JOINT TRANSDUCTION OF SEPARATE EXTRACHROMOSOMAL DRUG RESISTANCE DETERMINANTS IN STAPHYLOCOCCUS AUREUS E169

W. B. Grubb and R. J. O'Reilly

Department of Microbiology, University of Western Australia,
School of Medicine, Victoria Square, Perth,
Western Australia.

Received December 14, 1970

SUMMARY: An analysis of tetracycline, streptomycin, penicillin and cadmium resistance in Staphylococcus aureus E169 indicates that their determinants are all extrachromosomal. The determinants for penicillin and cadmium resistance are linked. Those for streptomycin and tetracycline resistance appear not to be linked, even though they can sometimes be co-transduced. A possible explanation is offered.

May, Houghton and Perret (1) found that Staphylococcus aureus E169 grown at 43°-44°C irreversibly lost Tc^R* and the ability to produce pase. They screened the treated cells for the loss of numerous other properties, including Sm^R, and found they were unaffected. On this basis, and because Tc^R and pase were lost at different rates, they concluded that the determinants for Tc^R and pase production were on separate autonomously-replicating cyto-plasmic particles.

Kasuga and Mitsuhashi (2) confirmed that in E169 Sm^R is stable and Tc^R is unstable. However, when transduced to a strain MS353, both are now stable. Of the Tc^R transductants, 93.3% were found to be also Sm^R, and of the Sm^R transductants, 84.6% were Tc^R. Co-transduction of Sm^R and Tc^R has been reported in other strains of S. aureus (3).

The evidence suggests that in E169 the determinants for Tc^R and Sm^R are extrachromosomal and chromosomal respectively. Their co-transduction could be occurring in a manner similar to that reported for the determinants for pase production and heavy metal ion resistance in S. aureus PS80 (4).

* Abbreviations; Sm, streptomycin; Tc, tetracycline; Pc, penicillin; pase, penicillinase; Cd, cadmium. Resistance or sensitivity is indicated by superscript R or S respectively.

In this strain the determinant for pase is chromosomal but can be duplicated on the plasmid which confers resistance to the heavy metal ions arsenate, mercury and cadmium. Consequently, the determinants for pase and heavy metal ion resistance can be co-transduced. A similar situation has been reported for erythromycin resistance and the plasmid carrying the markers for pase and Cd^R (5).

With these results in mind, we have re-examined the nature of the determinants for drug resistance in E169 to see if they are linked, and to ascertain whether they are located chromosomally or extrachromosomally.

MATERIALS AND METHODS

Bacterial strains. S. aureus strains E169, M1899 and M1614-3 were used. Their phage types are 52/52A/80/81, 29/52/80/KS6 and 52/52A/79/80 respectively. E169 is Tc^R, Sm^R,Pc^R, and Cd^R, M1899 is Sm^S, Tc^S, Pc^S and Cd^R and M1614-3 is Sm^S, Tc^S, Pc^S and Cd^S.

Elimination of drug resistance. The drug resistance of E169 after growth at elevated temperature (1), and in AF* (6) and EB (7), was compared with that of untreated controls. EB treatment was done in TSB, buffered at pH7.1 with 0.05M potassium phosphate buffer.

Screening procedure. Colonies were replicated (8,9) onto Oxoid Blood Base No. 2 containing either Tc (5 µg/ml), Sm (100 µg/ml), Pc (0,15 i.u./ml) or Cd $(NO_3)_2$ (100 µg/ml). Pase production was tested by the method of Dyke, Jevons and Parker (10).

Propagation and titration of phage. Typing phage 80 was propagated on E169 in TSB + 0.004M $CaCl_2$ + 0.3% agar layered on TSA (11). A 0.7% agar overlay was used to titre the phage.

Transduction (12). Growth from an overnight TSA slope of the recipient was suspended in 10 mls. of TSB + 0.008M $CaCl_2$ to a final concentration of approximately 5 x 10^8 cells/ml. This was warmed to 37°C and then 1 ml. of

* Abbreviations: AF, acriflavine; EB ,ethidium bromide; TSB and TSA, Difco tryptic soy broth and agar respectively.

1-2 x 10^9 p.f.u./ml. of transducing lysate added. After gentle shaking for 30 minutes at 37°C, 1 ml. of 5% sodium citrate was added, and the cells collected by centrifugation. The resuspended cells were left for 3-3½ hours at room temperature and then screened for transductants by spreading onto Difco brain heart infusion agar plates containing 0.5% sodium citrate and drugs at the previously stated concentration. When selection was made for Pc transductants, the cells were washed once before plating.

Ultraviolet (UV) irradiation. 3 ml. amounts of transducing lysates were irradiated in 9 cm Petri dishes for varying times with 50 μW/cm^2 UV in the 2300A to 2700A range.

RESULTS AND DISCUSSION

Sm^S variants of E169 have not been reported (1,2). However, we have found them in cultures of E169 grown at 37°C. Of 1009 colonies tested 7 were Sm^S. Three of these were phage typed and found to have the same pattern as the parent strain. Since the number of Tc^S and Pc^S cells in E169 increases with the incubation temperature (1) we grew E169 at a higher temperature than previously reported (1,2). From Table 1 it can be seen that growth at 44.5°C did not significantly increase the number of Sm^S cells.

Treatment with acridines has been found to eliminate Sm^R in strains of S. aureus (6,13). However, though we were unable to demonstrate this in E169, we were able to eliminate Pc^R with AF (Table 2). Previous attempts

TABLE 1. Effect of growth temperature on the drug resistance of E169.

Temperature	Colonies screened	Number of drug sensitive colonies			
		Tc	Sm	Pc	Cd
37°C	1178	3 (0.3%)	4 (0.3%)	8 (0.7%)	8 (0.7%)
44.5°C	805	439 (54.6%)	8 (1.0%)	61 (7.6%)	61 (7.6%)

TABLE 2. Effect of AF on the drug resistance of E169.

Concentration of AF	Colonies screened	Number of drug sensitive colonies			
		Tc	Sm	Pc	Cd
0	245	3 (1.2%)	2 (0.8%)	0	0
1.58 µg/ml.	118	0	0	118 (100%)	118 (100%)

to eliminate Pc^R in E169 were unsuccessful (1) and attempts to eliminate it in other strains with acridines have produced conflicting results (14).

Table 3 demonstrates that growing E169 in EB increases the number of cells sensitive to the four drugs tested. Though the increases for Tc^S and Sm^S are small, their numbers are significantly different, at the 1% level, to those in the untreated controls. The increase in the number of Pc^S and Cd^S cells is in accord with previous findings (7).

These results indicate that the determinant for Sm^R in E169 is extrachromosomal. To further confirm this we have done transduction studies with UV irradiated transducing lysates. Arber (15) has demonstrated that this treatment increases the transduction rate for chromosomal markers but decreases it for episomes. This technique has been used to determine if the markers for drug resistance in S. aureus are chromosomal or extrachromosomal (5,16, 17,18).

TABLE 3. Effect of EB on the drug resistance of E169.

Concentration of EB	Colonies screened	Number of drug sensitive colonies			
		Tc	Sm	Pc	Cd
0	537	2 (0.4%)	3 (0.6%)	8 (1.5%)	8 (1.5%)
6×10^{-6} M	377	18 (4.8%)	10 (2.7%)	100 (26.5%)	100 (26.5%)

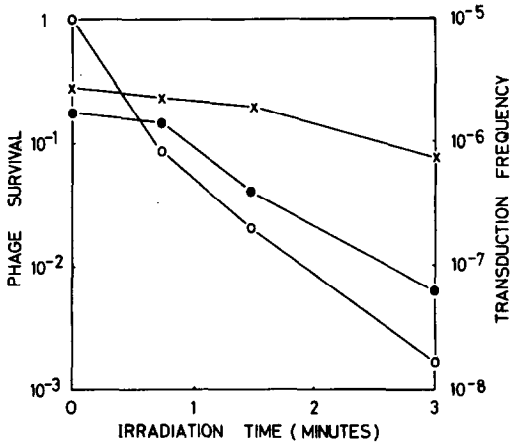

Fig. 1. The effect UV irradiation of phage lysates has on phage survival (O) and the frequency of transduction of Tc^R (X) and Pc^R (●). The phage lysate was prepared on E169 and the recipient was M1899.

In Fig. 1, M1899 was used as a recipient. As this strain is Cd^R and has a high mutation rate to Sm^R it could only be used as a recipient for Tc^R and Pc^R. M1614-3 was used as a recipient for Tc^R, Cd^R and Sm^R (Fig.2). As all Cd^R transductants were found to produce pase this was used as a measure of the Pc^R transduction rate in M1614-3. In both Fig. 1 and 2 the high

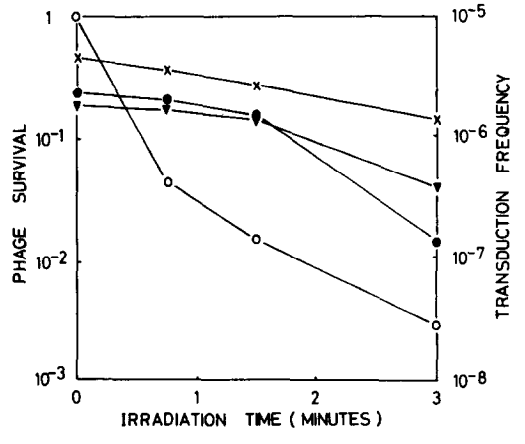

Fig. 2. The effect UV irradiation of phage lysates has on phage survival (O) and the frequency of transduction of Tc^R (X), Sm^R (▼) and Cd^R (●). The phage lysate was prepared on E169 and the recipient was M1614-3.

transduction rates (15) and the effect of UV irradiation on them, indicates that the determinants for Pc^R, Cd^R, Tc^R and Sm^R are all extrachromosomal.

These results confirm the independent extrachromosomal nature of the determinants for Pc^R and Tc^R (1). In addition they demonstrate that the determinants for Cd^R and Pc^R are linked and that the Sm^R determinant is extrachromosomal.

As they obtained high co-transduction rates for Tc^R and Sm^R Kasuga and Mitsuhashi (2) concluded the determinants for these were closely linked. However, this is not in keeping with the fact that they are not lost together. Also, we have not been able to obtain the same degree of co-transduction as Kasuga and Mitsuhashi (Table 4). This discrepancy is no doubt due to our having transduced with a virulent phage whereas they used a UV induced temperate phage.

TABLE 4. Co-transduction of Tc^R and Sm^R.

Donor strain	Recipient strain	Selected on	Number of colonies tested	% Resistant	
				Tc	Sm
E169	M1614-3	Tc	337	100	6.4
E169	M1614-3	Sm	184	9.9	100

It would appear then, that Sm^R and Tc^R are not co-transduced because their determinants are normally on the same linkage group. Co-transduction could be as a result of recombination between the two determinants as occurs with penicillinase plasmids (19). However, this does not account for the discrepancy in the co-transduction results and the failure of Sm^R and Tc^R to show joint elimination. A possible alternative explanation is that the two determinants are spatially near one another, perhaps because their attachment sites (19) are very close together. Consequently , both

determinants are sometimes enclosed in the capsid of a transducing phage, temperate and virulent phages varying in their ability to do this. Of the two possible explanations, the latter appears the more attractive.

ACKNOWLEDGEMENTS: We would like to thank Dr.J.W.May, (Monash University, Melbourne) for his interest in this work and for helpful discussion. We also thank Dr. P. Rountree (Royal Prince Alfred Hospital, Sydney) and Mr.L.R. Rowett (Royal Perth Hospital) for phage typing strains used in this work.

REFERENCES

1. May, J.W., Houghton, R.H., and Perret, C.J., J.Gen.Microbiol., 37, 157 (1964).
2. Kasuga, Tokuhiko, and Mitsuhashi, Susumu, Japan. J.Microbiol., 12, 269 (1968).
3. Kasuga, Tokuhiko, Hashimoto, Hajime, and Mitsuhashi, Susumu, J.Bacteriol., 95, 1764 (1968).
4. Asheshov, E.H., J.Gen.Microbiol., 59, 289 (1969).
5. Richmond, M.H., and Johnston,J.,Genet. Res., 13, 267 (1969).
6. Grubb, W.B., and May, J.W., Unpublished work.
7. Bouanchaud, D.H., Scavizzi, M.R., and Chabbert, Y.A., J. Gen. Microbiol., 54, 417 (1969).
8. May, J.W., and Houghton, R.H., Lab. Pract., 14, 168 (1965).
9. Lederberg, J., and Lederberg, E.M., J. Bacteriol., 63, 399 (1952).
10. Dyke, K.G.H., Jevons, M.P., and Parker, M.T., Lancet 1, 835 (1966).
11. Swanstrom, M., and Adams, M.H., Proc.Soc.Exp.Biol.Med., 78, 372 (1951).
12. Pattee, P.A., and Baldwin, J.N., J.Bacteriol., 82, 875 (1961).
13. Greceanu, V., and Iordanescu, S., Microbiol.,Parazitol.Epidemiol., 14, 321 (1969). Cited in Chem.Abstr., 73, 1162y (1970).
14. Richmond, M.H., Advan.Microbial Physiol., 2, 43 (1968).
15. Arber, W., Virology 11, 273 (1960).
16. Novick, R.P.,J.Gen.Microbiol., 33, 121 (1963).
17. Poston, S.M., Nature 210, 802 (1966).
18. Asheshov, E.H., Nature 210, 804 (1966).
19. Richmond,M.H., Biochem.J., 113, 225 (1969).