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Lac⁺ plasmids are responsible for the strong lactose-positive phenotype found in many strains of *Klebsiella* species

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SUMMARY

A variety of *Klebsiella* strains examined all show either a strong (ML^+) or a weak ($ML^{-/+}$) lactose-positive phenotype on MacConkey agar. ML^+ Klebsiellae have about 10 times the β-galactosidase activity of $ML^{-/+}$ strains in cultures both induced and not induced for this enzyme. Of 14 ML^+ strains of diverse origin tested, at least 13 carry a *lac* operon on a plasmid which can be transferred to *Escherichia coli*. The seven plasmids so far studied in detail all belong to the F compatibility group but are unable to promote their own transfer. To explain these results it is suggested that the *Klebsiella* group derive from a common ancestor with a chromosomal *lac* operon of low efficiency, which was made good by the acquisition of a *lac* operon from another bacterial strain, probably *E. coli*: the new *lac* genes remained as a plasmid, possibly because they could not be integrated in the new host.

INTRODUCTION

Plasmids carrying the genes of the *lac* operon have been found in two unrelated strains of *Klebsiella aerogenes* and both have been transferred to *Escherichia coli* K12 (Reeve & Braithwaite, 1970; Brenchley & Magasanik, 1972). This led us to collect *Klebsiella* strains from various sources and to test their ability to transfer *lac* genes, and our preliminary results are presented below. These suggest that *Klebsiellae* fall into two classes – those with a strong and those with a weak lactose-positive phenotype – and that the former owe this phenotype to the presence of a Lac plasmid.

MATERIALS AND METHODS

The *Klebsiella* strains tested are listed in Table 1; we are grateful to R. Dixon, J. P. Duguid, T. Hennessey and H. Matsumoto for supplying them. What is known about their origin may be found in the references given in Table 1 and in the NCTC and NCIB catalogues. One strain originally classed as a *Klebsiella* is now labelled *Citrobacter ballerupensis* (NCTC 7824).

The media used were L-broth and M9 minimal medium (see Reeve & Braithwaite, 1972). β-galactosidase was assayed as in Reeve & Braithwaite (1973), except that the bacteria were grown on 0·4 % citrate as carbon source and induction was for 2 h with 0·1 mM IPTG; this was sufficient to give maximal induction.

The *Klebsiella* strains were mated to *E. coli* K12 strain RE254 (F^- Lac^- His^- Trp^-

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Str^R Nal^R),* and when these matings failed to give any transfers, each *Klebsiella* strain was infected with FR5 (a complex plasmid in which F*gal* has become linked to resistance determinants of R1) and with derepressed mutants of R1 (R1*drd*16(o^c) and R1*drd*19(i^-)). Details of these transfer factors are given by Cooke, Meynell & Lawn (1970), Reeve (1970) and Reeve & Braithwaite (1970). The Klebsiellae carrying these factors were mated to RE254. Matings were incubated overnight on broth agar and loopfuls were spread on MacConkey lactose agar (Oxoid No. 3) containing 20 μg/ml Cm and 2 mg/ml Str, and on minimal lactose agar containing 2 mg/ml Str and 50 μg/ml each of histidine and tryptophan. The MacConkey plates selected for the transfer of FR5 or the R1*drd* plasmid and identified *lac*$^+$ recipients among them, and the minimal plates selected for *lac*$^+$ transfer.

Table 1. *Strains of* Klebsiella *group*

Strain	Type, origin, etc.	Source
A1	*K.a.* Ser. 54 from human faeces, Edinburgh	1
A3	*K.a.* Ser. 1, from human faeces, Edinburgh	1
D8	*K.pn.* mating strain (Matsumoto & Tazaki, 1970)	2
K.1.9	*K.a.* Ser. 1 (NCTC 9499)	1
K102	*K.a.* from human blood, Hammersmith Hospital, London	3
K122	*K.a.* from human urine, Hammersmith Hospital, London	3
NCTC 7242	*K.e.e.*	4
NCTC 7824	*Citrobacter ballerupensis*	4
NCIB 8017	*K.a.*	4
NCIB 8258	*K.a.*	4
NCIB 8595	*K.a.*	4
NCIB 8793	*K.a.*	4
V9A	*K.a.* from vole faeces, Scottish forest (Reeve & Braithwaite, 1970)	

Sources: 1, J. P. Duguid; 2, H. Matsumoto; 3, T. Hennessey; 4, R. Dixon. NCTC and NCIB nos are given for strains in these collections. Abbreviations: *K.a.*, *Klebsiella aerogenes; K.e.e.*, *Klebsiella edwardsii* var. *edwardsii; K.pn.*, *Klebsiella pneumoniae;* Ser., capsular serotype, where known.

RESULTS

When the *Klebsiella* strains were spread with a loop on MacConkey lactose agar so as to give a confluent 'well' area and some isolated colonies, each showed one of two phenotypes, much like the ML^+ (strong Lac^+) and $ML^{-/+}$ (weak Lac^+) phenotypes of V9A described by Reeve & Braithwaite (1972), and they will be given the same symbols. The ML^+ phenotype is characterized by deep pink colonies and well, with misting of the agar extending a few millimetres from the edge of areas of confluent growth and often surrounding large isolated colonies. $ML^{-/+}$ strains show paler pink colonies with an outer colourless zone, a pale pink well area and no misting of the agar. These phenotypes are seen after overnight growth at 37 °C, but the pink colour later disappears as acid is converted into acetylmethylcarbinol (Reeve & Braithwaite, 1972).

Of the 13 strains listed in Table 1 seven have the ML^+ and six the $ML^{-/+}$ phenotype, as shown in Table 2. When assayed for β-galactosidase, the ML^+ strains give enzyme activities (units/ml at OD_{550} 1·0) of 150–240 for induced and 1–4 for uninduced cells,

* Abbreviations and symbols: A, C, K, S, Su, R-factor determinants for resistance to ampicillin, chloramphenicol, kanamycin, streptomycin and sulfonamides respectively; Cm, chloramphenicol; Str, streptomycin; IPTG, Isopropyl-α-D-thiogalactoside. Gene symbols follow Taylor & Trotter (1972).

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while the corresponding figures for the $ML^{-/+}$ strains are 10–30 units induced and 0·3–0·6 units uninduced (Table 2). Thus the $ML^{-/+}$ strains possess an inducible β-galactosidase with about 10 % of the specific activity found in ML^{+} strains, both in uninduced and in induced cultures. Variants of some of these ML^{+} strains have been found in which a Lac plasmid has been lost, and they then acquired the $ML^{-/+}$ phenotype and level of β-galactosidase activity. It is therefore assumed that the $ML^{-/+}$ strains in Table 2 do not carry a Lac plasmid, and mating tests for the presence of such a plasmid have been confined to the ML^{+} strains.

Table 2. *Lac phenotypes and β-galactosidase activity*

ML^{+} strains			$ML^{-/+}$ strains		
Strain	β-galactosidase in:		Strain	β-galactosidase in:	
	Citrate	Citrate + IPTG		Citrate	Citrate + IPTG
A3	2·3	150	A1	0·2	14
K.1.9	1·5	210	D8	0·4	16
K102	2·0	150	K122	0·3	16
7824	4·4	240	7242	0·4	29
8258	1·5	235	8017	0·6	31
8793	1·1	195	8595	0·3	16
V9A	2·1	200			

Growth was in minimal citrate medium with or without 2 h induction by 0·1 mM IPTG. β-Galactosidase activity was calculated as units per ml corrected to a cell density of 1·0 at OD_{550}.

Table 3. *Characteristics of Lac plasmids from ML^{+} strains*

	Transferred to K12 by:			Compatible with F in:		
Host	Self	FR5	R1*drd*	Host	K12	Represses F or R1*drd*19
V9A	–	+	+	+	–	+
K.1.9	–	(+)	+	–	–	–
K102	–	–	+	–	–	–
A3	–	–	+	–	–	+
7824	–	(+)	+	–	–	+
8258	–	(+)	+	–	–	+
8793	–	–	+	NT	–	NT

+ indicates transfer, compatibility or repression, respectively, and – indicates the opposite. (+) indicates that when Lac^{+} has been transferred from a host carrying FR5 the two plasmids have been jointly transferred as if physically linked. Compatibility with F in K12 was tested by mating a Str^{s} strain of K12 carrying F*his* (F30) to RE254 carrying the Lac plasmid, selecting His^{+} [Str^{R}] progeny and testing them for their Lac phenotype. NT, not tested.

The results of mating these strains to *E. coli*, both before and after they had been infected with FR5 or an R1*drd* mutant, are summarized in Table 3. There was no transfer in the absence of FR5 or R1*drd* in the donor, but FR5 enabled four of the *Klebsiella* strains and one or both of the R1*drd* mutants enabled all of them to transfer *lac*+ to the recipient K12 strain. The question immediately arises as to whether the *lac*+ genes transferred are on a plasmid in the host or are mobilized from the chromosome by the

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transfer factor. R1*drd* can transfer genes near the *trp* operon in *E. coli* (Pearce & Meynell, 1968), so if it can mobilize *Klebsiella* genes at all it might be expected to mobilize the *trp* and *gal* regions more easily than the more distant *lac* region. However, several ML+ *Klebsiella* strains carrying R1*drd*16 failed to transfer *trp*+, *gal*+ or the genes for melibiose utilization to K12, suggesting that R1*drd* cannot mobilize the transfer of chromosomal genes from *Klebsiella* to *E. coli*. Table 3 also shows that, when F*his* is forced into K12 recipients of Lac+ from any of the *Klebsiella* strains listed, the Lac+ character is always lost. These results lead to the conclusion that the *lac*+ genes extracted from the ML+ *Klebsiellae* are all on plasmids in the host strains, and that these all belong to the same compatibility group as F.

The compatibility relationships of F and these Lac plasmids were not always the same in *E. coli* and the *Klebsiella* hosts. Thus the Lac plasmid of V9A was stable together with FR5 or F*gal* in V9A, and the two plasmids were transferred independently (Reeve & Braithwaite, 1970). However, FR5 and each of the other Lac plasmids show at least indirect evidence of incompatibility in their *Klebsiella* hosts: when FR5 was transferred to A3 and 8793, these acquired all the resistance determinants of FR5 but were unable to transfer the Lac plasmid or even FR5 to K12. K102 lost its Lac plasmid on infection with FR5; and K.1.9, 7824 and 8258 infected with FR5 either lost their Lac plasmid or always co-transferred resistance markers and *lac*+ to K12, whether selection was for FR5 or for Lac+. These results suggest that, with the exception of V9A, FR5 can only maintain itself in the host *Klebsiella* strains by either driving out the resident Lac plasmid or forming a physical union with it. This union could then lead to a complex able to transfer itself as in K.1.9, 7824 and 8258, or one in which genes essential for transfer had been lost as in A3 and 8793.

The Lac plasmid of V9A repressed sex factor activity of F and R1*drd*19(*i*−) (Reeve & Braithwaite, 1970), and tests for similar repressor ability by five of the other plasmids are given in the last column of Table 3. To our surprise, three of the new plasmids carried such a repressor gene – those of A3, 7824 and 8258. Two plasmids did not, while that from 8793 remains to be tested, since it has not yet been obtained in K12 together with a repressible sex factor.

A final question at this stage is whether the *lac* operon of low efficiency found in *Klebsiella* hosts which have lost their Lac plasmid is chromosomal. D8 transfers chromosomal genes in an ordered way to other *Klebsiella* strains (Matsumoto & Tazaki, 1970), and does not carry a Lac plasmid (Table 2). When a LacZ− Pro− mutant of D8 was mated to strains of V9A and K.1.9 which had lost their Lac plasmids and were of ML−/+ phenotype, close linkage was demonstrated between the *lac* and *pro* genes. In view of the close similarity in gene order between *E. coli* and *Klebsiella* D8 (Matsumoto & Tazaki, 1970, 1971), this is taken as strong evidence that the *lac* genes responsible for the ML−/+ phenotype are on the chromosome at the usual *lac* locus.

DISCUSSION

The *Klebsiella* strains examined fall clearly into two classes defined by their phenotypes on MacConkey lactose agar and their corresponding high and low specific activities of β-galactosidase. The seven ML+ strains, and also W70 (Brenchley & Magasanik, 1972), all possess a plasmid carrying an efficient *lac* operon, and loss of this plasmid (so far observed in A3, K102, K.1.9, V9A and W70) leaves a *lac* operon giving essentially the ML−/+ phenotype, similar to that of the ML−/+ strains listed in Table 2.

We have recently tested six further ML+ *Klebsiella* strains, and five of these also transferred a Lac plasmid to K12 after infection with R1*drd*; the sixth strain did not transfer either *lac*+ or R1*drd*, so its possession of a Lac plasmid is still in doubt. Thus at least 13 of the 14 ML+ strains so far examined possess a Lac plasmid. The seven plas-

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mids so far tested all belong to the same compatibility group as F, but are not associated with any resistance determinants in their *Klebsiella* hosts and are unable to promote their own transfer.

These results lead to the hypothesis that the *Klebsiella* group evolved from a common ancestor, of $ML^{-/+}$ phenotype, with a *lac* operon of low efficiency. Perhaps following a change of environment which made lactose a more important carbon source, this defect was rectified in the ML^{+} strains by the acquisition of a Lac plasmid, probably from *E. coli* in the mammalian intestine. These plasmids would then have originated from chromosomal genes mobilized by a transfer factor which appears subsequently to have been lost, and the lack of homology with the new host's chromosome may well have made their integration impossible. The $ML^{-/+}$ *Klebsiella* strains either never acquired such a plasmid or lost it later.

Note added in proof

A sample of strain NCTC 7824 recently obtained from the National Collection of Type Cultures has proved to be Lactose-negative, and this raises doubt whether the strain referred to as NCTC 7824 in Table 1 is correctly labelled. The latter is believed to have come from NCTC, but had passed through several laboratories before reaching the Nitrogen Fixation Unit at Sussex University, from whom we obtained it (Dr Ray Dixon, personal communication). Its earlier history is not fully recorded and it could perhaps have been mislabelled at some stage. Our tests show that it clearly belongs to the *Klebsiella* group (taken to include *Citrobacter*).

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