci which affect lactose utilization. We designate the gene for enzyme II (lac) *eluC*$^{+}$(*AL*), the 'AL' indicating that the mutant enzyme II (lac) has an altered affinity for lactose.

Strain RE1755A is lactose negative, galactose negative, and expresses a lactose inducible BGase-III. The mutation that allows lactose utilization was pleiotrophic in that it also rendered strain CT-1 galactose positive, and it prevented induction of BGase-III synthesis by lactose (Hall, 1979). It was originally suggested that the

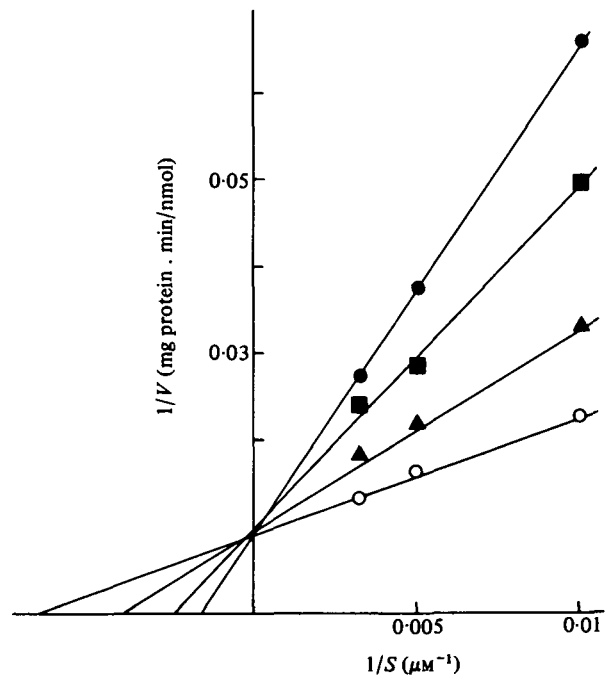

Fig. 3. Inhibition of lactose uptake by ONPG in strain RPD-2. Lactose grown RPD-2 cells were harvested in the early stationary phase of growth, and the rate of ^{14}C-lactose uptake was measured as previously described. Open circles = no ONPG present, closed triangles = 1 mM-ONPG present, closed squares = 3 mM-ONPG present, closed circles = 5 mM-ONPG present.

mutation was probably regulatory (Hall, 1979), and we now suggest that the mutation allowed the inducible expression of the *eluC* gene, the gene for enzyme II (lac). The analogy to the *bglR* mutation of *E. coli*, which permits the inducible synthesis of an enzyme II specific for β-glucosides (Prasad & Schaefler, 1974) has been pointed out previously (Hall, 1979). We therefore designate the site of the original mutation as *eluR*, and we follow the convention adopted for *E. coli* (Hall & Hartl, 1975), in designating the wild type allele present in strain RE1755A as *eluR*^{0} ('o' for original), and the allele present in CT-1 as *eluR*$^{+}$. Table 1 presents our gene designations with associated mutant phenotypes. With the exception of *ptsH* and *ptsI* we have no evidence for linkage of any of these genes.

(iv) *Relationship of the* elu *system to the cellobiose system*

Our interest in cellobiose originated from (i) the structural similarity between cellobiose (glucosyl-β-1,4-glucose) and lactose (galactosyl-β-1,4-glucose), and (ii) the fact that cellobiose is transported and phosphorylated by a PTS system (Schaefler & Malamy, 1969), and subsequently hydrolyzed by a phospho-glucosidase.

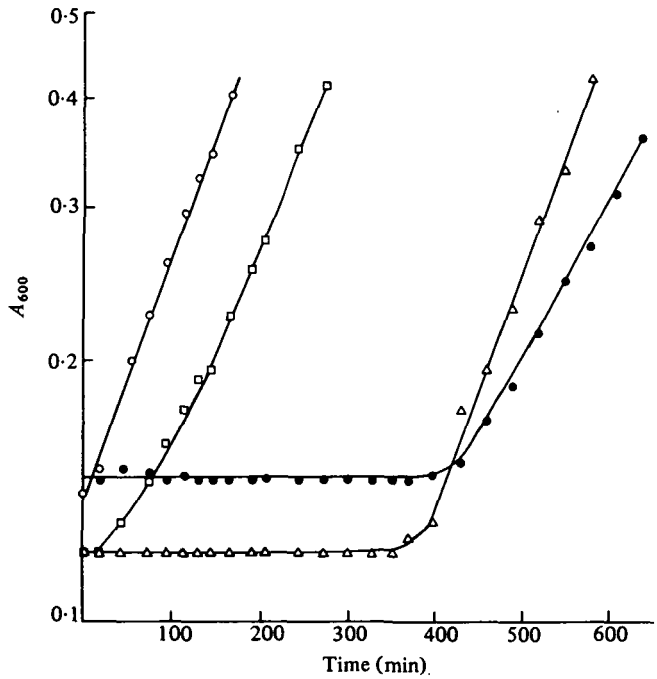

Fig. 4. Growth of strain CT-1 on 14 mM-lactose. Open circles = pregrown on lactose, triangles = pregrown on citrate, squares = pregrown on cellobiose, closed circles = pregrown on salicin.

Strain CT-1 undergoes an extended growth lag upon being shifted from glycerol, citrate, or succinate minimal media to lactose minimal medium (Hall, 1979; and Fig. 4). This growth lag is greatly reduced when cells are pregrown in cellobiose minimal medium (Fig. 4). The reduction in growth lag is specific to cellobiose, and does not occur when cells are pregrown in another β-glucoside, salicin.

When we measured the level of phospho-β-galactosidase in extracts of cellobiose-grown CT-1 cells we found that cellobiose induced synthesis of phospho-β-galactosidase nearly as efficiently as did lactose (extracts of cellobiose-grown cells contained $20{\cdot}6 \pm 3{\cdot}5$ units/mg compared with $32{\cdot}1 \pm 4{\cdot}9$ units/mg phospho-β-galactosidase activity for lactose-grown cells). We have used this observation to probe the question of co-ordinate control of the phospho-β-galactosidase gene and the *eluC* (enzyme II(lac)) gene. Table 5 shows that growth on cellobiose induced

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the phospho-β-galactosidase gene, but did induce the *eluC* gene while growth on lactose induced both genes.

These observations suggest that the phospho-β-galactosidase gene and the *eluC* gene are independently regulated. The reduction in growth lag upon being shifted from cellobiose minimal to lactose minimal medium would be accounted for by elimination of the need to synthesize phospho-β-galactosidase before lactose utilization could begin. The observations are consistent with the idea that cellobiose is an alternative gratuitous inducer of the phospho-β-galactosidase gene, but not an inducer of *eluC*.

Table 5. *Induction of phospho-galactosidase and transport activities in BG71*

Carbon source	Phospho-β-galactosidase	ONPG transport
Glycerol	0·76	< 0·25
Cellobiose	6·5	0·53
Lactose	8·2	9·3

Values are units per 10^9 cells.

Table 6. *Induction of phospho-β-glucosidase by lactose*

Strain	Medium	Phospho-galactosidase	Phospho-β-glucosidase
BG7	Citrate	0·58	3·5
BG7	Citrate + Lactose	3·5	19·3
BG71	Citrate	0·65	2·6
BG71	Citrate + Lactose	6·2	49·7

Values are in units per 10^9 cells.

This model does not imply that mutations in the *elu* genes should have any effect on cellobiose utilization. We had observed, however, that *eluB* mutants require 48 h to form colonies on cellobiose minimal medium, while strain CT-1 forms colonies within 24 h. This difference is not attributable to a difference in growth rate *per se*. *eluB* mutants grow at $0{\cdot}812 \pm 0{\cdot}04\ h^{-1}$ on cellobiose, a rate not significantly different from that of strain CT-1 ($0{\cdot}826 \pm 0{\cdot}03\ h^{-1}$). The *eluB* mutants, however, lag for 12 to 24 h before growing in cellobiose minimal medium, while strain CT-1 lags for less than half an hour.

Since the *eluB* mutations were apparently affecting the system for cellobiose utilization, we studied the effects of lactose on that system. O-nitrophenyl-β-D-glucoside (ONP-glu) is an analogue of cellobiose, just as ONPG is an analogue of lactose. Phosphorylated ONP-glu is not commercially available, thus phospho-glucosidase activity must be assayed in intact cells where the cellobiose-PTS system can phosphorylate the ONP-glu. Table 6 shows that lactose induced both phospho-glucosidase and phospho-galactosidase activities. The hydrolysis of ONP-glu by intact lactose grown cells also implies that lactose induces a β-glucoside-PTS transport system. This is consistent with earlier reports that lactose is an inducer

of the β-glucoside transport system in *Aerobacter* (*Klebsiella*) *aerogenes* (Schaefler & Schenkein, 1968).

Since the *eluB* mutations caused a lag in growth on cellobiose, it seemed possible that they might affect the efficiency with which cellobiose induced synthesis of the phospho-β-galactosidase. Table 7 shows that cellobiose is as good an inducer in *eluB* strains as in wild type, but that in *eluA* strains the level of phospho-β-galactosidase induced by cellobiose is reduced about three-fold.

Table 7. *Phospho-β-galactosidase activity in cellobiose grown strains*

Strain	Phospho-β-galactosidase activity
CT-1	9·5
CT-3	2·97
CT-4	2·75
CT-5	3·5
CT-6	11·5
CT-7	10·8
CT-8	10·8
CT-12	9·99
CT-13	11·6
CT-14	10·4

Values are in units per 10^9 cells.

4. DISCUSSION

It is possible that cellobiose is an alternative gratuitous inducer of the phospho-β-galactosidase, and that lactose is an alternative gratuitous inducer of the phospho-β-glucosidase and associated cellobiose-PTS transport system, and that the effects of the *eluA* and *eluB* mutations are coincidental or spurious. We prefer, however, a simpler explanation, namely that the phospho-β-galactosidase and the phospho-β-glucosidase (which hydrolyzes phosphorylated cellobiose) are one and the same enzyme. It has been previously observed that lactose is an inducer of the cellobiose transport system (Schaefler & Schenkein, 1968), and it would be expected that the gene for the hydrolytic enzyme and the genes for the transport system would be co-ordinately controlled. Our model would simply argue that neither the regulatory protein nor the hydrolase recognizes the difference between lactose and cellubiose. While it is true that the position of the C-4 hydroxyl is critical for most β-galactosidase and β-glucosidases, there are some enzymes that have both β-glucosidase and β-galactosidase activities (Barman, 1969). According to our model the cellobiose transport system would be unable to transport and phosphorylate lactose. Thus despite the fact that lactose induces the phospho-β-glucosidase/-galactosidase and cellobiose-PTS genes in strain RE1755A, it would be unable to utilize lactose. The *eluR* mutation which allows expression of the lac-PTS transport system would under these circumstances provide internal

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lactose-phosphate and allow the cell to take immediate advantage of lactose in the environment.

This model is clearly speculative. Rigorous testing of the model requires the measurement of phospho-β-glucosidase activity independently of transport. We hope that the required substrate, ONPglu-6-phosphate will be available shortly to permit such testing.

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