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Clonal analysis of the *lac* operons from *Klebsiella* M5al and the Lac plasmid (pRE2) from *Klebsiella* V9A

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Summary

The chromosomal *lac* region of the coliform bacterium *Klebsiella* M5al was cloned into the multicopy plasmid pBR322 to give pHE7 and pHE8. pHE8 contains 12·6 kb of M5al DNA, including its complete *lac* operon, and pHE7 contains 2·5 kb of M5al DNA and includes the complete *lacY* gene and a small segment of *lacZ*. The M5al operon has the same gene order as the *Escherichia coli lac* operon. The *lac* genes of the Lac plasmid of *Klebsiella* V9A were cloned into pBR322 to give pHE1 and pHE2, of approximately 39 and 43 kb. Both plasmids were unstable in an *E. coli RecA-* strain, in contrast to the stability of pHE8. Polyacrylamide gel electrophoresis tests suggested that the M5al β-galactosidase monomer is about 5% longer, i.e. has about 50 more amino acids, than that of the *E. coli Z* gene. Tests made on the enzymes coded by the *lac* operons of M5al, another *Klebsiella* strain (V9A) and its resident Lac plasmid, and several Lac$^+$ Enterobacteria, led to the conclusion that only *Escherichia coli* among the Enterobacteria contains an active *lacA* gene.

1. Introduction

Many wild strains of *Klebsiella* carry two inducible *lac* operons, one chromosomal and one plasmid-borne. The latter is induced by melibiose as well as lactose, can recombine with the *Escherichia coli lac* operon, and represses an I^- mutant of *E. coli lac*, suggesting that it is very closely related to the *E. coli* operon. In contrast, the chromosomal *lac* operon of *Klebsiella* is specifically repressed by melibiose (i.e. this is not catabolite repression), it has not been observed to recombine with the plasmid or *E. coli lac* genes, and its repressor does not repress the *E. coli* operon except, unexpectedly, in cells growing on melibiose. These results suggest that the *Klebsiella* chromosomal *lac* operon is much less closely related to the plasmid and *E. coli* operons than they are to each other (Reeve, 1979; Reeve & Braithwaite, 1974; Wilson, Wilson & Reeve, 1979). This makes DNA sequence comparisons between them of interest.

We have therefore cloned a *Klebsiella* chromosomal *lac* operon (from strain M5al) and the plasmid *lac* operon from *Klebsiella* strain V9A into the multicopy plasmid pBR322. Recently the *lacY* region of our M5al clone has been sequenced by McMorrow *et al.* (1989); and Riley and her associates have sequenced the complete *lac* operon, excluding most of the permease gene and material distal to it, from another *Klebsiella* strain, T17R1 (MacDonald & Riley, 1983; Buvinger & Riley, 1985*a* and *b*).

Here we describe our cloning results, summarize data on the probable size of the M5al β-galactosidase monomer, and describe tests which indicate that the *lac* operons in a number of Enterobacteria, including *Klebsiella*, do not contain an active thiogalactoside transacetylase gene (as coded by the *A* gene of the *E. coli lac* operon).

2. Materials and methods

(i) *Bacteria, plasmids and transposons* are listed in Table 1. RE476 was derived from C600ΔFI by mutating it to Srb$^-$ with phage Mucts, transducing to Srb$^+$ with P1 grown on JC5466 and selecting a derivative that did not carry Mu or P1.

(ii) *Media and chemicals.* Minimal medium contained 10·5 g K_2HPO_4, 4·5 g KH_2PO_4, 1 g tri-sodium citrate, 1 g $(NH_4)_2SO_4$, 0·2 g $MgSO_4$ and 0·02 g thiamine, per litre of H_2O. Required amino acids were added to 50 μg/ml. This is a good growth medium for *lac-proAB* deletion strains of K12. Antibiotics were used at 20 μg/ml in plates and 100 μg/ml in liquid medium for ampicillin (Ap), 20 μg/ml for chloramphenicol (Cm) and 40 μg/ml for kanamycin (Kn). L broth (LB) was made according to Miller (1972). For

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Table 1 A. *Bacteria*

Name	Characters, reference, origin
Klebsiella strains	
M5al	Wild-type, free of plasmids, Nif^{+}[1]
V9A: RE1403	Wild-type, source of pRE1 and pRE2 (Wilson *et al.* 1979)
V9A: RE1435	RE1403 cured of pRE1 and pRE2 (Reeve, 1976)
E. coli K12 strains	
K12 (λ)	Wild-type[2]
RE254	F^{-} His Trp Δ(*lacZ*) *strA nalA* (Reeve, 1976)
RE412	Lac^{-} derivative of ED8874, $r^{-}m^{+}$ *trpR* sup^{0} Δ(*gal-bio*) *strA*[3]
C600ΔFI	F$^{-}\Delta$XIII (*ProAB argF lac*) *argI* Thi $r^{-}m^{-4}$
RE476	C600ΔFI *recA* (This paper)
JC5466	F^{-} His Trp *recA* Spc^{r}[5]
RU2901	Rts1::Tn1725/*lacY galK* Leu Pro Thi *hsr hsm*[6]
Other Enterobacteria	
Citrobacter freundii (NTCC 9750)[4]	
Erwinia herbicola SCRI 427[7]	
Erwinia herbicola A93: completely lactose-negative[8]	
Erwinia herbicola A93^{+} spontaneous mutant of A93 to inducible Lac^{+}	
Enterobacter aerogenes (ATCC 13048)[6]	

Table 1 B. *Plasmids and transposons*

Name	Details, reference, source
pRE1	Tet plasmid in *Klebsiella* V9A (RE1403) (Wilson *et al.* 1979)
pRE2	Lac plasmid in *Klebsiella* V9A (RE1403) (Wilson *et al.* 1979)
pBR322	Tet^{r} Amp^{r} cloning vector (Bolivar *et al.* 1977*a*, *b*)
pHE1	*lac* genes from pRE2 cloned into pBR322 (This paper)
pHE2	*lac* genes from pRE2 cloned into pBR322 (This paper)
pHE3	M5al *lac* genes cloned into pBR322 (This paper)
pHE6	Subclone of pHE3 cloned into pBR322 (This paper)
pHE7	Subclone of pHE3 cloned into pBR322 (This paper)
pHE8	Subclone of pHE3 cloned into pBR322 (This paper)
Rtsl::Tn1725	Plasmid carrying transposon Tn1725 (Altenbruchner *et al.* 1983)
λ::Tn5	λ*b221 rex*::Tn5 *c1857* O_{am8} P_{am29} (Berg, 1977)

[1] From Ray Dixon, Unit of Nitrogen Fixation, Sussex University.
[2] From W. Hayes.
[3] From Noreen Murray, Dept. of Molecular Biology, Edinburgh University.
[4] From Simon Baumberg, Dept. of Genetics, Leeds University.
[5] From Mike Merrick, Unit of Nitrogen Fixation, Sussex University.
[6] From A. Jenkins, Dept. of Molecular Biology, Edinburgh University.
[7] From Michel Perombelon, Scottish Crop Research Institute, Dundee.
[8] From A. H. Neilson, Swedish Environmental Research Institute, Stockholm.

minimal and LB plates, Difco Bacto agar was added to 1·5%. ML agar was Oxoid MacConkey Lactose agar No. 3.

Indicator plates. XG was minimal agar containing 0·4% glucose and 40 μg/ml Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). XGI was XG with 10^{-4}M IPTG. XGI-Ap was XGI with 20 μg/ml of Ap. Bacterial colonies containing an inducible *lac* operon will be pale blue on XG and dark blue on XGI agar (Miller, 1972). TONPG agar was minimal agar with 0·2% sodium succinate as carbon course, 10^{-5} M IPTG and 10^{-3}M TONPG (orthonitrophenyl-β-D-thiogalactoside). Xgal, IPTG, ONPG, and TONPG were obtained from Sigma, London and restriction endonucleases from Boehringer.

(iii) *Cloning methods.* M5al chromosomal DNA partially digested with *Hin* dIII was ligated into the *Hin* dIII site of the multicopy plasmid pBR322 and the mixture was transformed into RE476, selecting on XGI-Ap plates to identify Lac^{+} clones. Cloning the plasmid *lac* operon from pRE2, the natural Lac plasmid of *Klebsiella* V9A, followed the same procedure. Restriction enzyme analysis of the pBR322 DNA clones and subcloning of restriction fragments followed Davis *et al.* (1980) and Yang *et al.* (1980).

(iv) *Transposon insertion mutagenesis. E. coli* K12 strain RE412 carrying pHE8 was infected with λ::Tn5 (Berg, 1977) on LB-ApKn plates to select for Tn transposition. Plasmid DNA from the colonies on these plates was used to transform RE476 to Ap^{r}Kn^{r}.

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Lac⁻ colonies were identified on ML-ApKn agar and classified as insertions into *lacY* or *lacZ* by their phenotypes on XGI and TONPG plates.

Tn1725 insertions were selected by the method of Altenbrucher *et al.* 1983. Ru2901 (Rts::Tn1725) was mated to RE476(pHE8) at 30 °C on a membrane placed on LB agar. The mating mixture was washed and grown overnight at 40 °C in minimal ApCm medium supplemented with arginine, proline and thiamine, to select for the presence of both pHE8 and Tn1725 in RE476. The plasmid DNA from this culture was used to transform RE476 to Ap^rCm^r, and Lac⁻ colonies were isolated and screened as for Tn5 insertion.

(v) *Estimating the molecular weights of the β-galactosidases.* Samples of each strain were grown in minimal glycerol medium with and without IPTG induction. The cells were centrifuged, resuspended in 40 μl Z-buffer (Miller, 1972) plus 2% SDS and a drop of chloroform, vortexed, centrifuged, and the supernatants were immersed in boiling water for $2\frac{1}{2}$ min. The protein subunits were resolved by SDS-polyacrylamide gel electrophoresis, using the discontinuous buffer system of Laemmli (1970), with 5% (stacking gel) and 7·5% (separating gel) acrylamide. The electrode buffer (pH 8·3) contained 0·025 M Tris, 0·192 M glycine and 0·1% SDS. The protein standards, from SIGMA, were: A, myosin (rabbit muscle) MW 205000; B, β-galactosidase (*E. coli*) MW 116000; C, phosphorylase B (rabbit muscle) MW 97400; D, albumin (bovine) MW 66000; E, albumin (chicken) MW 45000.

(vi) *Testing for the presence of an active Lac thiogalactoside transacetylase.* The enzyme activity was assayed as described by Miller (1972), in extracts of bacterial cultures grown overnight with and without 10^{-4} M IPTG, in minimal medium with carbon source 0·4% citrate for *Klebsiella* strains and 0·4% glycerol for other species. After suitable concentration, the cells were sonicated and centrifuged, a sample of the supernatant was assayed for β-galactosidase, and the remainder was heat-treated at 70 °C for 5 min and assayed for transacetylase activity following Miller's protocol.

3. Results

(i) *Cloning the* lac *genes of M5al into pBR322.* One Lac⁺ clone, labelled pHE3, was obtained by transformation into RE476, out of about 10000 tested on XG-Ap plates. RE476(pHE3) gives pale blue colonies on XG and dark blue colonies on XGI plates, and, since RE476 is deleted for the *lac-pro* region, pHE3 must carry the complete (inducible) *lac* operon of M5al. This was confirmed by enzyme assays (Table 2). The high basal level of enzyme in RE476(pHE3) also shows that the *lac* operon is in a multicopy plasmid.

(ii) *Restriction map and subcloning of pHE3.* pHE3 DNA was extracted and sized by gel electrophoresis, giving a total length of 22–23 kb, including about 18 kb of M5al DNA. The plasmid was restricted with *Eco* RI, *Bam* HI, *Hin* dIII and *Pst* I, using these enzymes singly and in pairs, and the fragments were sized by gel electrophoresis against λ restricted by *Eco* RI and *Hin* dIII.

Table 2. *β-galactosidase levels in M5al and clones in* E. coli

Strain	Uninduced	Induced
Klebsiella M5al	1·75	660
E. coli RE476	0·0	0·0
RE476(pHE3)	57	5800
RE476(pHE8)	88	5500
RE476(pHE1)	72	3990
RE476(pHE2)	99	4680

Growth was on minimal citrate for M5al and minimal glycerol for *E. coli* strains. Induction for 90 min with 1 mM IPTG. Enzyme activity expressed as units of enzyme per ml of cells at OD_{600} 1·0, following Miller (1972).

Fig. 1(top) gives the restriction map of pHE3, and includes the restriction sites of pBR322 (from Sutcliffe, 1978). pHE3 DNA digested with *Hin* dIII gave fragments of 15·6 and 2·5 kb, which were cloned into the *Hin* dIII site of pBR322 to give plasmids pHE6 and pHE7. A 12·6 kb *Eco* RI fragment of pHE3 was cloned into the *Eco* RI site of pBR322 to form plasmid pHE8. These three plasmids are shown in Fig. 1, and the map of pHE8 shows the restriction sites for *Bam* HI, *Eco* RI, *Hin* dIII, *Pst* I and *Sal* I. Two of the *Pst* I sites have been only approximately located.

Plasmids pHE6, pHE7 and pHE8 were transformed into RE476 and tested for the presence of *lac* genes. pHE6 and pHE7 give no β-galactosidase activity, so the *Hin* dIII site separating them must be in the *lacpoZ* region. Moreover, TONPG inhibits growth of cells carrying an active Lac permease (Smith & Sadler, 1971), but does not inhibit growth of RE476 carrying either pHE6 or pHE7, so *lacY* is also inactive in these plasmids. pHE8, however, carries an inducible *lac* operon with an active permease gene (Table 2).

(iii) *Transposon insertions into pHE8.* Tn5 and Tn1725 were inserted into the *lac* region of pHE8 and scored for *lacZ* and *lacY* activity as described in Methods. These insertion mutants were all found to be either Z^-Y^- or Z^+Y^-, none being Z^-Y^+. It follows that *Z* and *Y* are coordinately expressed, with transcription running from the operator through *Z* and then through *Y*, as in *E. coli*.

The locations of a number of these Tn5 and Tn1725 insertions were identified by isolating the plasmid DNA and studying the fragment sizes obtained after restriction with *Eco* RI and *Hin* dIII. Fig. 2 shows the positions of all transposons located and indicates which of these inactivated *Y* and *ZY*, respectively. The M5al *lac* in pHE8 is clearly arranged with *Y* on the right as shown in Fig. 2, and pHE7 (see Fig. 1)

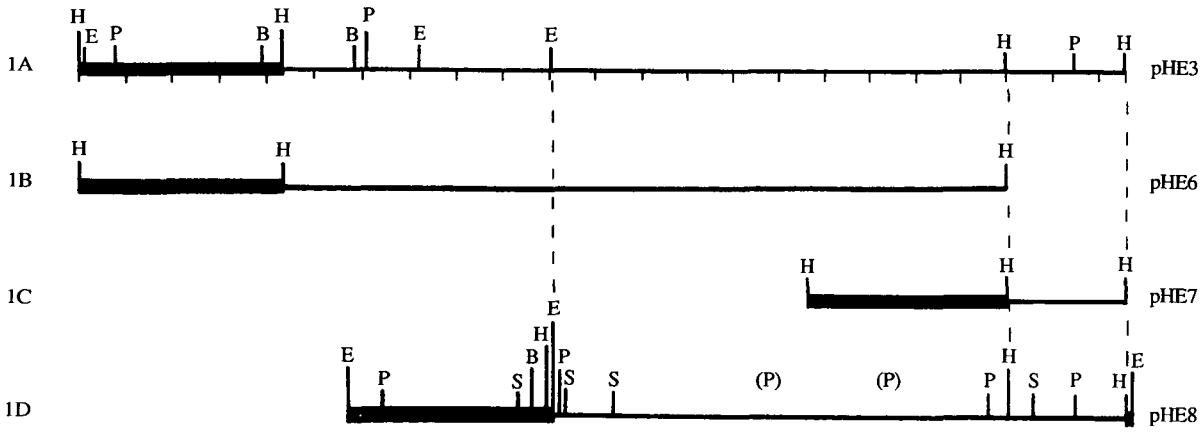

Fig. 1. Map of segment of M5al DNA cloned into pBR322 (top line, labelled pHE3), and the fragments of this segment which were subcloned into pBR322, labelled pHE6, pHE7 and pHE8. The M5al segments are shown aligned beneath each other, and the heavy bar on the left in each case represents pBR322 DNA. Restriction sites are shown for pHE3 and pHE8, and the scale marked below pHE3 is in kilobases. The restriction enzyme symbols are: B = *Bam* HI, E = *Eco* RI, H = *Hin* dIII, P = *Pst* I, S = *Sal* I.

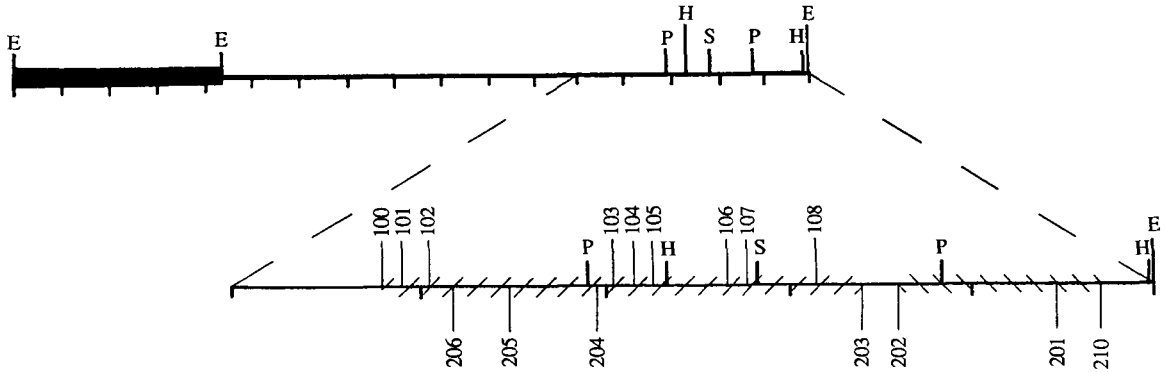

Fig. 2. Transposon insertions into pHE8 which inactivate *lacZY* (///) and lacY only (\\\\). The heavy bar is pBR322 DNA. The scale in kb is marked both under the upper line and under the expanded lower line. Numbers 100–108 are sites of Tn1725 insertion, numbers 201 upwards are sites of Tn5 insertion. P, H, S, P, H and E are restriction sites marked on both lines (symbols as in Fig. 1). The hatched areas mark the minimal lengths of the *Z* and *Y* genes.

must contain the complete *lac Y* gene and a portion of *Z*.

Of 40 Tn5 insertions tested, 36 were in *Y* and only 4 in *Z*. These were obtained in a single selection experiment and may not all have been independent isolates. Nevertheless, there seems to be a strong preference for Tn5 to insert into *Y* rather than *Z* in M5al *lac*, similar to that for Tn5 insertions into *E. coli lac*, found by Berg *et al.* (1980) and Miller *et al.* (1980) to be 5–7 *Y* insertions for one *Z* insertion, per unit length of DNA. No such preference was found among the 17 Tn1725 insertions into M5al *lac* (5 were in *Y* and 12 in *Z*).

(iv) *Cloning the* lac *genes from the V9A Lac plasmid, pRE2. Klebsiella* V9A carries two plasmids, pRE1 and pRE2. Both are non-conjugative, but can be transferred to *E. coli* by various conjugative plasmids. pRE1 carries the genes for inducible high-level resistance to tetracycline (Robertson & Reeve, 1972), while pRE2 carries both an *E. coli*-like *lac* operon and the genes for F1-type incompatibility (Reeve & Braithwaite, 1970, 1972). Unexpectedly, pRE2 is compatible with an F-prime in V9A but not in other *Klebsiellas* tested nor in *E. coli* K12 (Reeve & Braithwaite, 1974; Reeve, unpublished). Plasmids pRE1 and pRE2 were sized by Eckhart agarose gels and electron microscopy. pRE1 was estimated as 60 ± 5 kb and pRE2 as 180 ± 16 kb in length (data not shown).

Two Lac$^+$ clones – pHE1 and pHE2, of 39 and 43 kb – were obtained by ligating *Hin* dIII fragments of pRE2 DNA into pBR322. In RE476, these plasmids were found to carry an inducible *lac* operon with a high basal level of β-galactosidase (Table 2). Fig. 3 gives a restriction map of pHE1; but further analysis of this clone, including subcloning of the *lac* region, was hindered by its instability: either the complete plasmid or its *lac* region was rapidly lost in induced cultures and slowly in non-induced cultures. RE476(pHE1) gave mixed white Lac$^-$ and dark blue Lac$^+$ colonies of 2 mm diameter after 2 days on XG agar, but gave normal white and minute dark blue colonies on XGI plates. This suggests that the high permease and/or IPTG concentrations in induced

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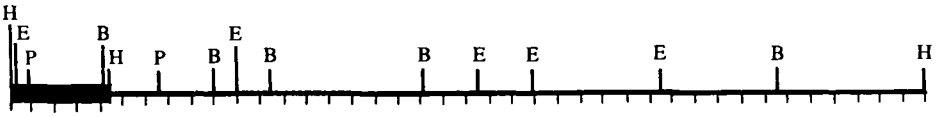

Fig. 3. Restriction map of pHE1. Details as for Fig. 1.

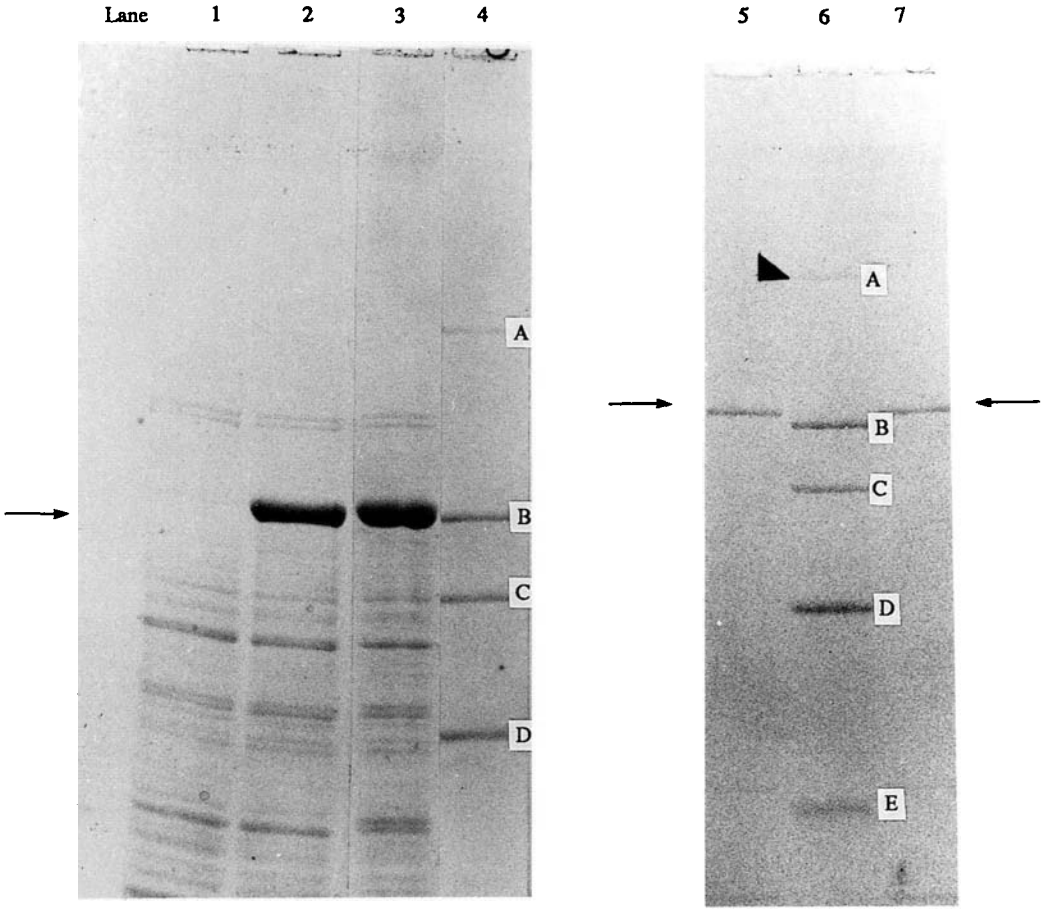

Fig. 4. Polyacrylamide gel electrophoresis of RE476 cell extracts carrying the cloned *lac* region of M5al. Lane 1, pHE8 uninduced cells; Lanes 2 and 5, pHE10 induced cells; Lanes 3 and 7, pHE8 induced cells. Lanes 1–4 and 5–7 are from different experiments, and lanes 5 and 7 have less protein loaded than lanes 1–3. The protein standards in lanes 4 and 6 have the following molecular weights (see methods): A, 205000; B, 116000; C, 97400; D, 66000; E, 45000. pHE10 is plasmid pHE8 with Tn5 inserted in *lacY* close to its left end (numbered 202 in Fig. 2), which codes for β-galactosidase but not Lac permease. Induction was by 10^{-4} M IPTG. The arrows mark the bands in each experiment which are assumed to contain the M5al β-galactosidase.

cells inhibit growth. RE476 carrying pHE3 or pHE8 was much more stable when tested under the same conditions.

(v) *Molecular weights of the β-galactosidase monomers.* These were estimated by SDS-polyacrylamide gel electrophoresis, as described in Methods, from extracts of RE476 carrying pHE3 or pHE10 (a derivative of pHE8 with Tn5 inserted into the *lacY* gene near its left end). Fig. 4 compares extracts from uninduced cells of RE476(pHE8) (lane 1), IPTG-induced cells of RE476(pHE10) (lanes 2 and 5) and IPTG-induced cells of RE476(pHE8) (lanes 3 and 7) with the protein standards (lanes 4 and 6). Lanes 1–4 show that the band marked by an arrow, not obtained from uninduced cells (lane 1), can be identified as the M5al β-galactosidase monomer. The standard marked B is the *E. coli* β-galactosidase monomer (MW 116000), and comparing band positions in lanes 5–7 the M5al monomer appears to be about 5% larger than that of *E. coli*, equivalent to some 50 extra amino acid residues. Buvinger & Riley (1985*a*) have recently sequenced the *lacZ* gene of another *Klebsiella* strain, T17R1, and find it has an excess of 34 nucleotides over that of K12.

(vi) *β-galactosidase (βG) and thiogalactoside transacetylase (TA) in Enterobacteria.* Three assays were made on IPTG-induced cultures of a number of lactose-positive strains, using *E. coli* K12 as the control in each assay. The results, summarized in Table 3, show (1) the total cell mass, (2) the

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Table 3. *Thiogalactoside transacetylase (TA) and β-galactosidase (βG) activities in Lac⁺ strains of Enterobacteria induced with IPTG at 1 mM*

Bacterial strain	Total units in 3 assays: Cell mass (OD_{600}) (1)	Total units in 3 assays: βG units ($\times 10^{-3}$) (2)	*E. coli* (%): βG (3)	*E. coli* (%): TA/βG (4)
E. coli K12	102	309	100	100
Klebsiella M5al	310	135	15	4
Klebsiella V9A (pRE2⁺)	61	104	57	1
Klebsiella V9A (pRE2⁻)	518	74	5	2
Citrobacter freundii NTCC 9750	84	39	16	2
Erwinia herbicola A93 (Lac⁺)	111	59	18	2
Erwinia herbicola SCRI 427	40	62	52	4
Enterobacter aerogenes ATCC 13038	140	8	2	0

corresponding total units of βG in the cultures assayed, (3) the ratios (2)/(1) expressed as a percentage of the corresponding ratio for *E. coli* and (4) the ratios TA/βG for each strain, expressed as a percentage of the ratio for *E. coli*. Thus column (3) estimates the relative specific activity of βG in induced cultures of each strain, compared with *E. coli*: these values range from 57% for *Klebsiella* V9A carrying its Lac plasmid (pRE2) to 5% for the same host strain cured of pRE2, and even less (2%) for an *Enterobacter aerogenes* strain. Column (4) shows that apart from *E. coli* none of the strains tested produced a significant level of TA activity, and no TA activity could be associated with pRE2. Our tests failed to reveal any plasmids in the strains tested, apart from pHE2 in the original V9A strain (F. E. H., unpublished).

4. Discussion

Klebsiella wild strains are unusual among the Enterobacteria in that they not only carry an inducible *lac* operon giving a much lower β-galactosidase activity than that of *E. coli* (by approximately an order of magnitude), but usually carry an *E. coli*-like *lac* operon on a plasmid, which presumably makes up for the deficiences of the chromosomal operon. The plasmid *lac* operon is much more closely related to that of *E. coli* than to the *Klebsiella* chromosomal *lac* operon (Reeve & Braithwaite, 1974), and this raises interesting questions about the evolutionary relationships of these three operons. In this paper we show by cloning and transposon insertion mutagenesis that the chromosomal *lac* operon of *Klebsiella* M5al has the same coordinate control and general organization as that of *E. coli*, the gene order being *I*, *po*, *Z*, *Y*, and that the β-galactosidase monomers of the two strains are very similar in size. These results agree with those of Buvinger & Riley (1985*a*, *b*), who have sequenced most of the *lac* operon of another *Klebsiella* strain, T17R1. Unexpectedly, they found that its *I* gene is transcribed in the opposite direction to that of *Z* and *Y*, in contrast to the situation in *E. coli*. It remains to be seen whether M5al *lac* and Lac plasmids of V9A and other *Klebsiellas* follow the pattern of strain T17R1 in this respect.

We found no evidence for an active *lacA*, which codes for the thiogalactoside transacetylase of *E. coli*, in M5al, V9A, the resident Lac plasmid of V9A, or the other Lac-positive Enterobacteria *Citrobacter freundii*, *Enterobacter aerogenes*, and two unrelated strains rather loosely labelled *Erwinia herbicola*. Alpers *et al.* (1965) found transacetylase in *E. coli* strains K12 and B but not in *Shigella dysenteriae*, *Salmonella typhimurium* or *Serratia marcescens*, and Guiso & Ullman (1976) and Cornelis (1981) found no detectable transacetylase activity and no cross-reaction with the *E. coli* protein in a *lac*-deletion strain of K12 carrying any one of a number of plasmids containing a *lac* operon. These plasmids were natural inhabitants of a variety of Enterobacteria. Taken together with our results this strongly suggests that, among the Enterobacteria, only *E. coli* strains contain an active thiogalactoside transacetylase gene, a conclusion which leaves the origin and function of this gene even more in doubt. The DNA regions immediately downstream of *lacY* in some of these bacteria, and particularly in M5al (cloned in pHE7), need to be sequenced.

Location tests by one of us (E. C. R. R., unpublished) strongly suggest that the *lac* operon in *Citrobacter freundii* NTCC 9750 is closely linked to *proA* and *proC*, placing it in the same chromosome location as *lac* in *E. coli*. But the *lac* operon has a different location, as yet undetermined, in *Klebsiella*, while it is closely linked to *metE* but not to either *pro* gene in *Erwinia herbicola* A93. So there are at least three different locations for the *lac* operon in the Enterobacteria. It is of interest that *Erwinia herbicola* strain A93 carries a completely silent *lac* operon with no detectable basal or inducible level of β-galactosidase; however, it readily mutates to an inducible lactose-positive state giving a measurable basal enzyme level. Its *lac* operon appears to have a

chromosomal location, since it is linked to *metE* (Reeve, unpublished). It is not at present possible to complete these location studies.

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