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The lactose system in *Klebsiella aerogenes* V9A

4. A comparison of the *lac* operons of *Klebsiella* and *Escherichia coli*

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SUMMARY

Klebsiella aerogenes V9A contains two *lac* operons, one chromosomal and one borne by the Lac plasmid, F_K*lac*. It is shown that the plasmid Lac repressor resembles that of *Escherichia coli* in that it represses the *E. coli* operon but is inactivated by melibiose. In contrast, the chromosomal Lac repressor of V9A is unable to repress the *E. coli* operon or the F_K*lac* operon, but is converted into an active repressor of both these operons on becoming bound to melibiose. The chromosomal Lac repressor is able to repress its own operon whether or not it is bound to melibiose, but is inactivated by isopropyl-β-D-thiogalactoside. These results support the hypothesis that the *Klebsiella* Lac plasmid was derived from an ancestral *E. coli* chromosome.

1. INTRODUCTION

A number of bacterial strains of the *Klebsiella* group have been found to carry a plasmid containing a *lac* operon which can be transferred to *Escherichia coli*. The plasmid has been cured from several of these strains, leaving the host with a chromosomal *lac* operon which is inducible but gives only about 10% of the specific activity for β-galactosidase found in the uncured host (Reeve & Braithwaite, 1973*b*). Tests on one of these plasmid-carrying strains of *Klebsiella*, V9A, showed that its galactoside permeases differ in several respects from those of *E. coli* (Reeve & Braithwaite, 1973*a*), and in addition both melibiose and raffinose potentiate specific repression of the *Klebsiella* lac genes. In *E. coli*, on the other hand, melibiose is an efficient inducer of the *lac* operon. These results led to the hypothesis that melibiose and raffinose bind to one or both of the *lac* repressor proteins in *Klebsiella* to produce an active complex, while the corresponding complex with the *lac* repressor of *E. coli* is inactive (Reeve, 1973*a*).

In this paper we show that melibiose inactivates the *lac* repressor of the *Klebsiella* plasmid when it is transferred to *E. coli*, that the *lac* repressor of the *Klebsiella* plasmid but not that on the *Klebsiella* chromosome represses the *E. coli lac* operon, and that the *Klebsiella* chromosomal *lac* repressor becomes able to repress the *E. coli lac* operon and the *Klebsiella* plasmid *lac* operon when complexed with melibiose. These results support the hypothesis put forward by Reeve (1973*b*) that the *Klebsiella* Lac plasmid originated from *E. coli*.

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2. MATERIALS AND METHODS

(i) *Bacteria*

(*a*) *Klebsiella* V9A: A His$^-$ mutant of V9A Thy$^-$ (Reeve & Braithwaite, 1972) and a descendant of this strain, cured of its Lac plasmid, whose origin will be described elsewhere. In this paper we shall refer to the cured host strain as V9A and the strain carrying its Lac plasmid as V9A (F_K*lac*)$^+$.

(*b*) *E. coli* K12: RE13 (F$^-$Met$^-$Lac$^+$) was obtained as W1485 from W. Hayes in 1961; X5097 (F$^-$Pro$^-$Lac$^-$Su$^-$Str^r carrying the *pro-lac* deletion XIII) was obtained from J. Scaife in 1970; RE254 is a Pro$^+$Lac$^-$Trp$^-$His$^-$Str^r recombinant of a cross between RE1 (F$^-$ProA$^-$Trp$^-$His$^-$LacY$^-$Str^r) and CA237 (HfrH Thi$^-$Lac$^-$Str^s carrying the *lac* deletion M445 and obtained from J. Scaife in 1968). RE254 carries the *lac* deletion M445, and has no β-galactosidase and no *lac* repressor activity.

(ii) *Plasmids*

F_K*lac* is the resident Lac plasmid of V9A (Reeve & Braithwaite, 1970, 1973*b*). F13 carries the wild-type *lac* genes of *E. coli* K12 and was obtained from E. Dubnau in 1963, carried in *Salmonella typhimurium* SL687. It is believed to be the F13 listed by Low (1972). JCFL0, from N.S. Willetts, is a constitutive (*lac I*$^-$) mutant of F*lac* from *E. coli* K12 (Achtman *et al.* 1971). FR5 carries F*gal* joined to the A, C, S and Su resistance determinants* of R1 (Reeve, 1970). FRL1 is a hybrid between FR5 and JCFL0, in which *gal* has been replaced by *lac* as a result of illegitimate recombination between the two plasmids in a RecA$^-$ strain of K12. FRL1 carries the genes of the F factor, the four resistance determinants from FR5, and the *lac* genes from JCFL0. It is LacI$^-$.

(iii) *Media and chemicals*

These were as described by Reeve & Braithwaite (1972) and Reeve (1973*a*). Vitamin-free Casamino Acids (Difco) was added to the minimal medium at 0·15 % for most assays.

(iv) *Assay of β-galactosidase*

In earlier tests this followed the protocol of Reeve & Braithwaite (1973*a*), in which samples were sonicated before assay. Later experiments used the protocol of Miller (1972), pp. 353–355, samples being first diluted 1/20 into chilled Z-buffer then treated with 2 drops of chloroform and 1 drop of 0·1 % SDS solution in water, per ml of assay mixture, instead of using toluene or sonication. This method was very convenient and gave consistent results which did not differ from those obtained after sonication. Miller's units, based on OD_{600} and 1·7 ml of reaction mixture, were converted to the units used in our previous papers by dividing by 11 for enzyme

* Abbreviations: cAMP, Adenosine 3′,5′-cyclic monophosphoric acid. IPTG, isopropyl-β-D-thiogalactopyranoside. TMG, Methyl-β-D-thiogalactopyranoside. SDS, sodium dodecyl sulphate. A, C, S, Su, resistance determinants for ampicillin, chloramphenicol, streptomycin, sulphonamides.

units per ml of culture and by 13 for units per ml adjusted to OD_{550} 1·0. These converted units are quoted in the tables.

3. RESULTS

The *lac* system in *Klebsiella* V9A(F_K*lac*)$^+$ is strongly repressed by melibiose, as is shown by the fact that cells induced by IPTG when growing in melibiose make only 10% as much enzyme as induced cells growing in citrate medium. Since V9A contains two *lac* operons, one in the chromosome and one in the Lac plasmid (F_K*lac*), this repression could be the result of an active complex being formed by binding of melibiose to the repressor protein of either or both operons. If the plasmid *lacI* protein forms an active product with melibiose, then the same repression should be observed when the Lac plasmid is tested in a strain of *E. coli* which has its own *lac* operon deleted.

Table 1. *Comparison of* $F_K$lac *with* Flac *from* E. coli

		β-galactosidase levels of cells growing in:		
Host strain	Plasmid	Glycerol	Glycerol + TMG	Melibiose
E. coli X5097	None	0·05	0·07	—
	F13	0·8	141	57
	F_K*lac*	4·8	78	118
		Citrate	Citrate + IPTG	Melibiose
Klebsiella V9A	F_K*lac*	2·4	200	1·9

The bacteria were growing in minimal medium on the carbon sources shown. Induction was by 1 mM TMG for *E. coli* and 0·1 mM IPTG for V9A, since IPTG is a more effective inducer than TMG in V9A. Induction was for 60 min. Assays were made on crude sonicates and enzyme activity was calculated as units per ml corrected to OD 1·0 (550 nm).

—, Not tested.

Table 1 compares the behaviour of F_K*lac* and the K12 plasmid F13 in the K12 *lac* deletion strain X5097. Both plasmid *lac* operons show the normal response to TMG and both are clearly induced by melibiose alone. The last line of the table shows that no induction occurs when V9A carrying F_K*lac* is grown on melibiose, although induction by IPTG occurs in cells grown on citrate. Repression of the *lac* system by melibiose in V9A must therefore be the result of an active complex formed between melibiose and the chromosomal *lac* repressor.

The characteristics of the chromosomal *lac* operon in V9A are shown in Table 2, which compares the induction behaviour of cells with and without the *Klebsiella* Lac plasmid present. Cured cells evidently contain an inducible *lac* operon, which has about 10% of the wild-type level of enzyme activity in both induced and uninduced cells. This *lac* operon is located on the chromosome since it has been shown to be linked to a proline locus (unpublished observations). Addition of melibiose at the same time as IPTG appears to cause an even more severe repression of the chromosomal than of the plasmid *lac* operon.

It proved unexpectedly difficult to carry the analysis further. V9A cured of F_K*lac* gives a pale pink phenotype on MacConkey lactose agar, making it hard to distinguish a lactose-negative mutant, and attempts to eliminate the chromosomal *lac* operon or obtain LacI$^-$ mutants have so far proved unsuccessful. Transfer of the chromosomal *lac* operon to *E. coli* would be even more difficult, and has not been attempted. There remained the possibility of putting a *lac* operon from *E. coli* into

Table 2. *Effect of* $F_K$lac *on β-galactosidase activity in* Klebsiella *V9A*

Additions to minimal citrate medium	β-Galactosidase level in V9A(F_K*lac*)$^+$	β-Galactosidase level in V9A
None	2·30	0·21
0·1 mM IPTG	96	7·8
0·1 mM IPTG + 0·4 % melibiose	13.7	0·78

Bacteria growing in minimal medium on citrate as carbon source received either no supplement or 0·1 mM IPTG either alone or together with 0·4 % melibiose (final concentration), 60 min before assay. Assay and units as for Table 1. Average of two experiments.

Table 3. *Behaviour of FRL1 in strains of* E. coli *K12*

Host strains ...	β-Galactosidase level in: RE13		RE254	
FRL1 ...	Absent	Present	Absent	Present
Cells not induced	1·9	43	0·13	560
Cells induced	320	760	0·11	—

Each strain was growing in minimal medium on glycerol as carbon source. For induction, 0·1 mM IPTG was added 60 min before assay. Assays and units as for Table 1. Averages of several experiments.

—, Not tested.

V9A and testing its response to melibiose. V9A(F_K*lac*)$^+$ has been shown to accept the plasmid FR5, carrying an F*gal* sex factor, without shedding F_K*lac*, although the two plasmids are incompatible in *E. coli* (Reeve & Braithwaite, 1973*b*). We therefore made the plasmid FRL1, carrying a LacI$^-$ F*lac* and the resistance genes of FR5 as markers (see Methods). FRL1 could be transferred into and re-extracted from V9A(F_K*lac*)$^+$ and V9A, and in addition it was found possible to infect V9A with the wild-type F*lac* plasmid F13. Results obtained with these plasmids are described below.

Table 3 shows the behaviour of FRL1 in two strains of *E. coli* K12, RE13 carrying a wild-type *lac* operon and RE254 which has its own *lac* genes deleted. Clearly the *lac* operon of FRL1 is constitutive in RE254 but inducible in RE13, confirming that FRL1 is LacZ$^+$LacI$^-$. Table 4 gives the β-galactosidase levels obtained in the cured V9A strain and the same strain infected with FLR1, when cells are grown on citrate or on melibiose as carbon source, and when they are induced with IPTG. This test differs from that in Table 2, where melibiose was added to cells growing in citrate

and induced by IPTG, since we now have a constitutive *lac* operon in the host. The cured V9A strain showed the expected behaviour (compare Table 2): IPTG gave a 20-fold increase of enzyme activity in cells growing on citrate but only a twofold increase for cells growing on melibiose. But the presence of FRL1 in the same strain gave an unexpected result: enzyme activity was expressed constitutively in cells growing on both carbon sources, IPTG having no effect, but the level of activity in melibiose was only about 17 % of that in citrate.

Table 4. *Behaviour of FRL1 in* Klebsiella

		β-Galactosidase levels in	
Carbon source	Addition	V9A	V9A(FRL1)+
Citrate	None	0·39	470
Citrate	0·1 mM IPTG	7·5	500
Melibiose	None	0·38	89
Melibiose	0·1 mM IPTG	0·67	73

Cells were growing in log phase on the carbon sources shown. IPTG was added 60 min before assay. Assays and units as for Table 1. Average of two experiments.

The constitutive level of activity in citrate indicates that the V9A chromosomal *lac* repressor does not repress the *E. coli lac* operon, so that the operator-binding specificities of the chromosomal *lac* repressors of *Klebsiella* and *E. coli* are clearly different. The lower enzyme level for FRL1-carrying cells growing in melibiose compared to citrate could be explained in two ways: either (1) melibiose exerts severe catabolite repression, or (2) melibiose binds to the V9A chromosomal *lac* repressor protein, so altering its conformation that it becomes able to repress the FRL1 *lac* operon. It is unlikely that melibiose exerts any appreciable catabolite repression in V9A (Reeve, 1973*a*), but further tests on the specific effect of melibiose on the *lac* system of cells carrying FRL1 have been carried out. For this purpose cells were grown to log phase in minimal medium on citrate, glucose or melibiose as carbon source, cAMP was added to aliquots of each culture, and its effect on β-galactosidase biosynthesis was measured.

cAMP needs to be used at 10 mM to produce an effect in *Klebsiella* (Reeve, 1973*a*), and at this concentration it causes a marked reduction in growth rate (by 40–60 %), as shown in Table 5 (*a*). Assuming that enzyme biosynthesis is affected in proportion to growth rate by this inhibition, we can compare the rates of β-galactosidase synthesis per unit increase in cell mass for the different treatments, as in Table 5 (*b*). Using this index, growth on glucose or melibiose as carbon source, in the absence of cAMP, gave rates of enzyme synthesis 14 % and 19 % of those in cells growing on citrate (column *a*). Addition of cAMP had little effect in cells growing on citrate, gave a sixfold increase with glucose and a twofold increase for melibiose (last column of Table 5 (*b*)). So cAMP released the repression by glucose completely but only reduced the melibiose repression by about 25 %. One of the experiments included in Table 5 is shown in Fig. 1, in which enzyme activity per ml is plotted against

cell mass (OD at 550 nm). There must be some uncertainty about the interpretation of these experiments in view of the effect of cAMP on growth rate, but it is clear that catabolite repression can account for at most a small part of the effect of melibiose on β-galactosidase biosynthesis, and we conclude that the main cause of repression is that the V9A chromosomal *lac* repressor becomes able to repress the *E. coli lac* operon when bound to melibiose.

Table 5. *Effect of cAMP on V9A(FRL1)*$^+$

(*a*) Effect on growth rate

Carbon source	cAMP:	% increase in OD_{550} (*a*) None	(*b*) 10 mM	Effect of cAMP (*b*/*a* %)
Citrate		90	56	62
Glucose		146	62	42
Melibiose		158	66	42

(*b*) Effect on β-galactosidase activity

Carbon source	cAMP:	$\Delta E/\Delta OD$ (*a*) None	(*b*) 10 mM	Effect of cAMP (*b*/*a* %)
Citrate		397	268	70
Glucose		57	370	640
Melibiose		75	159	210

The cells were growing in log phase in minimal medium supplemented with histidine, thymine and casamino acids, on the carbon sources indicated. Growth rate was measured over the period 60–120 min after addition of cAMP to aliquots of each culture. Assays were made 60 and 120 min after addition of cAMP to aliquots of each culture. ΔE is increase in units/ml of β-galactosidase and ΔOD is increase in optical density at 550 nm, during the period 60–120 min. For each assay samples were diluted 1/20 into Z-buffer and treated with chloroform and SDS (see methods). All figures quoted are the averages of six experiments.

Table 6 shows the β-galactosidase levels in the *Klebsiella* host growing on citrate uninduced, and after induction by IPTG with or without the simultaneous addition of melibiose, when the host carries four different combinations of its chromosomal *lac* operon with other *lac* operon(s). The first two columns show the effect of substituting an *E. coli lac* operon (on F13) for that of F_K*lac*: in both cases there is induction by IPTG and strong repression by melibiose, as we should expect, but a point of interest is that the basal level given by F_K*lac* is about 4 times as high as that given by F13, as occurred also in *E. coli* (Table 1). The last two columns show the effect of adding F_K*lac* to FRL1. The *lac* operon of FRL1, expressed constitutively in the presence of the V9A chromosomal *lac* repressor, becomes inducible in the presence also of the F_K*lac* repressor, but remains subject to repression by melibiose. This indicates that the F_K*lac* repressor is able to repress the *E. coli lac* operon if melibiose is not present. The repression by melibiose in the strain carrying the three *lac* operons must then be attributed to the combined effects of IPTG in inactivating the two plasmid *lac* repressors (melibiose would also have this effect) and of

melibiose in activating the chromosomal *lac* repressor so that it can now repress the two plasmid operons as well as its own operon.

Two alternative hypotheses could be put forward to explain the inhibitory effect by melibiose on *lac* induction by IPTG in *Klebsiella*. These are that melibiose

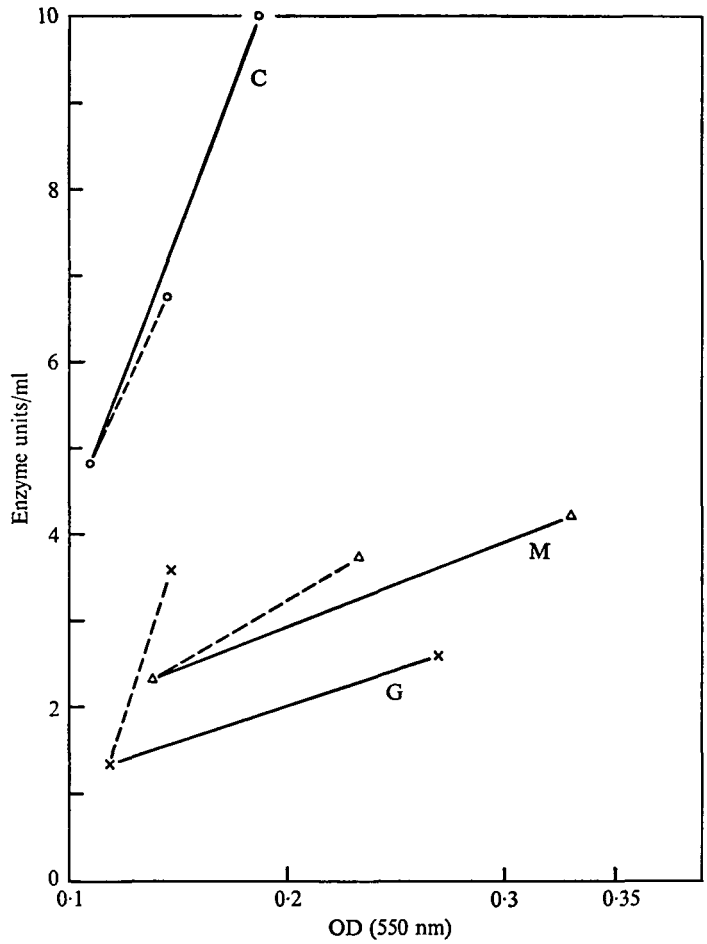

Fig. 1. Effect of cAMP on rate of synthesis of β-galactosidase and growth rate in cells of V9A cured of its own Lac plasmid but carrying FRL1, and growing in minimal medium on citrate, glucose and melibiose as carbon sources. Enzyme concentration (units per ml of culture) is plotted against cell mass (OD at 500 nm) for readings made 60 and 120 min after addition of cAMP to an aliquot of each culture. ○, Citrate (C); ×, glucose (G); Δ, melibiose (M); ——, no cAMP; — —, 10^{-2M} cAMP.

reduces the internal concentration of IPTG (1) by competing with IPTG for available sites of entry or (2) by inducing an enzyme (on the melibiose operon) which inactivates IPTG.

Regarding the first hypothesis, it is interesting that V9A possesses three distinct galactoside permeases capable of accumulating both lactose and melibiose, and one

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of these (GP. III) is blocked for uptake of lactose but not of melibiose by the presence of IPTG. However, when all three permeases are inactivated by mutation IPTG can still enter the cell and induce the *lac* operon(s) although neither lactose nor melibiose can be accumulated (Reeve & Braithwaite, 1973*a*); so it is unlikely that melibiose could block entry of IPTG.

The second hypothesis predicts that induction of the melibiose operon would have the same effect as the presence of melibiose in reducing the effectiveness of IPTG as a *lac* inducer. Galactose induces the melibiose operon (Reeve & Braithwaite, 1973*a*), but IPTG was found to be just as effective a *lac* inducer in cells growing on galactose as it is in cells growing on citrate as carbon source (unpublished results). In any event, neither hypothesis would explain the fact that cells of V9A (FRL1)$^+$ make β-galactosidase at a high rate when growing on citrate but at a much lower rate when growing on melibiose (Table 5*b*). The two hypotheses can therefore be discounted.

Table 6. *Effects of two and three* lac *operons in* Klebsiella *V9A host*

	Specific activity of β-galactosidase			
lac operons present:				
V9A chromosome	+	+	+	+
F_K*lac*	+	−	−	+
F13	−	+	−	−
FRL1	−	−	+	+
Induction:				
None	3·7	1·0	470	25
IPTG	240	470	500	560
IPTG + melibiose	10	30	73	89

The cells were growing in minimal medium supplemented with thymine, histidine and casamino acids, on citrate as carbon source. Assays were made 60 and 120 min after addition of IPTG (0·1 mM), or IPTG + melibiose (0·2 %), to aliquots of each culture. Averages of two experiments. Assays and units as in Table 5 except for column 3, where the data are taken from Table 4.

4. DISCUSSION

We can now specify the following characteristics of the three sets of *lac* genes compared – those of the chromosome and plasmid of V9A and the *E. coli lac* genes.

(1) The *lac* repressor protein of F_K*lac* resembles that of *E. coli* in that it represses the *E. coli* operon and is inactivated by melibiose.

(2) The V9A chromosomal *lac* repressor differs from the other two repressors in that it does not repress the *E. coli* operon unless bound to melibiose, and when so bound it represses all three operons.

(3) The V9A chromosomal *lac* operator differs from that of *E. coli* in that only the former is repressed by the V9A chromosomal *lac* repressor when melibiose is not present.

Other questions of repressor–operator interactions remain to be answered when suitable strains have been made, in particular: is the V9A chromosomal or plasmid

lac operon repressed by either of the two other repressors? However, it is clear that the *lac* operons of F_K*lac* and *E. coli* are similar in every characteristic we have so far been able to test, while both differ from the *lac* operon on the chromosome in activity level and repressor and operator specificity. This supports the previous hypothesis (Reeve, 1973*b*) that F_K*lac* originated from an ancestral *Escherichia coli* chromosome.

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