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Bacteriophage-Mediated Acquisition of Antibiotic Resistance by *Staphylococcus aureus* Type 88

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Antibiotic-resistant *Staphylococcus aureus* strains of phage type 88, lysogenic for phage 188, when grown in mixed culture with a nonlysogenic novobiocin-resistant strain, acquired novobiocin resistance at a high rate from the nonlysogenic strain. With most strains of phage type 88, there was no detectable transfer of resistance from lysogenic to nonlysogenic cells. Lysogenization with phage 188 of phage-sensitive strains conferred on the lysogenized cells the ability to acquire chromosome and plasmid resistance markers. The acquisition of novobiocin resistance in liquid cultures depended on the aeration of the culture, cell density, and the presence of Ca^{2+}. Pronase, and to a lesser degree other proteinases, increased the rate of acquisition of chromosome- and plasmid-determined resistance markers by cells lysogenic for phage 188.

A screening program for methicillin- and gentamicin-resistant *Staphylococcus aureus* strains isolated in New York City (11) indicated the presence of multiply resistant strains of phage type 88 in several hospitals. These strains have common characteristics such as rifampin resistance and the presence of a lysogenic phage designated phage 188 (11). Strains of phage type 88 possess a small plasmid which determines chloramphenicol resistance. In addition, there is also a very large plasmid present, with still unknown genetic determinants.

Data by Lacey (4, 5), Witte (12), and others indicate that transfer of antibiotic resistance in mixed cultures is often a very efficient method of genetic analysis of *S. aureus*. The mechanism of transfer in mixed cultures is not very well understood. According to Witte (12) and Lacey (5), it may be different from phage-mediated transduction, but Meijers et al. (7) consider transduction as the main mechanism of genetic transfer in mixed cultures.

Using mixed cultures, we attempted to make transfer antibiotic resistance determinants from multiply resistant lysogenic strains of type 88 to novobiocin-resistant nonlysogenic cells, sensitive to phage 188. These experiments led to the unexpected result of transfer of chromosome-determined novobiocin resistance from the nonlysogenic cells into lysogenic cells of type 88, with a very low rate of transfer of antibiotic resistance determinants from the lysogenic to the nonlysogenic cells. The presence of the lysogenic phage carried by strains of type 88 was required for the acquisition of antibiotic resistance from phage-sensitive donor strains.

MATERIALS AND METHODS

Strains. The methicillin- and gentamicin-resistant strains of experimental phage type 88 used in mixed cultures were isolated at New York Hospital, Bellevue Hospital, New York University Hospital, and Cabrini Hospital in New York City (11). Strain 916, isolated at Bellevue Hospital, was used in most experiments. Strain 766 is a novobiocin- and rifampin-resistant mutant of strain RN450 which has no detectable prophages (9). Additional strains are listed in Table 1. Strains lysogenized with phage 188 are designated by the laboratory number of the strain followed by the phage number in parentheses, e.g., 766(188).

Bacteriophages. Phage sensitivity was tested as previously described (3, 11) at routine test dilution and 100 times routine test dilution with the international set of typing phages and an additional set of experimental phages for nontypable strains. Phage induction of lysogenic strains was in LB broth (20 g of Tryptone [Difco Laboratories], 10 g of yeast extract [Difco], and 5 g of NaCl in 1,000 ml of water) in the presence of 1 μg of mitomycin C per ml. The plaque count was made with strain 766 as indicator. The host range of the phage preparations was determined with the propagating strains for the international and experimental phages used in our laboratory (11).

Lysogenic derivatives of sensitive strains were obtained from resistant colonies growing in plaques formed by phage 188. The reisolated colonies were tested for resistance to phage 188, for phage production upon induction with mitomycin C, and for sensitivity of the original strain to phages induced in its lysogenic derivatives. Nonlysogenic derivatives of strain 916 were obtained by nitrosoguanidine mutagenesis (6). Colonies grown on LB agar were inoculated with toothpicks in a preset pattern and transferred with a replicator with steel prongs to Novick's phage medium (8) overlayed with semisolid medium inoculated with 0.1 ml of a broth culture of the indicator strain

TABLE 1. Characteristics of strains tested

Strain	Strain susceptibility to following antibiotic[a]:												Lysogeny	Source of strain
	Novo	Rif	Cd^{2+}	Met	Gent	Ery	Clin	Pen	Str	Kan	Tet	Min	Chlor	
916	S	R	R	R	R	R	R	R	R	R	R	I	R	188	Bellevue Hospital
918	S	R	R	R	R	R	R	R	R	R	R	I	R	188	Bellevue Hospital
917	S	R	R	R	R	R	S	R	R	R	R	I	R	188	New York Hospital
919	S	R	R	R	R	R	S	R	R	R	R	I	R	188	New York Hospital
977	S	R	R	R	R	R	S	R	R	R	R	I	R	188	Cabrini Hospital
4818	S	R	R	R	R	R	R	R	R	R	R	I	R	188	New York University Hospital
4883	S	R	R	R	R	R	R	R	R	R	R	I	R	188	New York University Hospital
1220	S	R	R	R	R	R	R	R	R	R	R	R	R	ND[b]	916
766	R	R	S	S	S	S	S	S	S	S	S	S	S	ND	RN450
385[c]	R	R	S	S	S	S	S	S	S	S	S	S	S	ND	RN450 *pur-9*
1830	R	R	R	S	S	S	S	R	S	S	S	S	S	ND	Bureau of Laboratories (N.Y.C.)
RN1965	S	S	R	S	S	R	R	R	R	R	R	S	R	+[d]	R. Novick
PS84	S	S	R	S	S	S	S	R	R	S	R	I	S	184	Bureau of Laboratories (N.Y.C.)
PS90	S	S	R	S	S	R	S	R	R	S	R	I	R	+	Bureau of Laboratories (N.Y.C.)
1003	R[e]	S	S	S	S	S	S	S	S	S	R	S	S	188	916

[a] Abbreviations: Novo, novobiocin; Rif, rifampin; Met, methicillin; Gent, gentamicin; Ery, erythromycin; Clin, clindamycin; Pen, penicillin; Str, streptomycin; Kan, kanamycin; Tet, tetracycline; Min, minocycline; and Chlor, chloramphenicol. R, Resistant; I, intermediate resistance; and S, sensitive.

[b] ND, No detectable phage production after growth in the presence of 1 μg of mitomycin C per ml.

[c] Strain 385 required guanine.

[d] +, At least one lysogenic phage can be detected with indicator strains RN450 and 1830.

[e] Novobiocin resistance acquired in mixed culture from strain 766.

TABLE 2. Acquisition of novobiocin resistance by strains of type 88 in mixture with strain 766

Strain of type 88	No. of colonies that acquired resistance in following selective medium[a]:					
	NR-tet	NR-met	NR-gent	NR-chlor	NR-clin	NR-Cd^{2+}
916	780	694	745	180	165	232
917	843	902	784	176	191	283
918	806	782	809	158	162	187
919	907	857	921	182	179	268
977	851	953	793	207	186	283
4818	158	123	NT[b]	NT	NT	NT
4883	217	173	NT	NT	NT	NT

[a] For abbreviations, see Table 1, footnote *a*.
[b] NT, Not tested.

766. Lysogenic colonies form a halo around the site of inoculation, the halo being absent around nonlysogenic colonies. After reisolation, nonlysogenic colonies were tested for sensitivity to phage 188 and for lack of phage production after induction with mitomycin C.

Transduction procedures. Transduction procedures were performed as described previously (10). The transducing phage was added at a multiplicity of 0.1 to 0.2 PFU per colony-forming unit (CFU). In addition to direct selection on LB medium with the appropriate concentrations of antibiotics, 0.05 ml of the phage and cell mixture and 0.05 ml of the control culture were spread on LB agar without antibiotics. After incubation for 18 h, the cells from LB agar were suspended in LB broth to yield approximately 5×10^9 CFU/ml and were plated on selective media.

Transfer of resistance in mixed cultures. The strains used for mixed cultures were grown in LB broth to the mid-logarithmic phase (2×10^8 to 3×10^8 CFU/ml). For transfer on solid medium, 0.1 ml of the control cultures and 0.1 ml of an equal mixture of the two strains were plated on LB agar. After overnight incubation, suspensions of approximately 5×10^8 cells in LB broth were plated on LB medium with 3 μg of novobiocin and 2 μg of rifampin per ml (NR media) and the following concentrations of antibiotics: tetracycline, 2.5 μg/ml; erythromycin, 20 μg/ml; clindamycin, 5 μg/ml; methicillin, 10 μg/ml; penicillin, 10 μg/ml; gentamicin, 10 μg/ml; streptomycin, 50 μg/ml; kanamycin, 25 μg/ml; chloramphenicol, 30 μg/ml; and $Cd(NO_3)_2$, 2×10^{-5} M. In some instances, antibiotic media with novobiocin but without rifampin were used.

After incubation for 48 h at 37°C, colonies grown on selective media were transferred with toothpicks in a preset pattern on the same selective media and replicated on NR media with the other antibiotics. Representative colonies were reisolated on the selective medium and tested for antibiotic susceptibility by the method of Bauer et al. (1) and for phage sensitivity.

For experiments in liquid media, 5 ml of mid-logarithmic growth phase cultures was mixed in the presence of 3×10^{-3} M $CaCl_2$. The mixed cultures and control cultures were grown in Erlenmeyer flasks in a rotary shaker (120 rpm). The cultures were grown for 5 to 6 h to a cell density of 3×10^9 to 4×10^9 CFU/ml; 0.1 ml of inocula from the mixed culture and 0.1 ml of inocula from the control strains were then plated on NR selective media. In some experiments, cultures grown at different time intervals or overnight cultures grown at 80 rpm were used.

DNA-gel electrophoresis. In most instances, the method of Birnboim and Doly (2) adapted to *S. aureus* by S. J. Projan was used. This method allows the detection of both large and small plasmids. Several colonies were suspended in 75 μl of TES buffer (0.03 M Tris–0.005 M EDTA–0.05 M NaCl [pH 7.5]) with 20% sucrose and 15 μg of lysostaphin per ml. After 30 min of incubation at 37°C, an equal volume of 2% sodium dodecyl sulfate in 0.2 M NaOH was added and neutralized with 50 μg of 3 M sodium acetate (pH 4.8) per ml. After 20 min at 4°C and centrifugation (7 min in an Eppendorf centrifuge), the supernatant fluid was precipitated with 1.2 ml of cold ethanol. After centrifugation and suspension of 0.3 M sodium acetate (pH 7.0) followed by a second precipitation and centrifugation, the deposit was dissolved in 50 μl of TES and was subjected to overnight electrophoresis at 35 V with 0.7% agarose in Tris-borate buffer (pH 8.3).

RESULTS

Strains of type 88, 916, 917, 918, 919, and 977 were grown in mixed culture on LB agar with the nonlysogenic strain 766 (Table 2). No detectable growth was obtained with the control cultures. The highest rate of resistant colonies was observed on NR-tetracycline, NR-gentamicin, and NR-methicillin media. The ratio of resistant colonies selected on NR-tetracycline medium was 2×10^{-5} to 9×10^{-5}/CFU (total colony count on LB agar). Plating on NR-chloramphenicol, NR-clindamycin, and NR-$Cd(NO_3)_2$ media yielded approximately 20% of the colonies obtained on NR-tetracycline medium (Table 2). The number of resistant colonies was lower with strains 4818 and 4883 isolated at University Hospital. The colonies were reinoculated after a preset pattern on the same selective media and screened for the presence of additional resistance determinants by replication on the whole set of NR antibiotic media. Representative colonies were reisolated and retested. The colonies isolated on all selective media had the resistance spectrum of the multiply resistant strains of type 88. Only four tetracycline-resistant colonies ob-

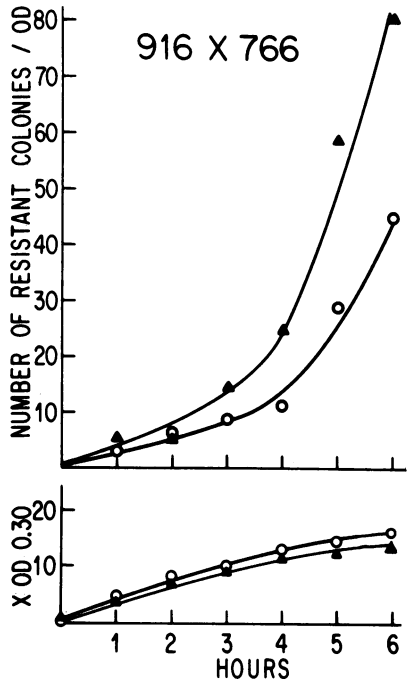

FIG. 1. Optical density (OD) determinations were made with cultures diluted 1/10. One milliliter of undiluted culture was plated on NR-tetracycline medium; after 3 h, both undiluted cultures and the 1/10 dilutions were plated. The results were expressed as the average number of colonies per plate (five plates) divided by the optical density. Symbols: O, no addition of pronase; ▲, addition of 100 μg of pronase per ml (selection on NR-tetracycline medium).

tained with strain 919 and two cadmium-resistant colonies obtained with strain 977 were susceptible to the other antibiotics tested. The presence of the resistance spectrum characteristic for strains of type 88 (Table 1) appears to indicate that the lysogenic strains of type 88 have acquired the chromosome novobiocin resistance marker from the nonlysogenic strain 766.

Additional experiments were carried out with strain 916 of type 88 in mixed cultures with strains 766 and 385. The ratio of resistant colonies on different media was similar to that observed in previous experiments (Table 2). The colonies tested had the resistance spectrum of strain 916.

Further tests on representative colonies involved phage sensitivity, antigenic structure and the presence of plasmid DNA. Colonies isolated on NR-tetracycline and NR-methicillin media were sensitive to phage 88 but resistant to phages of group III, to which strains 385 and 766 were sensitive. The resistant colonies were agglutinated by antisera I and III characteristic for strains of type 88 (11). Strains 385 and 766 were not agglutinated by these antisera. DNA-gel electrophoresis indicated the presence of a small chloramphenicol-resistant plasmid present in strains of type 88 (unpublished data). For colonies obtained with RN450 *pur*-9 strain 385, recombinants from mixed cultures of strains 916 and 385 did not require guanine. The above data are consistent with the acquisition of novobiocin resistance by strain 916.

Experiments in liquid media. The results obtained in LB broth depended largely on the experimental conditions, principally the presence of Ca^{2+} and the aeration of the culture. No detectable acquisition of resistance by strain 916 could be obtained in the absence of Ca^{2+}. Transfer was obtained at a concentration of 10^{-4} M $CaCl_2$, the rate of transfer increasing as the concentration was raised to 3×10^{-3} M. Ca^{2+} could be partially replaced by Mg^{2+} and Mn^{2+}, but not by Zn^{2+}. The effect of Ca^{2+} was less pronounced in transfer experiments on solid medium.

The rate of acquisition of novobiocin resistance was very low in stationary cultures. The rate of transfer was not affected when the cell concentration of stationary cultures was increased (12) by centrifugation of the culture and resuspension in 5% of the original volume.

In aerated cultures, the rate of acquisition of novobiocin resistance was low during the first 3 h of incubation but showed a sharp increase after 4 h (Fig. 1). After 5 to 6 h of incubation, the rate of acquisition of novobiocin resistance up to 5×10^{-5}/CFU could be observed (selection on NR-tetracycline medium). The rate of acquisition of novobiocin resistance increased to 8×10^{-4}/CFU after 18 h. The increase in the rate of acquisition of novobiocin resistance between the 4th and 5th h of incubation could not be demonstrated when the culture was diluted 1/20 at 3 h of incubation.

The ratio of strains 916 and 766 in mixed cultures was determined at zero time, 6 and 18 h of growth in LB broth, and 18 h of growth on LB agar. Appropriate dilutions were plated on LB agar (total cell count), LB agar with tetracycline (916 cells), and LB agar with novobiocin (766 cells). In LB broth, the ratio of 916 to 766 cells changed from approximately 1/1 to 25/1 to 35/1 after 6 h and 80/1 to 110/1 after 18 h. Similar results were obtained with mixed cultures on LB agar.

Supernatant fluid obtained after centrifugation of mixed cultures was tested for the ability to transfer novobiocin resistance to strain 916. Mixed cultures of strains 916 and 766 were grown for 3 and 6 h and centrifuged at 8,000 rpm for 15 min, and the supernatant fluid was added

to the cells of strain 916. No acquisition of novobiocin resistance or a very low rate of acquisition could be obtained after incubation of 2, 4, and 18 h. Determination of the phage concentration in the supernatant fluid, with strain 766 as indicator, gave values of 2×10^7 to 6×10^7 PFU/ml after 3 h and 3×10^6 to 8×10^6 PFU/ml after 6 h. The corresponding values with the supernatant fluid from control cultures of strain 916 alone were 3×10^6 to 7×10^6 PFU/ml after 3 h and 2×10^5 to 4×10^5 PFU/ml after 6 h.

DNase at relatively high concentrations (100 μg/ml) was added to mixed cultures of strains 916 and 766; it decreased the rate of acquisition of novobiocin resistance in stationary cultures by 40 to 70% but produced an increase of the rate of acquisition in aerated cultures. The effect varied greatly with the DNase preparations, some highly purified preparations having no detectable effect. It is possible that the effect of some DNase preparations is owing to impurities present in these preparations.

In attempts to inactivate DNase by pretreatment with pronase, a consistent increase of the rate of transfer of novobiocin resistance was observed. Further experiments indicated that pronase alone had the same activating effect, resulting in an increase of 130 to 300% of the rate of acquisition of novobiocin resistance. A detectable effect was obtained with concentrations as low as 1 μg/ml, but increased when the pronase concentrations were raised. Subsequently 100 μg of pronase (Calbiochem, 45,000 U/g) per ml was routinely added in transfer experiments. Other proteases such as chymotrypsin, proteinase K, and especially nagarase had an activating effect on the acquisition of novobiocin resistance.

The addition of pronase accelerated the appearance of novobiocin-resistant colonies (Fig. 1) but decreased slightly the optical density of the mixed cultures. The determination of the ratio of 916 to 766 cells after 6 h of incubation showed a higher ratio of 916 cells (120/1 to 180/1) than in the absence of pronase. The number of phage particles in the supernatant and the total colony count on nonselective medium were of the same order of magnitude as in the absence of pronase.

Lysogeny with phage 188 appears to be essential for the acquisition of antibiotic resistance. Strain 1220, a nonlysogenic derivative of strain 916 obtained by nitrosoguanidine treatment, became sensitive to phage 188 and showed no detectable phage production after induction with mitomycin C, strains 766 and 1830 serving as indicators (strain 1830 was found to be sensitive to all *S. aureus* phages tested in our laboratory). In mixed cultures of strain 1220 with strain 766, there was no detectable transfer of antibiotic resistance and the ratio of 1220 to 766 cells remained close to 1/1. Strain 1220 regained the ability to acquire novobiocin resistance after lysogenization with phage 188. The rate of acquisition was 20 to 30% of that observed with strain 916, possibly owing to a lower phage production (3×10^5 to 5×10^5 PFU/ml after 3 h incubation in mixed cultures).

Acquisition of antibiotic resistance by strains lysogenized with phage 188. Most experiments were carried out with strain 766 lysogenized with phage 188 (766[188]). Similar results were obtained with other strains lysogenized with phage 188. Mixed cultures of the lysogenic strains 766(188) and 916 gave a low rate of transfer of novobiocin resistance from strain 766(188) to strain 916 and of chromosome-determined gentamicin resistance associated with erythromycin resistance from strain 916 to strain 766(188) (3×10^{-8} to 8×10^{-8}/CFU). In mixed cultures of strains 1220 and 766(188), strain 766(188) acquired from strain 1220 plasmid-determined chloramphenicol resistance and several groups of associated chromosomal resistance markers, such as tetracycline and minocycline resistance, gentamicin and erythromycin resistance, and streptomycin and kanamycin resistance. There was no detectable transfer of novobiocin resistance from strain 766(188) to strain 1220. A more detailed account of these experiments will be given elsewhere.

In experiments with several *S. aureus* strains, it was found that strain 766(188) can acquire in mixed cultures both chromosome- and plasmid-determined resistance markers. The results with strains PS84, PS90, and RN1965 are summarized in Table 3. Strain RN1965 is genetically related to strain 766 and is lysogenic for phage P11. The plasmid content of the three strains was determined by DNA-gel electrophoresis (2). Strain RN1965 harbors six plasmids. Of these, three are penicillinase plasmids, since erythromycin, kanamycin, and cadmium resistance determinants were located on different penicillinase plasmids (R. Novick, personal communication). Strains PS84 and PS90 each harbor five plasmids, strain PS84 possessing both plasmid- and chromosome-determined tetracycline resistance (10).

Mixed culture experiments with nonlysogenic 766 cells indicate a low rate of transfer of antibiotic resistance markers from strain RN1965 to strain 766; from 2×10^{-8} to 5×10^{-9}, depending on the resistance marker. No transfer of antibiotic resistance was observed in mixed cultures of strain 766 with strains PS84 and PS90. The rate of transfer into strain 766(188) depended on the donor strain and the antibiotic selected (Table 3). In some instances, as selection for erythromycin resistance with the mixed

culture 766(188) and PS90, a ratio of resistant colonies up to 5×10^{-4}/CFU was observed. The rate of acquisition of tetracycline resistance was very high with all three donor strains, whereas the rate of acquisition of streptomycin resistance was high with strains RN1965 and PS90 and low with strain PS84. Erythromycin resistance was acquired at a much higher rate from strain PS90 than from strain RN1965.

The transfer of the antibiotic resistance markers from strain RN1965 was accompanied by the transfer of the corresponding plasmids. Individual plasmids could also be detected after the transfer of tetracycline resistance and cadmium resistance plus penicillinase activity from strain PS84 and tetracycline resistance, chloramphenicol resistance, and cadmium resistance plus penicillinase activity from strain PS90. No detectable plasmids were found after acquisition of streptomycin resistance from strain PS84 and streptomycin or erythromycin resistance from strain PS90.

Testing for coacquisition of nonselected antibiotic resistance markers in mixed cultures 766(188) and RN1965 indicated that 3% of the tetracycline-resistant colonies were also streptomycin resistant and vice versa. No coacquisition of chloramphenicol resistance has been observed. When selection was made for chloramphenicol resistance, a high rate of coacquisition of tetracycline and streptomycin resistance was observed. However, only one chloramphenicol-resistant colony was both streptomycin and tetracycline resistant. DNA gel electrophoresis indicated that the tetracycline, chloramphenicol, and streptomycin resistance markers remained linked to distinct plasmids. There was also coacquisition of cadmium resistance with erythromycin resistance and of erythromycin resistance with kanamycin resistance.

Strain 1003 is a derivative of strain 916 which apparently in a single step lost all resistance determinants with the exception of tetracycline resistance, yet retained the lysogenic phage 188. (A more detailed description of this strain will be given elsewhere.) In mixed culture experiments with a novobiocin-resistant derivative of strain 1003, the ratio of resistant colonies obtained with strain RN1965 was of the same order of magnitude as with the mixed culture 766(188) and RN1965, but the rate of coacquisition of resistance determinants was much lower (Table 3).

Transduction experiments were carried out with phage 188 induced in strain 766(188) (4×10^9 to 6×10^9 PFU/ml) with strains 916 and 1220 as acceptors. The rate of transfer of novobiocin resistance was very low (3×10^{-8} to 2×10^{-9}/PFU). There was no significant difference in the rate of transfer with lysogenic and nonlysogenic

TABLE 3. Transfer of antibiotic resistance in mixed cultures in LB broth with strains lysogenic for phage 188

Strains	Transfer of antibiotic resistance in following selective medium[a]:					
	NR-tet	NR-str	NR-chlor	NR-ery	NR-Cd^{2+}	NR-kan
766/188 + RN1965	1,536[b] (46 str)[c]	1,832 (51 tet)[c]	94 (15 str, 12 tet)[c]	390 (26 Cd^{2+})[c]	693 (5 ery)[c]	764 (14 ery)[c]
766/188 + PS90	1,290	1,634	284	1,734	325	NT[d]
766/188 + PS84	842 (39 mino)[e]	12	NT	NT	423	NT
1003 + RN1965	NT	1,493	79 (2 str)[c]	296 (4 Cd^{2+})[c]	430	743

[a] For abbreviations, see Table 1, footnote *a*.
[b] Number of colonies selected.
[c] Cotransfer of nonselected resistance markers.
[d] NT, Not tested.
[e] Cotransfer of minocycline resistance.

acceptor strains. In contrast with experiments with mixed cultures, the addition of pronase had no detectable effect on the rate of transfer of novobiocin resistance in transduction experiments.

DISCUSSION

It was found that multiply resistant strains of *S. aureus* of experimental phage type 88, lysogenic for phage 188, acquired chromosome-determined novobiocin resistance when grown in mixed culture with novobiocin-resistant strains 766 and 385 sensitive to phage 188. This system was extended to other chromosome- and plasmid-determined resistance markers by using acceptor strains lysogenic for phage 188. The direction of transfer of antibiotic resistance was predominantly from cells sensitive to phage 188 to the lysogenic cells. The rate of acquisition of resistance depended to a high degree on the markers and strains used.

The acquisition of resistance determinants in liquid cultures required aeration and the presence of Ca^{2+} and was activated by proteases. Lysogeny of the acceptor strain and sensitivity to phage 188 of the donor strain appear to be essential for the acquisition of antibiotic resistance in mixed cultures. This is indicated by the lack of the ability to acquire antibiotic resistance by strain 916 after the loss of the prophage 188 (strain 1220) and the acquisition of antibiotic resistance markers in mixed cultures by strains lysogenised with phage 188. The very low rate of bidirectional transfer in mixed cultures of lysogenic strains 916 and 766(188) is consistent with these observations.

The requirement for aeration could be explained by the higher degree of phage sensitivity of the actively growing donor cells. The activating effect of proteases is more difficult to explain. The increased ratio of 916 to 766 cells compared with mixed cultures without proteases could suggest a higher degree of lysis of the donor cells.

In contrast to results obtained with mixed cultures, transduction experiments with phage 188 or the addition of supernatant fluid of mixed cultures to acceptor strains resulted in a very low rate of transfer of antibiotic resistance. It is, therefore, improbable that a typical transduction process as suggested by Meijers et al. (7) is sufficient to explain the acquisition of antibiotic resistance by lysogenic cells. The relatively high rate of transfer in mixed cultures could possibly be explained by the need for cell contacts, as suggested by the requirement for a high cell density or by the continuous generation of relatively unstable genetic material, possibly after contact of the phage particles with the lysogenic acceptor cells.

A comparison of the data obtained with strains of type 88 with published data on transfer of genetic material in mixed cultures is difficult, owing to differences in experimental conditions and of the strains used by different investigators. High cell concentrations and the presence of Ca^{2+} were required in the experiments of Lacey (4, 5) and Witte (12). Lacey used aerated cultures, whereas Witte obtained efficient transfer in stationary cultures. In most experiments reported in the literature (4, 6, 7, 12), the transfer of genetic material occurred mainly from lysogenic to nonlysogenic cells, although bidirectional transfer has been reported (7). In recent experiments, Lacey (5) observed a high rate of acquisition of antibiotic resistance by cells lysogenized with phage 55. The transfer was limited to plasmid-determined antibiotic resistance markers and occurred after a short time interval of incubation. The short interval of contact between donor and acceptor cells led Lacey to the assumption that the lysis of the donor cells by phage 55 is not involved in this type of transfer, but rather the presence of prophage 55 facilitates genetic transfer by cell contact. Strains lysogenic for phage 188 acquire both chromosome- and plasmid-determined resistance markers and the lysis of the donor cells is apparently required for the acquisition of resistance determinants. The acquisition of chromosome-determined resistance markers could be useful in the genetic analysis of multiply resistant strains of *S. aureus* which often contain chromosome-determined resistance markers (10; unpublished data).

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