SHORT COMMUNICATIONS

Transfer of Chromosomal Genes between *Staphylococci* in Mixed Cultures

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In *Staphylococcus aureus*, plasmid-borne genes may be transferred both from cell-free lysates and from donor cultures to recipient organisms by transduction (Novick & Morse, 1967; Lacey, 1971*a, b*). Although chromosomal genes may be transduced from cell-free lysates at low frequency (e.g. Asheshov, 1966; Dyke, Parker & Richmond, 1970), the transfer of these genes has not been reported between cultures. It is shown here that three chromosomal genes – resistances to novobiocin, streptomycin and to rifampicin – may be transferred between pairs of cultures when incubated together.

METHODS

Strains. The donor strain (609) used in these experiments is the same strain from which plasmid-determined genes (neomycin or tetracycline-resistance) were transferred to recipient cultures by mixed culture incubation (Lacey 1971*a, b*). From 609 and the recipient strain used in the following experiments (6936), spontaneous one-step mutants resistant to either streptomycin (at 100 µg/ml), to novobiocin (at 5 µg/ml) or rifampicin (at 10 µg/ml) were isolated by plating the wild strains on nutrient agar containing these concentrations of each drug. Novobiocin and rifampicin resistances were transferred from 609 to 6936 *str-r*, and streptomycin resistance to 6936 *nov-r*.

Transfer. In each transfer experiment, 1 ml of overnight broth cultures of the donor and recipient cultures were added to 100 ml nutrient broth (Difco) containing 0.01 M-calcium chloride and incubated with aeration at 37 °C. The donor and recipient strains were also incubated singly. After 20 h, the cultures were plated on the appropriate selective medium which was examined after incubation for 48 h at 37 °C for resistant recipients.

RESULTS AND DISCUSSION

Resistant mutants of both strain 609 and 6936 were never observed in proportions as great as 10^{-9} of control cultures and were usually $< 10^{-10}$. After growth with a donor culture, the proportion of resistant recipient clones was much higher; about 10^{-6} for novobiocin resistance, and 10^{-7} for streptomycin and rifampicin resistance (Table 1). The frequency is defined as the number of resistant recipients per total recipients. The colonial appearance, sensitivity to antibiotics other than those investigated here and phage patterns of a proportion of the resistant recipients resembled those of the recipients 6936 *str-r* and 6936 *nov-r*. The level of resistance acquired by the recipients was the same as that in the donor. There seemed little doubt that the resistance in the recipient was acquired by transfer of the genes from the donor. The frequency of transfer of tetracycline and neomycin resistance by plasmids is also shown in Table 1.

Table 1. *Frequency* of acquisition of resistance in the recipient (6936) after incubation with donor culture (609)*

Resistance determinant of donor transferred	Genetic locus	Proportion of resistant recipients after 20 h incubation with the donor	Proportion of resistant recipients in control cultures
Neomycin	Plasmid	1.2×10^{-4}	$< 10^{-12}$
Tetracycline	Plasmid	3.5×10^{-4}	$< 10^{-12}$
Novobiocin	Chromosomal	1.0×10^{-6}	5×10^{-10}
Streptomycin	Chromosomal	2.2×10^{-7}	5×10^{-11}
Rifampicin	Chromosomal	9.4×10^{-8}	1×10^{-10}

* The mean of three experiments performed on different occasions.

Although filtrates of 609 *nov-r*, 609 *str-r* and 609 *rif-r* did not contain agents able to transfer resistance to 6936, the transfer between cells was considered to be by transduction for the following reasons: (i) calcium ions were necessary for the transfer which was abolished by the addition of citrate; (ii) either removal of phage P609 from each of the donors, denoted as 609 (P609)⁻ or the use of a P609-resistant mutant of 6936 as recipient prevented transfer. On relysogenization of 609 (P609)⁻ with P609, transfer occurred at frequencies comparable to that from 609 donor strains (for methods see Lacey, 1971 *b*).

The transferred genes were thought to be chromosomal since (a) the cultures did not segregate sensitive organisms at high frequency and (b) the effect of u.v. light on the transduction frequency of lysates was characteristic of chromosomal genes. Cultures 609 *str-r*, 609 *nov-r*, 609 *rif-r*, 2 cultures of 6936 *str-r*, *nov-r* in one the novobiocin resistance, and in the other the streptomycin resistance had been transferred from 609, and in addition cultures 6936 *nov-r*, *rif-r* were examined. Each of these cultures was grown overnight at 42 °C and stored on slopes at room temperature for 3 months, and then replicated on nutrient agar containing either 100 µg streptomycin/ml, 5 µg novobiocin/ml, or 10 µg rifampicin/ml. From about 4000 colonies of each culture examined, no sensitive segregants was detected.

Transduction experiments were by the method of Dyke *et al.* (1970) from mitomycin-C induced lysates prepared from each culture (Lacey, 1971 *b*). The recipient was 6936 wild strain. The effect of u.v. exposure on the transduction frequencies of each of the three genes was similar from both 609 and 6936 and was characteristic of chromosomal genes (Asheshov, 1966; Novick, 1966). The transduction frequency of novobiocin resistance showed a slight (maximum four-fold) increase, that for streptomycin resistance a 20-fold increase, and that for rifampicin more than a 100-fold. Furthermore mutants of this type are likely to be chromosomal since they must have resulted from changes in genes already in the bacteria.

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