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Segregation of co-transduced streptomycin and tetracycline resistance in *Staphylococcus aureus*

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SUMMARY

Extrachromosomal streptomycin and tetracycline resistance in *Staphylococcus aureus* E169 has been transduced to three different recipients. An analysis of the transductants demonstrates that: (1) in most cases the behaviour of the markers in the transductants is similar to that in the donor; (2) the markers are co-transduced at frequencies of 2–44 % and the frequency is highest when the selected marker is streptomycin resistance; (3) in the co-transductants the markers segregate independently, suggesting that they are unlinked.

1. INTRODUCTION

May, Houghton & Perret (1964) reported that *Staphylococcus aureus* E169 loses its tetracycline resistance (Tc^R) when grown at 43–44 °C. As streptomycin resistance (Sm^R) and numerous other markers were not lost they concluded that the Tc^R determinant was on a plasmid.

In transduction studies with an ultraviolet (u.v.) induced phage from E169, Kasuga & Mitsuhashi (1968) have demonstrated that Tc^R and Sm^R are co-transduced at frequencies of 85–93 % depending on which marker is selected. They concluded that Sm^R and Tc^R are closely linked.

More recently, transduction has been carried out with a virulent phage and the effect on the transduction rate of irradiating the phage with UV light studied (Grubb & O'Reilly, 1971). These results indicate that both the Sm^R and Tc^R determinants of E169 are extrachromosomal. It was also found that streptomycin sensitive (Sm^S) cells could be isolated from E169. They occurred at a much lower frequency than tetracycline sensitive (Tc^S) cells, and independently of them, indicating that the Sm^R and Tc^R determinants are not closely linked. However, as they could be co-transduced at frequencies of 6–10 % it was suggested that unlinked extrachromosomal determinants can be co-transduced and that the rate of co-transduction varies for temperate and virulent phages.

On the evidence available it seems unlikely that the Sm^R and Tc^R determinants are closely linked in E169. However, it is possible that they can occasionally

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become associated by a mechanism similar to that suggested by Richmond (1967) for penicillinase plasmids. If this is the case then some association of the two markers should be evident in the co-transductants. With this in mind co-transductants have been examined to see if the markers are co-ordinately lost or segregate independently.

2. MATERIALS AND METHODS

Bacterial strains. The donor in the transduction experiments was *S. aureus* E169 which is Tc^R, Sm^R and phage type 52/52A/80/81. Recipient strains M1078, M1085 and M1614–3 are all Sm^S, Tc^S, and of phage type 29/52/52A/80, 29/52/52A/80 and 52/52A/79/80 respectively.

Transduction. Transduction was carried out with staphylococcal typing phage 80 (Grubb & O'Reilly, 1971).

Selection of transductants for testing. Isolated colonies were picked from the transduction plates and multipoint replicated (May & Houghton, 1965) onto Oxoid Blood Agar Base No. 2 + 0·5% sodium citrate (BABcit), BABcit + *Tc* (5 μg/ml) and BABcit + *Sm* (25 μg/ml). Transductants which were either Tc^R, Sm^R, or resistant to both antibiotics were picked and plated onto BABcit + *Tc*, BABcit + *Sm* and BABcit + *Sm* and *Tc* respectively. Isolated colonies were then picked from these plates.

Stability of transduced markers. Both the donor and the transductants were grown at 37 °C and elevated temperature. The cultures were then plated out and isolated colonies picked and multipoint replicated onto BABcit, BABcit + *Tc* and BABcit + *Sm*.

Growth at elevated temperature. 100 ml flasks containing 10 ml of meat infusion broth (Cruickshank, 1960, p. 190) were inoculated with about 10^7 cells of an overnight broth culture. The flasks were shaken for 6–7 h at the highest temperature which permitted growth.

3. RESULTS

From Table 1 it can be seen that irrespective of which organism is used as a recipient, Tc^R is transduced at a higher rate than Sm^R and the co-transduction rate is highest when selection is made on *Sm*.

The results in Table 2 are in accord with previous findings. May *et al.* (1964), Kasuga & Mitsuhashi (1968) and Grubb & O'Reilly (1971) have all demonstrated that Tc^S cells can be readily isolated from E169 when it is grown at elevated temperature. Although, Grubb & O'Reilly (1971) have demonstrated that Sm^S cells can be isolated from E169, this was at a low frequency, and so it is not surprising that Sm^S colonies have not been detected in the small number of colonies screened here.

The stability of Tc^R and Sm^R in transductants has been compared with their stabilities in E169 by selecting two representatives of each type of transductant and growing at 37 °C and at elevated temperature. In designating transductants, the usual convention of placing the recipient strain after the donor strain has been

Table 1. *Transduction rates with E169*

Recipient	Selection	Transduction frequency	% resistant	
			Tc	*Sm*
M1614–3	*Tc*	$9{\cdot}6 \times 10^{-6}$	100	6·0
	Sm	$4{\cdot}5 \times 10^{-6}$	29·0	100
M1078	*Tc*	$1{\cdot}1 \times 10^{-5}$	100	1·9
	Sm	$4{\cdot}2 \times 10^{-7}$	44·2	100
M1085	*Tc*	$1{\cdot}1 \times 10^{-5}$	100	2·0
	Sm	$2{\cdot}6 \times 10^{-7}$	25·3	100

Table 2. *Stability of* Tc^R *and* Sm^R *in E169*

Growth temp. (°)	Colonies screened	No. of colonies resistant	
		Tc	*Sm*
37	52	52	52
44·5	52	28	52

Table 3. *Stability of* Tc^R *and* Sm^R *in E169/M1085*

Transductant	Growth temp. (°)	Colonies screened	No. of colonies resistant	
			Tc	*Sm*
Tc–1	37	52	52	—
	43·5	52	13	—
Tc–2	37	52	51	—
	43·5	52	12	—
Sm–1	37	52	—	52
	44	52	—	52
Sm–2	37	52	—	52
	44	52	—	52

adopted. This is followed by the selection marker and in the case of co-transductants by the co-transduced marker.

The results for transductants which are either Tc^R or Sm^R are shown in Tables 3–5. It can be seen that although the maximum temperature of growth varies for individual transductants, Sm^R and Tc^R have similar stabilities as in E169. The results with E169/M1085 and E169/M1614–3 co-transductants are essentially similar (Tables 6 and 7). However, for three of the four E169/M1078 co-transductants the results are different in that the Sm^R and Tc^R are both very unstable (Table 8).

A feature which is common to all co-transductants is that Sm^R and Tc^R behave as independent markers. Even in the three E169/M1078 co-transductants in which Sm^R and Tc^R are both very unstable, the two markers segregate independently. This point is further emphasized when E169/M1614–3 co-transductants which are still Sm^RTc^R after growth at 43 °C are picked, re-grown at elevated temperature

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Table 4. *Stability of* Tc^R *and* Sm^R *in E169/M1078*

Transductant	Growth temp. (°)	Colonies screened	No. of resistant colonies	
			Tc	*Sm*
Tc–1	37	52	52	—
	43·5	52	10	—
Tc–2	37	52	46	—
	43·5	52	11	—
Sm–1	37	52	—	52
	43·5	52	—	52
Sm–2	37	52	—	52
	43·5	52	—	52

Table 5. *Stability of* Tc^R *and* Sm^R *in E169/M1614–3*

Transductant	Growth temp. (°)	Colonies screened	No. of colonies resistant	
			Tc	*Sm*
Tc–1	37	52	52	—
	43	52	17	—
Tc–2	37	52	52	—
	43	52	13	—
Sm–1	37	52	—	52
	43	52	—	52
Sm–2	37	52	—	52
	43	52	—	52

Table 6. *Stability of* Tc^R *and* Sm^R *in E169/M1085 co-transductants*

Transductant	Growth temp. (°)	No. of colonies				
		Screened	Tc^RSm^R	Tc^RSm^S	Tc^SSm^R	Tc^SSm^S
TcSm–1	37	52	52	0	0	0
	44	52	5	0	44	3
TcSm–2	37	52	52	0	0	0
	44	52	46	0	5	1
SmTc–1	37	52	50	1	1	0
	44	52	17	0	35	0
SmTc–2	37	52	52	0	0	0
	44	52	12	0	40	0

and then screened for drug resistance (Table 9). It can be seen that Sm^R and Tc^R still behave as independent markers.

All the transductants that have been studied were phage typed and found to have the same phage patterns as the recipients.

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Table 7. *Stability of* Tc^R *and* Sm^R *in E169/M1614–3 co-transductants*

Transductant	Growth temp. (°)	No. of colonies				
		Screened	Tc^RSm^R	Tc^RSm^S	Tc^SSm^R	Tc^SSm^S
TcSm–1	37	52	52	0	0	0
	43	52	18	0	32	2
TcSm–2	37	52	50	0	1	1
	43	52	17	1	29	5
SmTc–1	37	52	50	1	0	1
	43	52	15	0	36	1
SmTc–2	37	52	52	0	0	0
	43	51	13	0	38	0

Table 8. *Stability of* Tc^R *and* Sm^R *in E169/M1078 co-transductants*

Transductant	Growth temp. (°)	No. of colonies				
		Screened	Tc^RSm^R	Tc^RSm^S	Tc^SSm^R	Tc^SSm^S
TcSm–1	37	52	52	0	0	0
	44	52	14	0	38	0
TcSm–2	37	52	6	20	16	10
	44	52	1	14	6	31
SmTc–1	37	52	3	20	19	10
	44	52	1	3	1	47
SmTc–2	37	52	5	3	3	41
	44	52	0	0	2	50

Table 9. *Re-testing of E169/M1614–3 co-transductants which had not shown segregation*

Strain	Growth temp. (°)	No. of colonies				
		Screened	Tc^RSm^R	Tc^RSm^S	Tc^SSm^R	Tc^SSm^S
TcSm 1a	42	52	10	0	42	0
TcSm 1b	42	52	10	0	41	1
TcSm 2a	42	52	3	0	49	0
TcSm 2b	42	52	2	0	50	0
SmTc 1a	42	52	10	0	42	0
SmTc 1b	42	52	20	0	31	1
SmTc 2a	42	52	19	0	33	0
SmTc 2b	42	52	27	0	25	0

4. DISCUSSION

The co-transduction frequencies obtained here are much lower than those reported by Kasuga & Mitsuhashi (1968), possibly because of the different phages used. Kasuga & Mitsuhashi induced a phage in E169 with UV, whereas typing phage 80 has been used in these experiments.

The instability of the Tc^R in the recipients studied here is in contrast to the results of Kasuga & Mitsuhashi (1968). They found that the Tc^R of E169 was stable

when transduced to strain MS353. As the stable Tc^R of strain MS146 was unstable when transduced to a Tc^S variant of E169 they concluded that the stability of Tc^R is a function of the host. Although Tc^R is extrachromosomal and unstable in E169 its stability in MS353 and MS146 could be accounted for in one of two ways. Either the Tc^R is still extrachromosomal but very stable in MS353 and MS146, or it is integrated into the chromosome of these strains. The work of Asheshov (1969) and Richmond & Johnston (1969) suggests that at least some staphylococcal plasmids can become associated with the chromosome. However, in neither case was permanent integration demonstrated. Stable integration has only been demonstrated for deleted plasmids (Novick, 1967).

Some variation in the behaviour of extrachromosomal determinants in different recipients is demonstrated by E169/M1078 transductants. In three of the co-transductants Sm^R is very unstable, whereas in the remaining co-transductant and in the Sm^R transductants it is stable. The reason for this is not immediately apparent.

The most interesting aspect of these results is the independent behaviour of the Tc^R and Sm^R in the co-transductants. There would appear to be three possible explanations for their independent behaviour: (1) they are co-transduced as separate markers; (2) the two determinants become occasionally closely associated and though they are transduced as such they readily dissociate again; (3) co-transduction may be the result of double infection by transducing phages. This last alternative is unlikely because the multiplicity of infection was less than one and the co-transduction rates are too high to envisage two transducing phages infecting the one cell.

The second alternative requires some mechanism whereby different determinants can become associated. To date three different mechanisms have been reported for the drug-resistance determinants of staphylococci. Two of these mechanisms involve a chromosomal location for one of the markers. Asheshov (1969) has demonstrated that the plasmid marker for mercury and cadmium resistance can duplicate the chromosomal penicillinase determinant, and Richmond & Johnston (1969) have shown in another strain that chromosomal erythromycin resistance can be lost concomitantly with the plasmid markers for cadmium and penicillin resistance. For either of these mechanisms to apply to the Tc^R and Sm^R in E169 would require a chromosomal location for one of these determinants. However, previous work indicates that both Tc^R and Sm^R are extrachromosomal (Grubb & O'Reilly, 1971). The evidence for this is based on the instability of the markers and the effect on the transduction rate of UV irradiating the transducing lysate. When E169 is grown at 37 °C both Tc^S and Sm^S cells can be isolated at a frequency of 0·3 %. When grown at 44·5 °C, 54·6 % of the population is Tc^S and 1·0 % Sm^S. After growth in the presence of ethidium bromide Tc^R and Sm^R are lost at frequencies of 4·8 and 2·7 % respectively. When the transducing lysate is irradiated with increasing doses of UV light the reduction in the transduction rate for both Tc^R and Sm^R is characteristic of extrachromosomal determinants (Arber, 1960). These results indicate that neither marker has a permanent chromosomal location.

However, the possibility of a transient chromosomal location for these markers cannot be discounted. The remaining mechanism whereby different determinants can become associated has been demonstrated for extrachromosomal determinants. Richmond (1967) has shown that penicillinase plasmids can become associated to form penicillinase diploids. Although this has not been demonstrated for other types of plasmid it is possible that the Tc^R and Sm^R can become associated. This association must be an unstable association as evidenced by the dissociation of the two markers in the co-transductants and the failure so far to detect co-ordinate loss of the two markers in E169. The other possibility is that in E169 there are three types of plasmid; Tc^R, Sm^R, and Tc^RSm^R. This would explain why in E169 Tc^S cells are still Sm^R but would not explain the segregation of the markers in the co-transductants.

The remaining alternative is that the Tc^R and Sm^R are co-transduced as unlinked markers. Such an explanation would require some mechanism, whereby a phage can enclose two unlinked determinants. If the determinants are widely separated within the cell it is difficult to envisage how this could occur. However, if they are close together then it may be possible for a phage to occasionally enclose more than one extrachromosomal determinant. If, as has been suggested (Jacob, Brenner & Cuzin, 1963; Novick, 1967), extrachromosomal determinants are attached to the cell membrane then maybe the attachment sites for these determinants are very close.

Obviously more experimental evidence is required to explain the relationship of Tc^R and Sm^R in E169 and the mechanism whereby they are co-transduced.

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