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Transfer characteristics of two resistance determinants in a wild strain of *Klebsiella aerogenes* (V9A)

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SUMMARY

Klebsiella aerogenes strain V9A carries determinants A_K and T_K, giving resistance to ampicillin and tetracycline, and a plasmid F_K*lac*, but no active sex factor. F- and I-type sex factors were able to transfer T_K from V9A to *Escherichia coli* K12 and between strains of K12, and T_K behaved as a separate plasmid with its own replicon. A_K could not be transferred, except possibly by a sex factor carrying its own A determinant, but the evidence for such transfer was inconclusive. It is suggested that A_K is either a chromosomal gene or is in a plasmid with a cell attachment in *Klebsiella* not represented in *E. coli* K12. A_K produces a β-lactamase.

1. INTRODUCTION

A wild strain of *Klebsiella aerogenes* (V9A), found in faeces from a forest vole population, has been shown to harbour a plasmid F_K*lac* with unusual properties: it carries the genes of the Lactose operon and an active *fi*$^+$-type sex-factor repressor gene, shows mutual exclusion with F-factors in *Escherichia coli* K12, and is nevertheless completely unable to promote conjugation, presumably because it is defective in some of the necessary genes (Reeve & Braithwaite, 1970). The host strain is also resistant to tetracycline and ampicillin, and this paper describes transfer studies on the T and A resistance determinants.

2. MATERIALS AND METHODS

(i) *Bacterial strains*

The origin of *Klebsiella* V9A was described by Reeve & Braithwaite (1970). This bacterium is a non-motile Gram-negative rod with a thin capsule, and has the diagnostic characters of the II c_1 group of Cowan & Steel (1966). Its IMViC pattern (− − + +) and its ability to ferment a number of sugars with acid and gas production identify it as *Klebsiella aerogenes*. It is prototrophic and grows well at 37 °C, and is resistant to A† and T but not to C, S, Su, colistin, nitrofurantoin or nalidixic acid. Thy$^-$, Gal$^-$ and Trp$^-$ mutants were derived and used as required in the various mating tests.

The other bacterial strains used are all derivatives of *Escherichia coli* K12, and are listed in Table 1. F-specific phage was f 2.

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† Abbreviations: A, C, K, T, S and Su indicate resistance determinants for ampicillin, chloramphenicol, kanamycin, tetracycline, streptomycin and the sulphonamides; and these symbols will also be used for the antibiotics themselves where there is no danger of confusion. Gene symbols otherwise follow the recommendations of Demerec *et al.* (1966). *drd* indicates a derepressed mutant of a sex factor.

The following sex factors were used as aids to transfer:

F*gal* is F'8.

FR5 is F*gal* ACSSu, obtained by combining resistance genes from R 1 with F'8. This sex factor complex was made by Dr J. O. Bishop by a sequence of P 22 transductions and matings. It behaves as a single physical unit in transfer, and does not carry K or the sex factor of R 1 (from J. O. Bishop). FR5-1 and FR5-2 are two ampicillin-sensitive mutants of FR5, obtained by mutagen treatment.

I*drd*-K is a derepressed mutant of R 144, which has an I-type sex factor and a K determinant (from Elinor Meynell).

I*drd trp cys*B is a derepressed mutant of a sex factor complex carrying the I sex factor, *col*Ib and the *trp cysB* region of the K12 chromosome (from G. G. Meynell).

Table 1. *Bacterial strains derived from* Escherichia coli *K12*

Stock no.	Genotype	Other name
RE 1	*proA trp his lac strA* F^-	J 62
RE 9	*proA thr leu thi lac gal strA* F^-	AB 712
RE 110	*pyrD gal strA* F^-	MS 3
RE 178	*proA trp his arg thr leu thi lac gal strA* F^-	—
RE 245	$\nabla[pro_B lac]_{XIII}$ *strA* F^-	X 5097

Sugar markers other than *lac* and *gal* and phage characters are omitted. A sex factor present in a strain is shown in brackets, e.g. RE 1 (F*gal*).

(ii) *Media*

Broth was L-broth (Lennox, 1955), supplemented with thymine at 80 μg/ml. MacConkey agar was Oxoid No. 3. Minimal medium was that of Vogel & Bonner (1956) with sugar added at 0·2 % (w/v). Thymine at 80, amino acids at 50 and vitamins at 10 μg/ml were added as required. These media were solidified with 1·5 % Difco Bacto Agar to make broth and minimal agar. Filtered antibiotic solutions were added to melted agar media just before pouring the plates.

(iii) *Mating experiments*

Fully grown broth cultures were diluted together 10^{-2} into broth and incubated 16 h for overnight matings. Log phase donor cells in broth at about 2×10^8/ml and fully grown recipient cells were diluted together 10^{-1} into broth and incubated 1–3½ h for shorter matings. Mating mixtures were incubated as a thin layer of medium at 37 °C without shaking. Transfer frequencies were measured by plating serial dilutions on selective plates, using thymine requirement or sensitivity to streptomycin or nalidixic acid to counter-select the donor. Alternatively, loopfuls of undiluted mixture were serially cross-streaked on selective plates in the usual way for obtaining single colonies (Gillies & Dodds, 1965, fig. 10). With this method, low-frequency transfer (separate colonies in the plate well) and high-frequency transfer (confluent growth in the well and in the first one or two cross-streak regions and many single colonies elsewhere on the plate) could be clearly distinguished. Low-frequency transfer was always associated with f2-resistance and indicated repression of sex factor activity. MacConkey agar containing A, C, K or T at 20 μg/ml and streptomycin at 2000 μg/ml was used to select for transfer of resistance determinants from V9A to K12. Colonies were purified on the same plate types before being further characterized.

Other non-standard methods are described in the text or tables.

3. RESULTS

(i) *Transfer of resistance from* Klebsiella *V9A to* Escherichia coli *K12*

All attempts to transfer antibiotic resistance directly by conjugation were unsuccessful. Various transfer factors were therefore introduced into the *Klebsiella* and infected clones were then mated to K12 strains. Typical results of matings without and with a sex factor present are given in Table 2, and lead to the following conclusions:

Table 2. *Transfers from* Klebsiella *V9A carrying a sex factor to* Escherichia coli *K12*

Sex factor in donor	Recipient	Transfers per recipient of: Sex factor	T	A	T/Sex factor transfers (%
None	Several	—	$0/5 \times 10^{9}$	$0/5 \times 10^{9}$	—
F*gal*	RE 9	$6{\cdot}5 \times 10^{-5}$	$4{\cdot}8 \times 10^{-7}$	$0/1{\cdot}5 \times 10^{8}$	0·7
	RE 9	$5{\cdot}0 \times 10^{-5}$	$2{\cdot}2 \times 10^{-7}$	$0/3 \times 10^{8}$	0·4
FR5*	RE 178	$1{\cdot}9 \times 10^{-5}$	$7{\cdot}4 \times 10^{-7}$	.†	4·0
	RE 9	$7{\cdot}5 \times 10^{-6}$	$2{\cdot}0 \times 10^{-7}$	.†	2·7
	RE 9	$4{\cdot}8 \times 10^{-6}$	$1{\cdot}5 \times 10^{-7}$	.†	3·0
I*drd*-K	RE 1	$1{\cdot}2 \times 10^{-1}$	$5{\cdot}0 \times 10^{-4}$	$0/5 \times 10^{8}$	0·4
	RE 1	$2{\cdot}1 \times 10^{-1}$	$6{\cdot}8 \times 10^{-4}$	$0/5 \times 10^{8}$	0·3
I*drd trp cysB*	RE 1	$5{\cdot}4 \times 10^{-2}$	$1{\cdot}0 \times 10^{-7}$	$0/5 \times 10^{8}$	2×10^{-4}
	RE 1	$4{\cdot}5 \times 10^{-2}$	$2{\cdot}5 \times 10^{-8}$	$0/5 \times 10^{8}$	5×10^{-5}

Matings with no sex factor in the donor lasted 16 h, all other matings lasted 3½ h.

* FR5 is F*gal*ACSSu.

† Cannot be measured because of A determinant in sex factor.

(1) Transfer requires the presence of a sex factor, indicating that V9A does not itself carry an active sex factor.

(2) Derepressed I-type sex factors transfer themselves with high efficiency (5–20 % of recipients infected in 3½ h), while F-factors (F*gal* and FR5) transfer with low efficiency ($< 10^{-4}$ per recipient). Since the same F-factors transfer rapidly between K12 strains, we attribute their low transfer rate in V9A to the presence of a sex-factor repressor gene of *fi*$^{+}$-type in the host (Reeve & Braithwaite, 1970).

(3) The four sex factors all transfer T, at characteristic rates which range from 0·4 % to 4 % for three of them. The fourth factor, I*drd trp cysB*, transfers T very infrequently (usually $< 10^{-4}$ % of its own transfer frequency). It is remarkable that two derepressed *col*I-type sex factors should differ 1000-fold in their abilities to transfer the T determinant.

(4) The three sex factors which do not themselves carry an A gene all fail to transfer the *Klebsiella* A determinant.

(ii) *Further transfer of T determinant between K12 strains*

Two transfer tests are summarized in Table 3. Each donor is a K12 strain simultaneously infected with both T and FR5 from V9A carrying this sex factor. In both matings the sex factor was transferred, during 60–90 min, to about 10^{4} times as many recipients as when V9A(FR5) was the donor and mating was for 3½ h. Clearly the sex factor has regained its high infection efficiency on transfer to K12. T is transferred at the same relative rate (4–0·4 % that of sex factor transfer) as with the V9A donor. T thus behaves as a separate plasmid which does not become linked to the sex factor on transfer from *Klebsiella*. T can also be transferred between K12 strains by the other sex factors listed in Table 2 and by F-primes such as F*his* and F*lac*.

Table 3. *Transfer of T between strains of K12*

Donor	Recipient	Transfer per recipient of: Sex factor	Transfer per recipient of: T	T/sex factor (%)
RE 110	RE 178	$9{\cdot}4 \times 10^{-2}$	$3{\cdot}8 \times 10^{-3}$	4·0
RE 178	RE 9	$3{\cdot}4 \times 10^{-1}$	$1{\cdot}4 \times 10^{-3}$	0·4

Both donors had received T and F*gal* ACSSu direct from V9A. Mating was for 60 and 90 min, respectively, in the two tests.

(iii) *Transfer of* Klebsiella *A determinant* (A_K)

Three of the sex factors tested could not mediate the transfer of A_K, and selection for its transfer by the fourth sex factor (FR5) was not possible because of the masking effect of the determinant (A_F) contained in FR5. Nevertheless, FR5 appears to be able to induce transfer of A_K, as shown in Table 4. When V9A(FR5) is mated to a Lac$^-$ K12 strain and T recipients are selected, some of these are also Lac$^+$ because they inherit F_K*lac*. The T Lac$^-$ recipients almost always carry FR5 (marked by A, C and sensitivity to f 2), while the T Lac$^+$ recipients rarely carry FR5, because the two plasmids cannot normally coexist in the same K12 cell. Absence of FR5 from these clones is shown by the absence of C and the fact that they cannot act as donors of T or Lac$^+$. However, 5 of the 21T Lac$^+$ clones tested in Table 4 were also resistant to ampicillin, so that they must have received either A_K or A_F, the latter dissociated from the rest of the FR5 complex since no other FR5 genes were present.

Table 4. *Phenotypes of T recipients from mating V9A(FR5) × RE245 : K12 Lac$^-$ Str^r*

Phenotype: T	A	C	Lac	f2	No. of colonies
+	−	−	+	−	16
+	+	−	+	−	5
+	+	+	−	+	15

T recipients were selected and purified on MacConkey agar containing tetracycline (20 μg/ml) and streptomycin (2000 μg/ml). The numbers of Lac$^+$ and Lac$^-$ colonies tested are not proportional to the numbers obtained.

For T, A and C, + and − indicate resistance and susceptibility. f2: F-specific phage test. + and − indicate sensitivity and resistance.

On the assumption that A_K has been transferred, we can make K12 strains having the three A genotypes A_F, A_K and $A_F A_K$, as shown in Table 5, where their levels of resistance to ampicillin are compared. The last two columns of the table give the A genotypes assuming (1) TA Lac$^+$ recipients carry A_K, or (2) they carry A_F. In the latter case strain (c) might carry either one or two doses of A_F (cf. Anderson, 1968).

The fact that the three strains all have clearly different resistance levels supports the first hypothesis, that strain (b) has inherited A_K and not A_F, the two determinants giving different levels of resistance in K12. To provide a more decisive proof, a K12 strain carrying FR5 was treated with the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and two ampicillin-sensitive mutants of the sex factor, FR5-1 and FR5-2 were selected. Both mutants retained the other characteristics of FR5: high infection frequency, sensitivity to F-specific phage of infected cells, and transfer of F*gal* and the determinants C, S and Su as a unit. Each mutant factor was introduced into V9A, which was then mated to K12.

All A-transfers should now be A_K. Both matings gave normal transfer frequencies of T, F_K*lac* and the sex factor, but no ampicillin resistance was transferred. This unexpected result could be explained on either of the two hypotheses discussed above, since both FR5 mutants might have lost the A_F gene entirely, and with it the ability to mobilize A_K for transfer. The evidence for transfer of A_K thus remains indecisive.

Table 5. *Ampicillin resistance in K12 RE 178 carrying A determinants*

Plasmids present	No. of cells tested	% survival on ampicillin (μg/ml.) 50	100	200	Presumed genotype 1	2
(*a*) FR5	540	87	4	0·0	A_F	A_F
(*b*) T,A	130	89	64	0·0	A_K	A_F
(*c*) T,A,FR5	650	98	89	87	A_FA_K	$2A_F$?

Overnight broth cultures were diluted and plated on Oxoid Nutrient Agar containing 0, 50, 100, 200 μg/ml ampicillin. Means of five plate counts are given as percentage of the mean count on Nutrient Agar alone. FR5 is sex factor F*gal*ACSSu. Strains are: (*a*) RE 178 infected with FR5 from K12 donor, (*b*) RE178 TA Lac$^+$ recipient from V9A(FR5) which had lost Lac$^+$ spontaneously, (*c*) strain (b) infected with FR5 from K12 donor.

It may be noted that Perret's iodine test (Anderson & Lewis, 1965) shows that bacterial strains carrying either A_K or A_F produce a β-lactamase. Comparison of the characteristics of the two enzymes may settle the question of whether TA Lac$^+$ recipients have inherited A_K.

4. DISCUSSION

Klebsiella aerogenes strain V9A carries two resistance determinants, T_K and A_K, and a plasmid F_K*lac* but no active sex factor. T_K is transferred to K12 and between strains of K12 by both F- and I-type sex factors, which must first be introduced into the host *Klebsiella*, but it does not appear to become associated with the sex factor after transfer, and is also transferred independently of A_K and F_K*lac*, since it is frequently transferred without either. T_K thus behaves as an independent plasmid, possessing its own replicon and able to transfer during conjugation but without any sex factor activity. In these respects it resembles certain R-determinants (Anderson, 1968), and we can assume that it does not have a chromosomal location.

The *Klebsiella* determinant A_K produces a β-lactamase, and in this respect resembles R-factor determinants for ampicillin resistance. But it could not be transferred with the help of three sex factors which did not themselves carry an A determinant, and the evidence is indecisive that it is transferred by FR5(F*gal*ACSSu), which does possess an A determinant. Two ampicillin-sensitive mutants of FR5 were unable to mobilize A_K. It should be noted, however, that the very rare occurrence of an ampicillin-resistant recipient has been observed in matings with V9A donors carrying a sex factor without an A determinant. These might represent A_K transfers, but it has yet to be proved that it is A_K which has been transferred, and not, for example, a mutation in the sex factor or the Lac plasmid. These cases will be described in another paper. On the present evidence, A_K may be either a chromosomal gene or on a separate plasmid which cannot normally be transferred to K12, possibly because its attachment site in *Klebsiella* does not exist in K12.

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REFERENCES

ANDERSON, E. S. (1968). The ecology of transferable drug resistance in the Enterobacteria. *Annual Review of Microbiology* **22**, 131–180.

ANDERSON, E. S. & LEWIS, M. J. (1965). Drug resistance and its transfer in *Salmonella typhimurium*. *Nature, Lond.* **206**, 579–583.

COWAN, S. T. & STEEL, K. J. (1966) *Manual for the identification of Medical Bacteria*. Cambridge University Press.

DEMEREC, M., ADELBERG, E. A., CLARK, A. J. & HARTMAN, P. E. (1966). A proposal for a uniform nomenclature in facterial genetics. *Genetics* **54**, 61–76.

GILLIES, R. R. & DODDS, T. C. (1965). *Bacteriology Illustrated*. Edinburgh: Livingstone.

LENNOX, E. S. (1955). Transduction of linked genetic characters of the host by bacteriophage. *Virology* **1**, 190–206.

REEVE, E. C. R. & BRAITHWAITE, J. A. (1970). F_K*lac*, an episome with unusual properties found in a wild strain of a *Klebsiella* species. *Nature, Lond.* (in the Press).

VOGEL, J. H. & BONNER, D. M. (1956). Acetylornithinase of *Escherichia coli*: partial purification and some properties. *Journal of Biological Chemistry* **218**, 97–106.