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# Aminocyclitol-Modifying Enzymes Specified by Chromosomal Genes in *Staphylococcus aureus*

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A genetic analysis of aminocyclitol resistance in two strains of *Staphylococcus aureus* was performed. Resistance of strain FK170, isolated in Zurich, was due to the production of a 3'-phosphotransferase [APH(3')]. Strain 5532, isolated in London, produced a 2"-phosphotransferase [APH(2")] and a 6'-*N*-acetyltransferase [AAC(6')]. Plasmid deoxyribonucleic acid (DNA) was isolated by isopycnic centrifugation from the two parent strains, as well as from susceptible variants and from resistant transductants of both strains. Comparative analysis of plasmid DNA by centrifugation in sucrose gradients revealed that strain FK170 harbored a 2.7-megadalton tetracycline R-plasmid and a 36-megadalton cryptic plasmid. Strain 5532 contained an 18.5-megadalton penicillinase plasmid. No evidence for plasmid location of the markers for aminocyclitol resistance could be obtained.

It is well known that antibiotic resistance in *Staphylococcus aureus* is frequently determined by genes that are part of resistance plasmids. The best known example is the plasmid-determined resistance in staphylococci to penicillin mediated by a penicillinase (21, 23). In many tetracycline- or chloramphenicol-resistant strains of *S. aureus*, evidence for plasmid inheritance of resistance markers has been presented (15, 16, 23). Occasionally, however, strains which evidently carry the markers for resistance to penicillin (1, 26, 28) or to tetracycline (15) integrated in the staphylococcal chromosome have been found. Resistance to kanamycin and other aminocyclitols (3, 15), to gentamicin (12, 31, 32), and to tobramycin (17, 29) has always been observed to be determined by R-plasmids of various sizes in staphylococci.

In this paper, we report the existence of two *S. aureus* strains in which we were unable to find any evidence of plasmid-determined resistance to aminocyclitol antibiotics. One of these strains was resistant to kanamycin and other aminocyclitols due to the production of an aminocyclitol 3'-phosphotransferase [APH(3')]. This enzyme differed from the APH(3') enzymes usually found in gram-negative organisms (13). The other strain produced an aminocyclitol 2"-phosphotransferase [APH(2")], as well as a 6'-*N*-acetyltransferase [AAC(6')], and was resistant to gentamicin, tobramycin, sisomycin, and other aminocyclitols (2, 7, 18).

## MATERIALS AND METHODS

**Bacterial strains.** The staphylococcal strains studied are listed in Table 1, along with their susceptibilities to representative aminocyclitol antibiotics and the aminocyclitol-modifying enzymes produced. *S. aureus* FK170, in addition to having the characteristics mentioned in Table 1, was resistant to tetracycline, erythromycin, and trimethoprim-sulfamethoxazole and produced penicillinase. Strain 5532 (27) was resistant to aminocyclitols and penicillins (penicillinase), but susceptible to other antimicrobial agents. Both strains belonged to phage group III.

**Determination of drug resistance.** Minimal inhibitory concentrations were determined by serial twofold dilutions of antibiotic in Mueller-Hinton agar (BBL Microbiology Systems) or Mueller-Hinton broth (BBL). The minimal inhibitory concentration technique corresponded to the International Collaborative Study procedure (9). In some experiments, drug resistance was determined by the U.S. disk diffusion test (20).

**Examination of genetic stability.** Spontaneous loss of antibiotic resistance was investigated in cultures grown overnight at 37°C in brain heart infusion broth. Induced loss of resistance was examined in cultures treated with acriflavine or ethidium bromide, in cultures grown at 45°C, or in broth cultures stored for 1 month or longer at 37°C (14). An indicator method was used to screen for penicillinase-negative mutants of *S. aureus* (21, 25). Joint elimination of other resistance markers in negative variants was examined with the disk diffusion test.

**Enzymatic procedures.** Crude extracts containing aminocyclitol-modifying enzymes were prepared with the lysostaphin method as described (30). Enzymatic activities were measured by the phosphocellulose paper binding assay (6). The system for phosphorylation by extracts of *S. aureus* FK170, FK429, and the transductant RN450 Kan was identical to that described by Kayser et al. (14). Phosphorylation and acetylation by lysates of *S. aureus* 5532 and derivatives and the transductant Ps53Gen were done according to standard procedures (6, 7, 18).

TABLE 1. *Origin of S. aureus isolates, production of modifying enzymes, and susceptibility to representative aminocyclitol antibiotics*

| Strains[a] | Origin, relevant characters, and references | Minimal inhibitory concentration[a] (μg/ml) of: | | | | | Aminocyclitol-modifying enzyme produced |
|---|---|---|---|---|---|---|---|
| | | Kan | Gen | Tob | Ami | Net | |
| FK170 | Wild type, Zurich (this paper) | >256 | 0.03 | 0.03 | 16 | 0.06 | APH(3') |
| FK427 | APH(3')-negative variant of FK170 (this paper) | 4 | 0.03 | 0.03 | 2 | 0.06 | None |
| FK428 | Tetracycline-susceptible variant of FK427 (this paper) | 4 | 0.03 | 0.03 | 1 | 0.06 | None |
| FK429 | Tetracycline-susceptible variant of FK170 (this paper) | >256 | 0.03 | 0.03 | 16 | 0.06 | APH(3') |
| 5532 | Wild type, London (27) | >256 | 250 | 125 | 8 | 8 | APH(2") AAC(6') |
| S25 | APH(2")-negative variant of 5532 (27) | 64 | 0.7 | 4 | 4 | 0.1 | AAC(6') |
| 5532 P'ase⁻ | P'ase-negative variant of 5532 (this paper) | ND[b] | ND | ND | ND | ND | APH(2") AAC(6') |
| S25 P'ase | P'ase-negative variant of S25 (this paper) | ND | ND | ND | ND | ND | AAC(6') |
| Ps53 | Propagating strain for phage 53 | 1.0 | 0.12 | 0.06 | 0.06 | 0.06 | None |
| Ps53 Gen | Gentamicin-resistant transductant of Ps53 (27) | >256 | 128 | 128 | 8 | 8 | APH(2") AAC(6') |
| RN450 | Plasmid/prophage-free variant of Ps47 (23) | 0.5 | 0.06 | 0.03 | 0.12 | 0.03 | None |
| RN450 Tet | Tetracycline-resistant transductant of RN450 (this paper) | 0.5 | 0.06 | 0.03 | 0.12 | 0.03 | None |
| RN450 Kan | Kanamycin-resistant transductant of RN450 (this paper) | >256 | 0.03 | 0.06 | 8 | 0.12 | APH(3') |
| RN11 | Host of reference plasmid PI258 (23) | ND | ND | ND | ND | ND | None |
| RN1304 | Host of reference plasmid T169 (23) | ND | ND | ND | ND | ND | None |

[a] Kan, kanamycin; Gen, gentamicin; Tob, tobramycin; Ami, amikacin; Net, netilmicin; Tet, tetracycline; P'ase, penicillinase.
[b] ND, Not done.

**Transduction procedure.** Transduction was performed with phage 53 of the phage typing set. The phage was adapted to donor strains by serial single-plaque passages. High-titer phage lysates were prepared, and antibiotic resistance was transferred to appropriate receptors as described in the text. The procedure corresponded to the method used in our laboratory (14).

**DNA procedures.** Cleared lysates of the strains under study were prepared by a method similar to that described by Novick and Bouanchaud (23). The lysates were mixed with saturated CsCl to give a final density of 1.54, and ethidium bromide was added to give a final concentration of 500 μg/ml. Approximately 8.5 ml was centrifuged to equilibrium in a 50 Ti fixed-angle rotor at 42,000 rpm for 40 to 60 h at 20°C. (for more details see reference 15). When *E. coli* K-12 J53 (R15) served as a source of the reference deoxyribonucleic acid (DNA), the methods of Clewell and Helinski (4) for isolation of plasmid R15 were closely followed.

To characterize plasmid DNA, centrifugation in linear sucrose gradients (11 ml, 15 to 30%) was carried out. The gradients were prepared in TES buffer [0.05 M tris(hydroxymethyl) aminomethane–0.05 M ethylenediaminetetraacetic acid–0.05 M NaCl, pH 8.0], 0.1 to 0.2 ml of plasmid-containing solution was layered on top of the gradients, and centrifugation was carried out in an SW41 rotor (Beckman Instruments, Inc.) at 20°C for 180 to 240 min. Sedimentation constants and molecular weights of individual molecules were compared with data of reference plasmids and calculated by the methods of Hudson et al. (11) and Clowes (5). The molecular form of plasmid DNA peaks—covalently closed circular or open circular—were determined by comparing the sedimentation pattern of partially denatured plasmid solutions with unimpaired preparations or by sedimenting the material in an alkaline sucrose gradient (10).

Rapid screening for plasmid DNA with little starting material was performed, with slight modifications, as described for gram-positive organisms (8). Single colonies were suspended in 10 μl of electrophoresis buffer (pH 8.2) in small tubes. A 15-μl amount of the lysis mixture was added. The mixture was composed as described (8), but contained 30 μg of lysostaphin per ml instead of lysozyme. After incubation for 15 min at 37°C, the total volume was transferred to a slot of a standard vertical slab gel apparatus. The gel concentration was 0.7% agarose type I (Sigma Chemical Co.). The DNA was electrophoresed for 1 h at 30 V and then for 4 to 5 h at 80 to 100 V.

## RESULTS

**Experiments to obtain antibiotic-susceptible variants.** The first indication that resistance to aminocyclitols in *S. aureus* FK170 might

be governed by a chromosomally located gene came from the observation of the considerable stability of this resistance character. Phosphotransferase-negative variants could not be isolated spontaneously or by treatment of cultures with various curing agents known to affect plasmid replication. In total, 35,500 colonies were examined and screened for loss of kanamycin resistance with negative results. However, storage of broth cultures for 40 days at 37°C yielded five enzyme-negative variants among 14,500 clones examined in a single experiment. No joint elimination of other resistance markers in the kanamycin-susceptible variants was observed. Aging of cultures was also successful in obtaining tetracycline-susceptible variants. Resistance to erythromycin and production of penicillinase, however, were remarkably stable characters in FK170. No susceptible variant clones could be found among more than 30,000 colonies examined.

**Physical characterization of plasmid DNA in strains FK170 and derivatives.** Because of the relative stability of resistance to aminocyclitols in strain FK170 and in variants derived from it, additional experiments were conducted to establish whether resistance was plasmid mediated or not. Dye-buoyant density centrifugation was used to isolate plasmid DNA from cleared lysates. Plasmid DNA was shown to be present in all strains. Plasmid DNA was then layered onto sucrose gradients to characterize individual molecules (Fig. 1). Strain FK170 contained two distinct plasmids. The smaller plasmid was shown to be a tetracycline resistance plasmid, since the Tet-susceptible variant FK429 did not contain it and the molecule was observed in RN450 after transfer of Tet resistance from FK170 (Fig. 2). In separate experiments, this plasmid sedimented together with the reference plasmid $T_{169}$ of strain RN1304 (23) and thus had a molecular weight of $2.7 \times 10^6$. The larger plasmid present in FK170 had a molecular weight of $36 \times 10^6$. This plasmid also was found in all derivatives of FK170 and therefore seemed not to be essential for the resistant phenotype.

To exclude the possibility that a kanamycin resistance plasmid would have the same size as this "cryptic" plasmid and thus would band at the same position in sucrose gradients, we tried to isolate variants which had lost the cryptic plasmid. Cultures of FK170 and FK428 were stored for up to 100 days at 37°C, and individual clones were isolated. The presence of plasmid DNA in these clones was examined with rapid agarose gel electrophoresis procedure. Among 176 clones of both strains, no variants devoid of the 36-megadalton (Md) plasmid were observed. Clones of FK170, which had lost the 2.7-Md tetracycline resistance plasmid, however, were found frequently.

To obtain additional evidence of the chromosomal location of the APH(3′) gene, resistance to kanamycin was transduced from FK170 into RN450. No plasmid DNA was detected in the RN450 transductants producing APH(3′).

**Physical characterization of plasmid DNA in strain 5532 and derivatives.** Separation of plasmid DNA from chromosome DNA in CsCl-ethidium bromide density gradients gave results which strongly suggested the chromosomal location of the determinants for the production of APH(2″) and AAC(6′). Although plasmid DNA was present in 5532 and in the APH(2″)-negative variant S25, no covalently closed circular DNA was detected in penicillinase-negative derivatives of these strains (Fig. 3). This suggested that the plasmid peaks in the wild-type strain and in the APH(2″)-negative variant were due to a penicillinase plasmid. Comparison of $^3$H-radiolabeled plasmid DNA with differentially labeled DNA from the reference strain RN11 in neutral sucrose gradients showed that both molecules had identical sedimentation characteristics. The penicillinase plasmid of 5532 thus proved to have a size of about 18.5 Md. Transfer of APH(2″) together with AAC(6′) from 5532 into Ps53 resulted in a gentamicin-resistant transductant of Ps53 (27). Isolation of plasmid DNA from this transductant and from Ps53 and comparative analysis of the DNAs by differential sedimentation revealed that both strains contained identical plasmids, about 19 Md in size (Fig. 4). The biological function of the plasmid in Ps53 is unknown. It is probably impossible for it to be a penicillinase plasmid, since the Ps53 *bla* locus has been established to be chromosomal (26).

## DISCUSSION

Most determinants of antibiotic resistance in *S. aureus*, as in *Enterobacteriaceae*, are thought to be located on staphylococcal plasmids. Occasionally, however, strains have been found which carry typical plasmid resistance markers integrated into the staphylococcal chromosome.

In this paper, we describe the apparent chromosomal location of genes controlling the production of aminocyclitol-modifying enzymes in two such strains. The kanamycin-resistant strain FK170 harbored a small 2.7-Md and a large 36-Md plasmid. Since a kanamycin-susceptible variant also contained the two plasmids and since plasmid DNA could not be detected in kana-

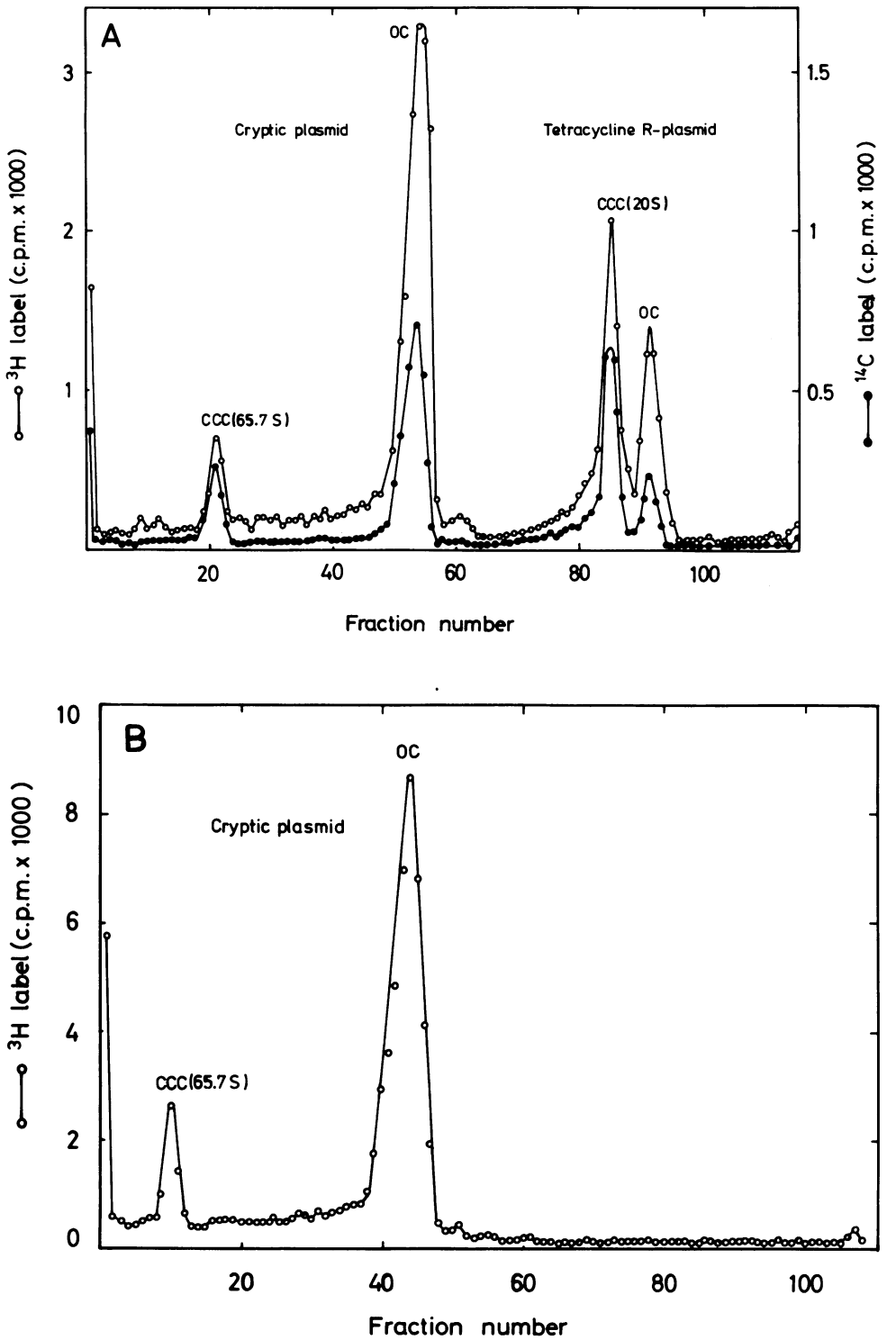


FIG. 1. *Neutral sucrose gradients (15 to 30%; from right to left) of plasmid DNA. Symbols: (A) ○, $^{3}$H-labeled S. aureus FK170; ●, $^{14}$C-labeled S. aureus FK427 [APH(3')-negative variant of FK170]; (B) ○, $^{3}$H-labeled S. aureus FK429 (Tet-susceptible variant of FK170). CCC, Covalently closed circular; OC, open circular.*

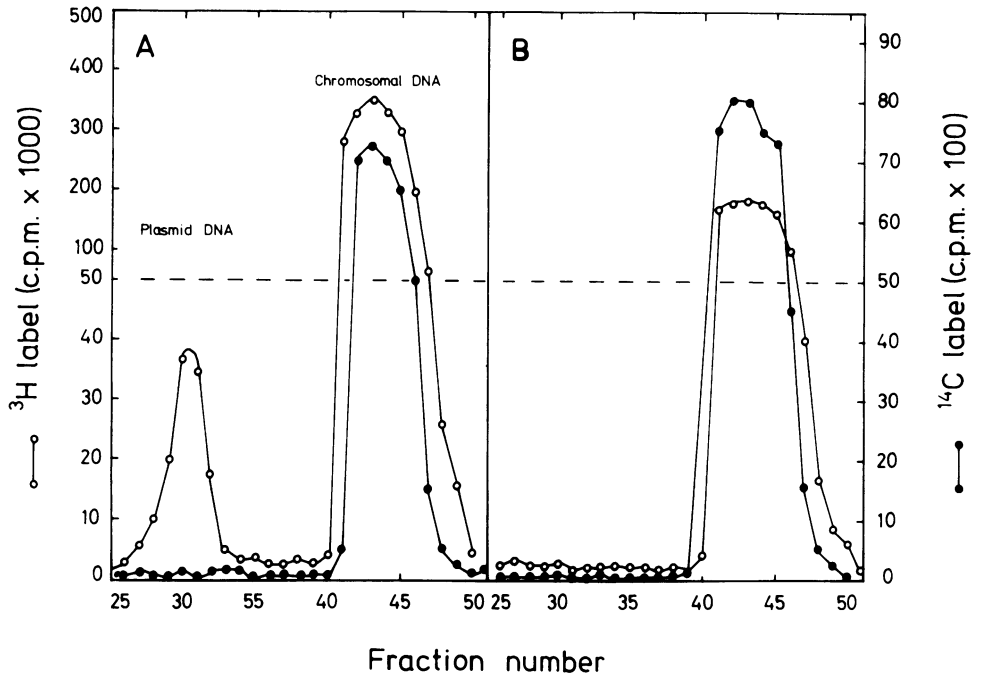


FIG. 2. *Dye-CsCl density gradients of bacterial lysates. Density increases from right to left. Symbols: (A)* ○, *$^3$H-labeled S. aureus RN450 Tet;* ●, *$^{14}$C-labeled S. aureus RN450; (B)* ○, *$^3$H-labeled S. aureus RN450 Kan;* ●, *$^{14}$C-labeled S. aureus RN450.*

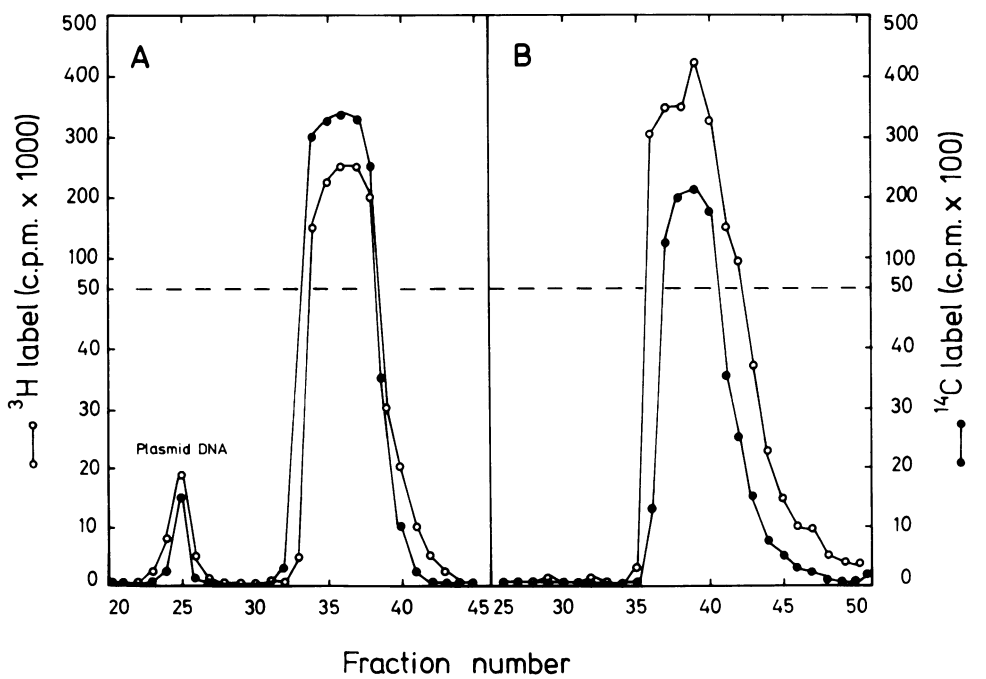


FIG. 3. *Dye-CsCl density gradients of bacterial lysates. Density increases from right to left. Symbols: (A)* ○, *$^3$H-labeled S. aureus 5532;* ●, *$^{14}$C-labeled S. aureus S25 [APH(2″)-negative variant of 5532]; (B)* ○, *$^3$H-labeled S. aureus FK426 (penicillinase-negative variant of 5532);* ●, *$^{14}$C-labeled S. aureus FK475 (penicillinase-negative variant of S25).*

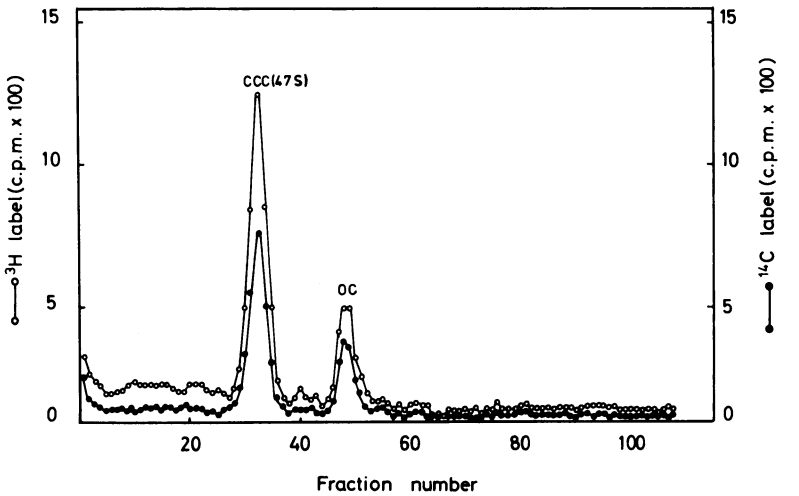


FIG. 4. *Neutral sucrose gradients (15 to 30%; from right to left) of plasmid DNA. Symbols: ○, $^3$H-labeled S. aureus Ps53; ●, $^{14}$C-labeled S. aureus Ps53 Gen (gentamicin-resistant transductant of Ps53). CCC, Covalently closed circular; OC, open circular.*

mycin-resistant tranductants, evidence of chromosomal location of the marker for production of APH(3') is strong. The small plasmid of FK170 has been found to code for resistance to tetracycline. The biological functions of the larger plasmid are unknown. It seems unlikely to us that this cryptic plasmid has anything to do with resistance to penicillin or erythromycin, because these phenotypic traits are remarkably stable in strain FK170. It is known that large plasmids in staphylococci are generally very unstable (15). The failure to eliminate the cryptic plasmid by prolonged aging of a culture for more than 3 months points to the possibility that this plasmid might contain genetic information vital for FK170.

Chromosomal location of the markers for production of APH(2") and AAC(6') in *S. aureus* 5532 is likely, since the only plasmid found in this strain was shown exclusively to specify the production of penicillinase. Cotransfer of the determinants of APH(2") and AAC(6') to Ps53 (27) suggested the close location of the genes on the chromosome. Recently, attempts to separate APH(2") from AAC(6') in enzymatic extracts of *S. aureus* R Palm by several techniques were not successful (18). It was suggested, therefore, that one and the same enzyme could phosphorylate as well as aceytylate aminocyclitols. On the other hand, Dowding (7) was able to separate phosphorylating from acetylating activity by polyacrylamide gel electrophoresis in his strain. The genetic experiments conducted with strain 5532 do not resolve the situation in this culture, since they are consistent with both hypotheses.

It is now becoming more and more apparent that bacterial replicons are able to interact and recombine in a variety of ways that may not involve conventional base homologies. The occurrence of apparently nonhomologous recombinational events in *S. aureus* has been observed repeatedly, especially in the case of antibiotic resistance determinants. Such events, involving, among other things, insertion of plasmid fragments into the chromosome, transfer of DNA sequences from the chromosome into plasmids (19, 22, 24), or the stable integration of entire plasmids into the chromosome (26) can be most easily interpreted by translocatable elements or insertion sequences. Indeed, a clear-cut example of genetic translocation in *S. aureus* involving the 5.2-kilobase transposon Tn*551* has recently been described (24). It could be that illegitimate recombination of genes, plasmids, or plasmid fragments, coding for resistance to aminocyclitols, has also occurred in the *S. aureus* strains FK170 and 5532. Further experiments to demonstrate translocation of aminocyclitol resistance in these strains are now being performed.

## LITERATURE CITED

1. **Asheshov, E. H.** 1966. Chromosomal location of the genetic elements controlling penicillinase production in a strain of *Staphylococcus aureus*. Nature (London) **210**:804-806.
2. **Brown, D. F. J., F. H. Kayser, and J. Biber.** 1976. Gentamicin resistance in *Staphylococcus aureus*. Lancet **ii**:419.
3. **Chopra, I., P. Bennett, and R. W. Lacey.** 1973. A variety of staphylococcal plasmids present as multiple copies. J. Gen. Microbiol. **79**:343-345.
4. **Clewell, D. B., and D. R. Helinski.** 1970. Properties of a supercoiled deoxyribonucleic acid-protein relaxation complex and strand specificity of the relaxation event. Biochemistry **9**:4428-4440.
5. **Clowes, R. C.** 1972. Molecular structure of bacterial plasmids. Bacteriol. Rev. **36**:361-405.

6. **Davies, J., M. Brzezinska, and R. Benveniste.** 1971. R-factors: biochemical mechanisms of resistance to aminoglycoside antibiotics. Ann. N.Y. Acad. Sci. **182**:226–233.
7. **Dowding, J. E.** 1977. Mechanisms of gentamicin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **11**:47–50.
8. **Eckhardt, Th.** 1978. A rapid method for the identification of plasmid deoxyribonucleic acid in bacteria. Plasmid **1**: 584–588.
9. **Ericson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing. Acta Pathol. Microbiol. Scand Sect. B. Suppl. 217.
10. **Falkow, S., L. S. Tompkins, R. P. Silver, P. Guerry, and D. J. LeBlanc.** 1971. The replication of R-factor DNA in *Escherichia coli* K-12 following conjugation. Ann. N.Y. Acad. Sci. **182**:153–171.
11. **Hudson, B., D. A. Clayton, and J. Vinograd.** 1968. Complex mitochondrial DNA. Cold Spring Harbor Symp. Quant. Biol. **33**:435–442.
12. **Jaffe, H. W., H. M. Sweeney, C. Nathan, R. A. Weinstein, S. A. Kabins, and S. Cohen.** 1980. Identity and interspecific transfer of gentamicin-resistance plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Infect. Dis. **141**:738–747.
13. **Kayser, F. H., M. Devaud, and J. Biber.** 1976. Aminoglycoside 3′-phosphotransferase IV: a new type of aminoglycoside-phosphorylating enzyme found in staphylococci. Microbios Lett. **3**:63–68.
14. **Kayser, F. H., J. Wuest, and P. Corrodi.** 1972. Transduction and elimination of resistance determinants in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **2**:217–223.
15. **Kayser, F. H., J. Wuest, and P. Santanam.** 1976. Genetic and molecular characterization of resistance determinants in methicillin-resistant *Staphylococcus aureus*. J. Med. Microbiol. **9**:137–148.
16. **Lacey, R. W., and J. Grinsted.** 1973. Genetic analysis of methicillin-resistant strains of *Staphylococcus aureus*: evidence for their evolution from a single clone. J. Med. Microbiol. **6**:511–526.
17. **Le Goffic, F., A. Martel, M. L. Capman, B. Baca, P. Goebel, H. Chardon, C. J. Soussy, J. Duval, and D. H. Bouanchaud.** 1976. New plasmid-mediated nucleotidylation of aminoglycoside antibiotics in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **10**:258–264.
18. **Le Goffic, F., A. Martel, N. Moreau, M. L. Capman, C. J. Soussy, and J. Duval.** 1977. 2″-O-Phosphorylation of gentamicin components by a *Staphylococcus aureus* strain carrying a plasmid. Antimicrob. Agents Chemother. **12**:26–30.
19. **Lindberg, M., and R. P. Novick.** 1973. Plasmid-specific transformation in *Staphylococcus aureus*. J. Bacteriol **115**:139–145.
20. **Matsen, J. M., and A. L. Barry.** 1974. Susceptibility testing: diffusion test procedures, p. 418–427. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
21. **Novick, R. P.** 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. J. Gen. Microbiol. **33**:121–136.
22. **Novick, R. P.** 1967. Penicillinase plasmids of *Staphylococcus aureus*. Fed. Proc. **26**:29–38.
23. **Novick, R. P., and D. Bouanchaud.** 1971. Extrachromosomal nature of drug resistance in *Staphylococcus aureus*. Ann. N. Y. Acad. Sci. **182**:279–294.
24. **Novick, R. P., I. Edelman, M. D. Schwesinger, A. D. Gruss, E. C. Swanson, and P. A. Pattee.** 1979. Genetic translocation in *Staphylococcus aureus*. Proc. Nat. Acad. Sci. U.S.A. **76**:400–404.
25. **Novick, R. P., and M. H. Richmond.** 1965. Nature and interactions of the genetic elements governing penicillinase synthesis in *Staphylococcus aureus*. J. Bacteriol. **90**:467–480.
26. **Pattee, P. A., N. E. Thompson, D. Haubrich, and R. P. Novick.** 1977. Chromosomal map locations of integrated plasmids and related elements in *Staphylococcus aureus*. Plasmid **1**:38–51.
27. **Porthouse, A., D. F. J. Brown, R. G. Smith, and T. Rogers.** 1976. Gentamicin resistance in *Staphylococcus aureus*. Lancet **i**:20–21.
28. **Poston, S. M.** 1966. Cellular location of the genes controlling penicillinase production and resistance to streptomycin and tetracycline in a strain of *Staphylococcus aureus*. Nature (London) **210**:802–804.
29. **Rosendorf, L. L., and F. H. Kayser.** 1974. Transduction and plasmid deoxyribonucleic acid analysis in a multiply antibiotic-resistant strain of *Staphylococcus epidermis*. J. Bacteriol. **120**:679–686.
30. **Santanam, P., and F. H. Kayser.** 1976. Tobramycin adenylyltransferase: a new aminoglycoside inactivating enzyme from *Staphylococcus epidermidis*. J. Infect. Dis. **134**:S33–S39.
31. **Soussy, C. J., D. H. Bouanchaud, J. Fouace, A. Dublanchet, and J. Duval.** 1975. A gentamicin resistance plasmid in *Staphylococcus aureus*. Ann. Microbiol. (Paris) **126B**:91–94.
32. **Wood, D. O., M. J. Carter, and G. K. Best.** 1977. Plasmid-mediated resistance to gentamicin in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **12**: 513–517.