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Transferable Plasmids Mediating Multiple-Antibiotic Resistance in *Streptococcus faecalis* subsp. *liquefaciens*

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In strain HK187 (*Streptococcus faecalis* subsp. *liquefaciens*) four plasmids were isolated. The molecular weights, as analyzed in neutral sucrose gradients and by electron microscopy, were found to be about 36×10^6, 31×10^6, 26×10^6, and 4×10^6. Plasmid 3, also designated pFK14, with a molecular weight of 26×10^6, was found to be responsible for resistance to chloramphenicol and erythromycin and also for high-level resistance to streptomycin and lincomycin. In mixed cultures these four resistances could be transferred with high frequency to a plasmid-free recipient, *S. faecalis* strain JH2-2.

Recent investigations have shown that there exist a large number of plasmids in enterococci (4, 6, 21, 27). Many of these plasmids carry resistance to antibiotics, and some plasmids have been shown to transfer between enterococcal strains (13, 24). Transduction, well known for group A streptococci (19), has not been demonstrated within enterococci and also failed with strains we tested (20), but there seems to be a mechanism of transfer akin to conjugation (14).

We investigated multiple-antibiotic resistance in a strain of *Streptococcus faecalis* subsp. *liquefaciens* isolated from a clinical specimen. Elimination of resistance to erythromycin (Em), chloramphenicol (Cm), streptomycin (Sm), and lincomycin (Lm) suggested that the genetic markers of these resistances might be linked with plasmids.

This suggestion was confirmed by the isolation of plasmid deoxyribonucleic acid (DNA) from the wild-type strain and antibiotic-susceptible variants and by analysis of the DNA in neutral sucrose gradients and electron microscopy. Multiple resistance could be transferred to appropriate receptor strains by mixed cultivation of donor and recipient.

The results of our examinations will be reported in detail in this paper.

MATERIALS AND METHODS

Media and reagents. Strains were grown in either brain heart infusion (BHI) broth or BHI agar (Baltimore Biological Laboratory [BBL], Cockeysville, Md.).

Mueller Hinton broth and Mueller Hinton agar (BBL) were used for antibiotic susceptibility testing. All radioisotopes were purchased from the Radiochemical Center, Amersham, England. Sigma Chemical Co., St. Louis, Mo. provided the following reagents: bovine pancreatic ribonuclease (type I-A) and deoxyribonuclease I; lysozyme from egg white (grade I); cytochrome *c* from horse heart (type VI).

Sephadex G25-fine was bought from Pharmacia Fine Chemicals, Uppsala, Sweden. Rifampin (Rimactane) and Sarkosyl NL97 were gifts from Ciba-Geigy, Basel, Switzerland. All other chemicals and antibiotics were purchased from standard commercial sources.

Bacterial strains. *S. faecalis* subsp. *liquefaciens* strain HK187 was isolated from a clinical specimen. The species was identified both by the Sherman criteria and by biochemical tests (8), such as hyrolysis of gelatin and acid production from sorbitol, mannitol, and sucrose. Lancefield group D was verified by the Fuller serological test (9). HK187 is resistant to Em, Cm, tetracycline, and gentamicin and exhibits high degrees of resistance to Sm and Lm (see Table 2). The plasmid-free *S. faecalis* strain, JH2-2, has been described previously by Jacob and Hobbs (14). The strain is susceptible to Em, Cm, and tetracycline, shows a low degree of resistance to Sm and Lm (see Table 3), and is resistant to more than 50 μg of rifampin per ml. R. Novick provided *Staphylococcus aureus* RN11, carrying the penicillinase plasmid 258.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was performed by the standardized agar diffusion method of Bauer et al. (2), using Mueller Hinton agar plus 5% (vol/vol) human blood from the blood bank. When necessary, the minimal inhibitory concentrations (MICs) of antimicrobial agents were determined by the broth dilution method in MH broth, as proposed by Ericsson and Sherris (7).

Antibiotic resistance "curing" procedure. An overnight broth culture was diluted $1{:}10^5$ into BHI broth containing a range of concentrations of either ethidium bromide (EB), acriflavine, or rifampin.

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After 18 h of incubation, cultures that contained a concentration of agent just subinhibitory to cell growth were plated onto BHI agar. Control cultures, grown in BHI broth with no chemical agent added, were also plated. Treated and nontreated cultures were screened for antibiotic-susceptible variants by the replica plating method on plates containing 12.5 μg of Tc per ml and 25 μ of Cm or Em per ml. After purification, antibiograms for variants were determined by the agar diffusion method.

Labeling and lysis of cells. Cells, grown overnight in BHI broth, were diluted to approximately 10^8 ml/ in fresh BHI broth containing either 12.5 μCi of [*methyl*-^{3}H]thymidine (19 Ci/mmol) per ml or 3.3 μCi of [2-^{14}C]thymidine (59 mCi/mmol) per ml in 8 ml of medium. The cultures were grown to about 10^9 cells/ml in a water bath shaker.

Lysis of cells by method 1. The following lysis procedure and dye-buoyant density centrifugation have been described previously (14). An 8-ml radiolabeled culture was washed twice in TES buffer [0.05 M NaCl, 0.05 M tris(hydroxymethyl)aminomethane, 0.005 M disodium ethylenediaminetetraacetate, pH 8] at 4°C. The cell pellet was suspended in 0.25 ml of TES buffer containing 100 mg of sucrose per ml. This was followed by the addition of 0.25 ml of the same buffer containing 2 mg of lysozyme per ml, 1 mg of ribonuclease per ml, and 100 mg of sucrose per ml. The suspension was incubated for 30 min at 37°C and cooled for 5 min in ice. The cells were lysed by the addition of 0.25 ml of Sarkosyl (2.4% in water) and gently mixed. After the addition of 0.5 ml of TES buffer at room temperature, the lysate was slowly drawn in and out of a 1-ml pipette 20 times (sheared lysate).

Lysis of cells by method 2. A second method for cell lysis was as follows. A 50-ml amount of radiolabeled culture was washed as described. The cell pellet was resuspended in 9 ml of TES buffer followed by 1 ml of lysozyme (4 mg/ml in TES buffer). The suspension was incubated for 60 min at 37°C. The cells were lysed by the addition of 0.2 ml of Sarkosyl (25% in water) and gently mixed.

CsCl-EB centrifugation. Sheared lysate (1.15 ml) prepared by method 1 was added to a mixture of CsCl (4.609 g) dissolved in 3 ml of TES buffer and 1 ml of EB (4.8 mg/ml) in TES buffer. Sheared lysate from method 2 (1.75 ml) was added to 4.609 g of CsCl dissolved in 3 ml of TES and 0.25 ml of EB solution (10 mg/ml in TES). The samples were then centrifuged for 60 h at 42,000 rpm in a Ti 50 rotor at 20°C in a Beckman L5-65 ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Tubes were punctured, and 4-drop fractions were collected in tubes with an apparatus designed by Tan (26). A 10-μl sample of each fraction was pipetted onto a glass fiber disk (GF/B; Whatman, Inc., Clifton, N.J.), dried, washed, and counted for radioactivity. The plasmid fractions were pooled, and EB was removed by extraction with an equal volume of isopropanol. After dialysis against TES buffer (pH 8) or desalting with a Sephadex G25-fine column (prepared with TES buffer, pH 8, in a Pasteur pipette), the DNA samples were stored frozen (−20°C).

Sucrose gradient velocity centrifugation. Linear 5 to 20% neutral gradients of sucrose in TES buffer, pH 7.5, were prepared both with a gradient-mixing apparatus and with the deep-freezing method (1). DNA samples of between 30 and 100 μl were layered onto a gradient. Centrifugation was performed in an SW 50.1 rotor (6 by 5 ml), using a Beckman L5-65 ultracentrifuge, at 35,000 rpm and 20°C. Fractions of 0.1 ml were collected directly on glass fiber disks, dried, washed, and counted for radioactivity (see above). Molecular weights were calculated as previously described (1).

Electron microscropy. The supercoiled circular (covalently closed circular [CCC]) DNA molecules isolated by the dye-buoyant density gradient procedure were dialyzed against TES buffer, pH 8, and allowed to spontaneously convert to open circular (OC) molecules by storage for about 2 weeks at 4°C.

The spreading of plasmid DNA was performed by the Kleinschmidt technique (17). DNA (100 μl in TES buffer, pH 7.5) was added to 2 μl of a cytochrome *c* solution containing 4 M tris(hydroxymethyl)aminomethane-hydrochloride, 0.1 M ethylenediaminetetraacetate, and 5 mg of cytochrome *c* per ml, pH 8.2, and spread on a 0.3 M ammonium acetate hypophase, pH 7.5. Carbon-coated grids (400 mesh) were used to pick up the film. The grids were then washed with water for a few minutes, dehydrated in ethanol, and air dried. The grids were then rotary shadowed at an angle of 7° with carbon-platinum (1,100 Hz, as measured on a quartz thin-crystal monitor from Balzers AG [Balzers, Liechtenstein]).

Transfer of antibiotic resistance to a plasmid-free recipient. Strains were grown overnight in BHI broth. After dilution to 10^8 cells/ml with fresh broth, the donor (strain HK187) and recipient (strain JH2-2) cultures were mixed in the ratio 1:10 and incubated at 37°C. After an appropriate time, dilutions of the mixture were plated on BHI agar containing antibiotics to select for the recipient, the donor, and the recipient containing a plasmid.

Radioisotope counting. Glass fiber disks containing samples were dried and then washed with cold 5% (wt/vol) trichloroacetic acid followed by ethanol. After drying they were counted in 10 ml of scintillation fluid [3.6 g of 2,5-diphenyloxazole, 90 mg of 1,4-bis(5-phenyloxazolyl)benzene per liter, in toluene] in plastic scintillation vials. Counting was performed in a Packard Tri-Carb liquid scintillation spectrophotometer (Packard Instrument Co., Downers Grove, Ill.).

RESULTS

Elimination of antibiotic resistances in strain HK187. Intercalating agents such as EB or acriflavin are known to eliminate plasmids from host cells, probably by selective inhibition of plasmid DNA replication (22, 25) and/or due to the organization (11) and conformation (10) of plasmids within the cell being different from those of the chromosome. Plasmid replication is blocked when intercalating agents are used at

subinhibitory concentrations to bacterial growth (curing). In strain HK187, spontaneous loss of resistance to Cm and Em was increased under the effect of intercalating agents (Table 1). Most of the susceptible colonies had concomitantly lost resistance to Cm and Em. Six antibiotic-susceptible variants tested exhibited a decrease in resistance to Sm and Lm (Table 2). There were only two colonies found that had not lost the Cm and Em resistances together. The first, selected in acriflavin, had lost Em resistance (Cm^r/Em^s) and was designated S1; the second, selected in a control experiment (i.e., without the addition of an intercalating dye), had lost Cm resistance (Cm^r/Em^s) and was designated S2. Whereas strain S2 showed no change in resistance to Em, Sm, and Lm compared with HK187, the MIC of Lm against strain S1 was also decreased. The MIC of Sm was not altered (Table 2).

TABLE 1. *Instability of antibiotic resistance markers in S. faecalis subsp. liquefaciens strain HK187*

Treatment	Colonies	
	No. tested/expt	Susceptible to Cm and Em (%)
None	2,270	0.22
EB (10×10^{-6} M)	4,050	6.0
Acriflavin (3.125 μg/ml)	600	3.3
Rifampin (50 μg/ml)	1,000	1.0
Storage (20°C for 5 mo)	1,900	86.8

In all of the elimination experiments, no colonies could be detected that had lost resistance to tetracycline and/or gentamicin.

Centrifugation results. Spontaneous loss of resistance to Cm and Em and the increase of loss under intercalating agents led to the assumption that resistance to these antibiotics may be located on plasmids. We therefore compared a radiolabeled lysate from each of six antibiotic-sensitive variants with a differentially radiolabeled lysate of strain HK187. A plasmid fraction could be detected in all six variants, as Fig. 1 shows for the variant strain HK194. If resistance to Cm and Em is plasmid borne, it is probable that the resistance genes are on the same plasmid, since these resistances, with very few exceptions (e.g., S1 and S2), were always lost together. Two explanations are possible for the retention of plasmid DNA in the variants. Either there is more than one plasmid in strain HK187 or the genes for resistance to Cm and Em are occasionally de-

TABLE 2. *MICs of wild-type strain HK187 and antibiotic-susceptible variants*

Strain	MIC of antibiotic (μg/ml)[a]					
	Em	Cm	Sm	Lm	Gm	Tc
HK187	100	100	10,000	1,000	100	50
HK194[b]	1.56	6.25	625	50	100	25
S1	1.56	100	10,000	50	100	25
S2	100	6.25	10,000	1,000	100	50

[a] Gm, Gentamicin; Tc, tetracycline.
[b] Five further variants tested all had the same MIC.

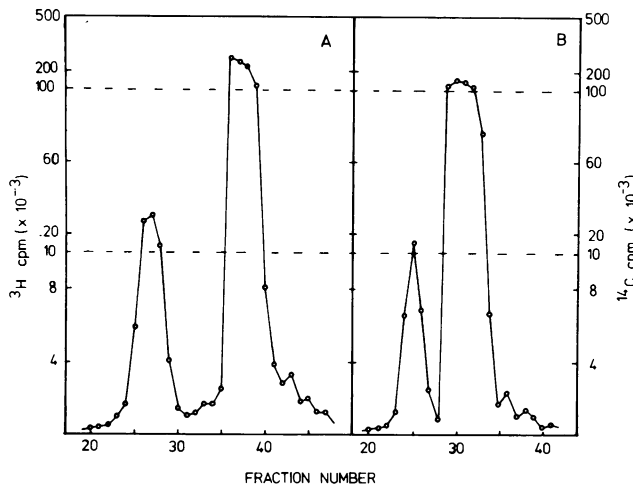


FIG. 1. *CsCl-EB equilibrum density gradient analysis of DNA from strains HK187 and HK194. Radiolabeled cultures were lysed by method 2 (see the text), and 1.75 ml of each lysate was centrifuged to equilibrum in a CsCl-EB density gradient. Fractions (4 drops) were analyzed for radioactivity, and the results were plotted as shown. Note change of scale at 10×10^3 and 100×10^3 cpm. (A) ^{3}H-labeled DNA of strain HK187; (B) ^{14}C-labeled DNA of strain HK194.*

leted from a single plasmid. That the former explanation is correct was verified by neutral sucrose gradient analysis of plasmid DNA of strain HK187 (Fig. 2). From the sedimentation pattern, we suggest that there are four plasmids in strain HK187. The fast-sedimenting DNA in fractions 4-5, 7-8, and 10-11 and the slower-sedimenting DNA of fractions 32-33 represented CCC molecules of plasmids 1, 2, 3, and 4, respectively. The DNA bands in fractions 18-24 and 36-37, on the other hand, were due to OC molecules of plasmids 1, 2, and 3 (fractions 18-24) and plasmid 4 (fractions 36-37). The suggestion was confirmed by the results obtained from deoxyribonuclease-treated plasmid DNA of strain HK194 (see Fig. 3). Limited deoxyribonuclease treatment led to a diminution in the size of CCC peaks and a corresponding increase in the size of OC peaks. Further confirmation was gained by taking electron micrographs from the different DNA fractions, which showed that fractions 4-11 and 32-33 indeed contained mainly CCC molecules, whereas fractions 18-24 were composed of OC DNA forms.

As plasmid 3 (pFK14) was absent in strain HK194, we suggest that this plasmid mediates resistance to Cm and Em. In addition, we suggest that the high level of resistance to Sm and Lm is located on plasmid 3.

Molecular weight determination of CCC plasmid DNA, using neutral sucrose gradients. For molecular weight determination, we compared our plasmids with a reference DNA in a neutral sucrose gradient (Fig. 4). The penicillin resistance plasmid, 258, from *S. aureus* RN11 has a contour length of 9.4 μm (23), which corresponds to a molecular weight of 19.5×10^6, assuming that 1 μm corresponds to 2.07×10^6 (18). The sedimentation coefficient of the CCC form of the reference plasmid then may be calculated by using the empirical formula: $s_{20,w} = 7.44 + 0.00243 \times MW^{0.58}$ (12) (where $s_{20,w}$ is the sedimentation coefficient corrected to water at 20°C, and MW is the molecular weight). By comparing the distances of each unknown plasmid peak from the top of a linear gradient with the distance of the reference peak, the *s* values of the unknowns were determined (3, 5). Using these *s* values (see Fig 4) and the formula mentioned above, the approximate molecular weights of plasmids 1 to 4 were calculated to be 36×10^6, 31×10^6, 26×10^6, and 4×10^6, respectively.

Plasmid DNA from strain S1 cosedimented with plasmid DNA from strain HK187. In contrast, in strain S2 the CCC form of plasmid 3 sedimented more slowly than that in HK187 (sedimentation coefficient = 53S), suggesting that the plasmid had suffered a deletion of

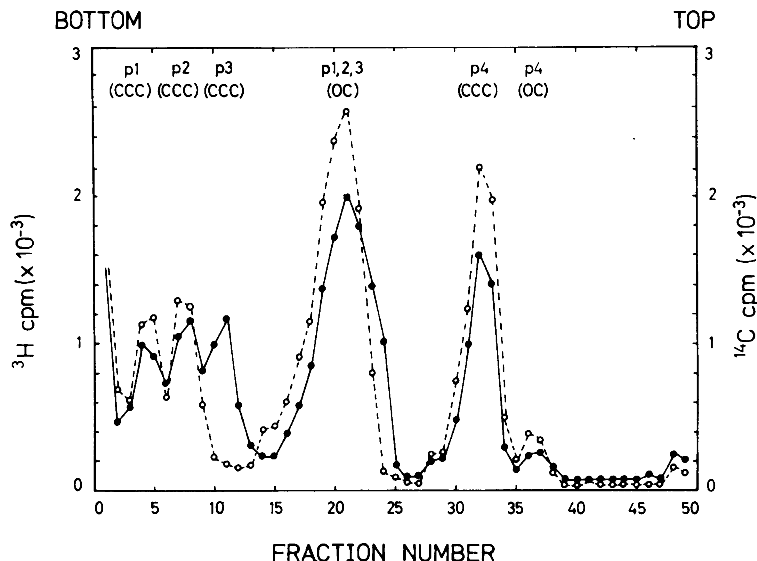


FIG. 2. *Neutral sucrose gradient analysis of plasmid (p) DNA from strains HK187 and HK194. Plasmid DNA was isolated from separate lysate preparations by using a CsCl-EB density gradient. After dialysis, 50 μl of ^{3}H-labeled strain HK187 plasmid DNA was layered together with 25 μl of ^{14}C-labeled strain HK194 plasmid DNA onto a 5 to 20% sucrose gradient and centrifuged at 35,000 rpm at 20°C for 115 min. Fractions (0.1 ml) were collected and counted for radioactivity. Sedimentation was from right to left. Symbols: (●) ^{3}H, (○) ^{14}C.*

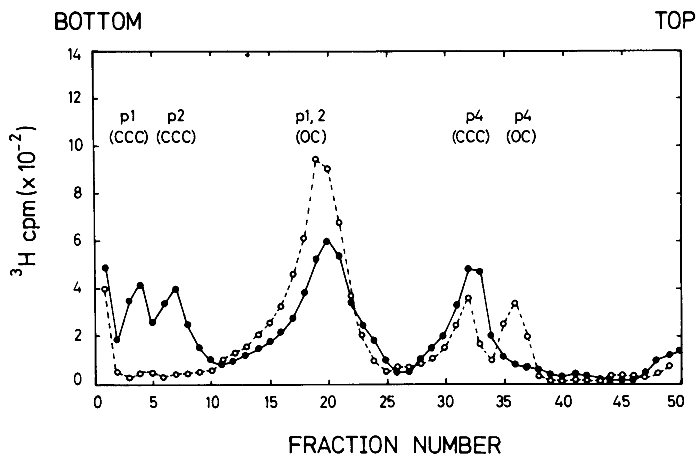


FIG. 3. *Neutral sucrose gradient analysis of deoxyribonuclease (DNase)-treated plasmid (p) DNA from strain HK194. A 50-μl amount of ^{3}H-labeled HK194 plasmid DNA was treated with an equal volume of DNase I in 25 mM $MgSO_4$ (final concentration of DNase I, 5×10^{-5} M) for 8 min at 25°C. To stop DNase I activity, 50 μl of disodium ethylenediaminetetraacetate (60 mM, pH 8) was then added. A sample of 50 μl was layered onto a 5 to 20% sucrose gradient and centrifuged at 35,000 rpm at 20°C for 115 min. As a control, another sample of HK194 plasmid DNA was treated in the same manner, but DNase I was replaced by 25 mM $MgSO_4$. Fractions of 0.1 ml of each gradient were collected and counted separately for radioactivity. The two sets of data were plotted on the same figure for ease of comparison. Symbols: (○) DNase-treated ^{3}H-labeled HK194 plasmid DNA, (●) untreated ^{3}H-labeled HK194 plasmid DNA.*

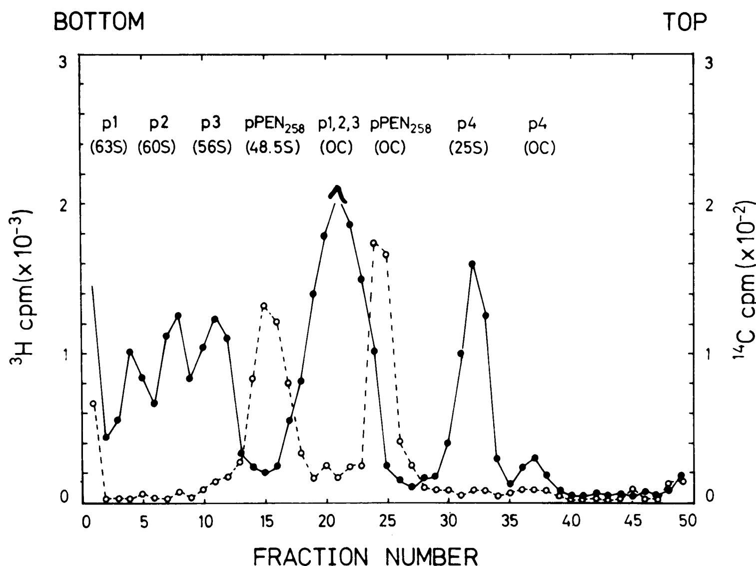


FIG. 4. *Neutral sucrose gradient analysis of plasmid (p) DNA from strain HK187, with RN11 as reference. Plasmid DNA was isolated from S. faecalis HK187 as described in the text and from S. aureus RN11 as described elsewhere (16). After dialysis of plasmid DNA, 50 μl of ^{3}H-labeled strain HK187 DNA was layered together with 50 μl of ^{14}C-labeled strain RN11 DNA onto a 5 to 20% sucrose gradient and centrifuged at 35,000 rpm at 20°C for 115 min. Fractions (0.1 ml) were collected and counted for radioactivity. Sedimentation was from right to left. PEN_{258}, Penicillin resistance plasmid of strain RN11. Symbols: (●) ^{3}H, (○) ^{14}C.*

about 3×10^6 daltons of DNA. No difference in sedimentation could be seen in plasmids 1, 2, and 4 (data not shown).

Electron microscopy. Plasmid fractions from CsCl-EB buoyant density gradients were stored at 4°C to allow spontaneous conversion of the CCC plasmid form to the OC form. DNA was spread according to the Kleinschmidt tech-

nique (17) when photographs were to be taken for measuring the contour length. OC forms of plasmids were photographed together with a test grid (0.883 μm) and enlarged on paper. With the overall enlargement from the test grid, the average contour length and the standard deviation of plasmid 1 was 18.2 ± 0.24 μm (11 molecules measured; range, 17.6 to 18.8 μm). The average length of plasmid 2 was 15.0 ± 0.39 μm (13 molecules measured; range, 14.6 to 15.6 μm), that of plasmid 3 was 12.93 ± 0.29 μm (17 molecules measured; range, 12.3 to 13.5 μm), and that of plasmid 4 was 1.67 ± 0.04 μm (14 molecules measured; range, 1.6 to 1.9 μm). Using the assumption that 1 μm corresponds to 2.07×10^6 (18), the molecular weights for plasmids 1 to 4 were calculated to be $(37.75 \pm 0.5) \times 10^6$, $(31.1 \pm 0.8) \times 10^6$, $(26.8 \pm 0.6) \times 10^6$, and $(3.46 \pm 0.08) \times 10^6$. These molecular weights are in good agreement with those calculated by sedimentation analysis. Figure 5 shows OC forms of plasmids 1 to 4.

Transfer experiments. To prove that resistances to Em, Cm, Sm, and Lm are located on plasmid 3, we tried to transfer antibiotic resistance from strain HK187 to the plasmid-free recipient, *S. faecalis* JH2-2 (14). Kinetics of acquisition of transferred resistance by strain JH2-2, when selection was for Cm resistance transfer, are shown in Fig. 6. A similar result

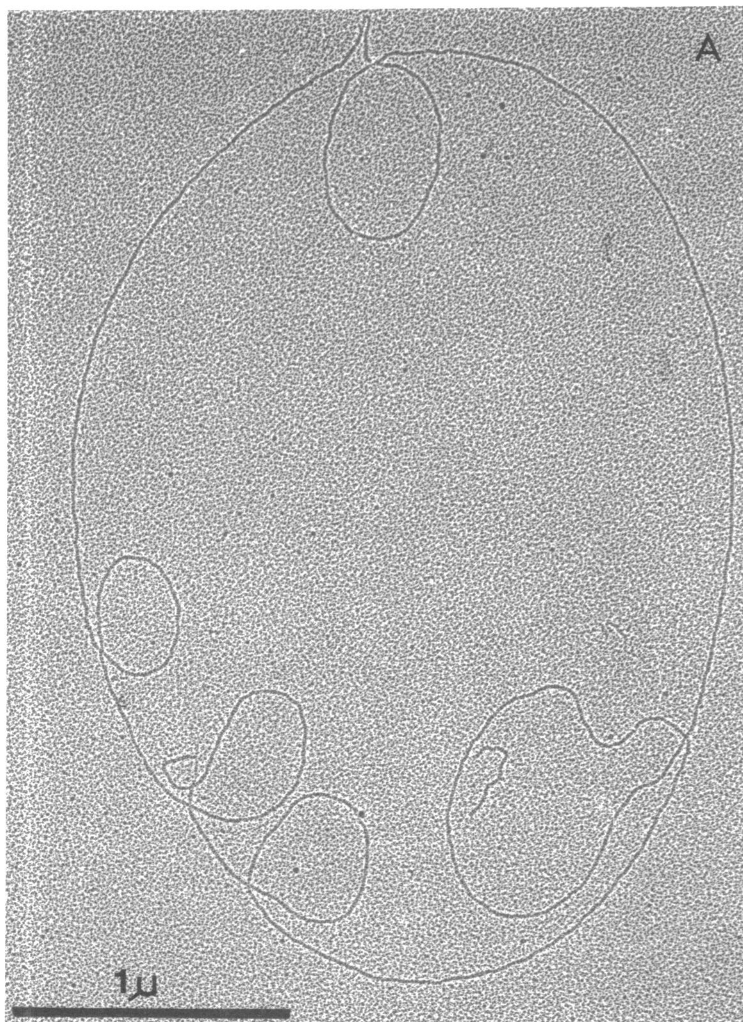
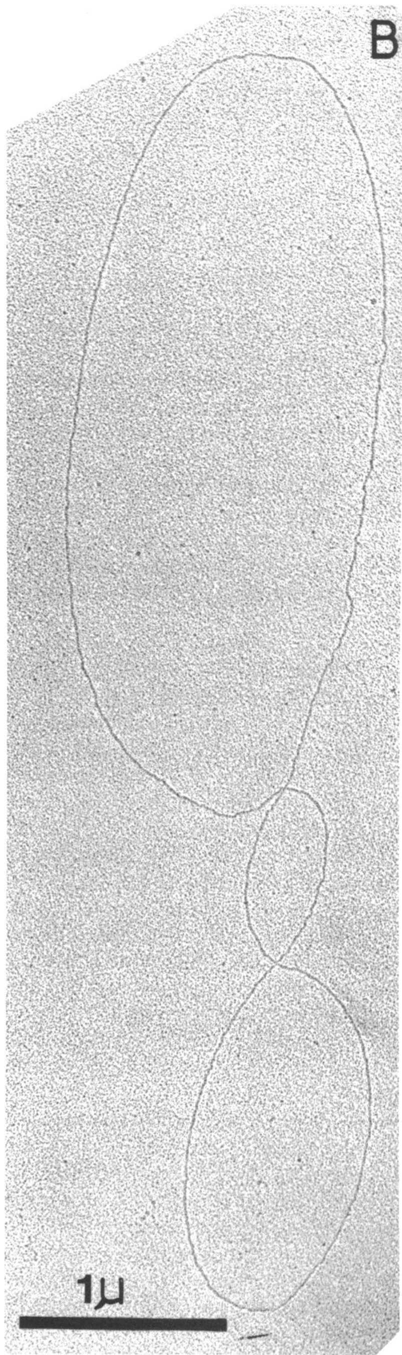
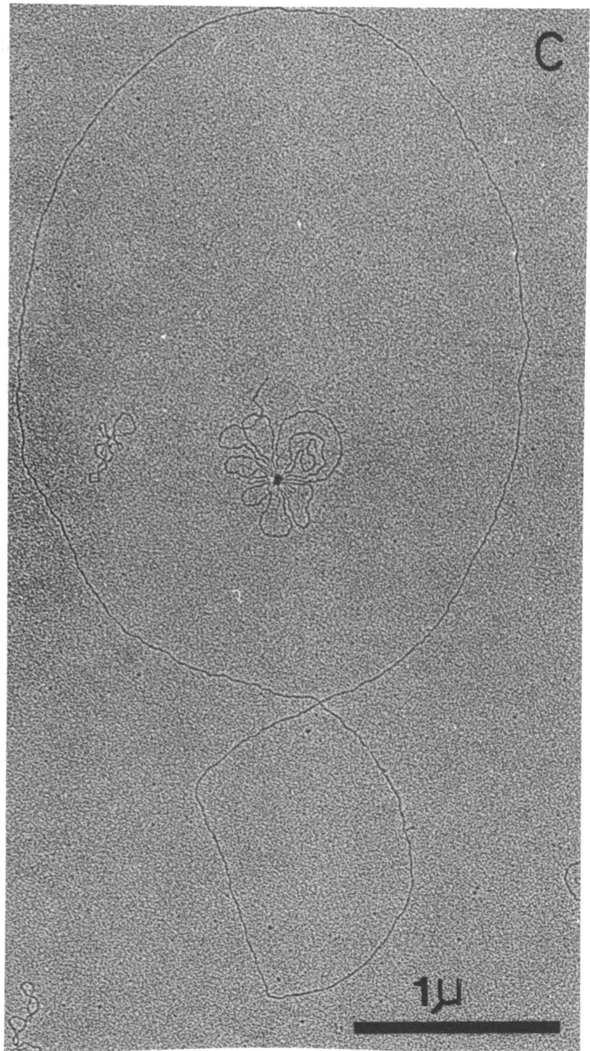


FIG. 5. *Electron micrographs of plasmids isolated from strain HK187. OC plasmid DNA molecules were shadowed with platinum-carbon, and photographs were taken in a Philips EM 201 electron microscope. (A) Plasmid 1, (B) plasmid 2, (C) plasmid 3, (D) plasmid 4.*





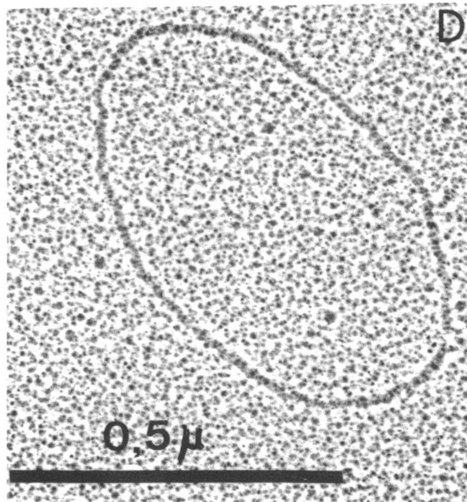


FIG. 5. B–D

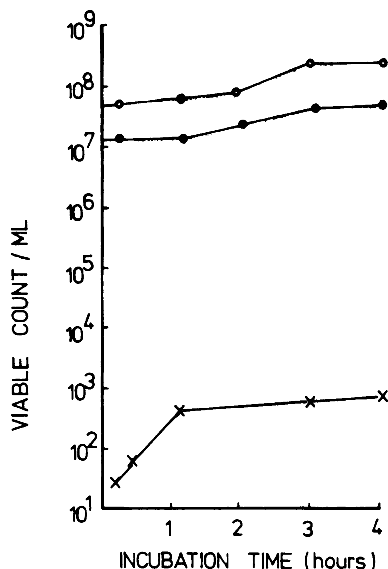


FIG. 6. *Kinetics of acquisition of transferable resistance by strain JH2-2. A mixed culture of strains HK187 and JH2-2 was incubated at 37°C; at the times indicated samples were withdrawn, and appropriate dilutions were plated onto BHI agar to select for donors (●), recipients (○), and recipients having acquired antibiotic resistance (×). Selection was on 25 μg of Cm per ml for donors, on 50 μg of rifampin per ml for recipients, and on 25 μg of Cm and 50 μg of rifampin per ml for recipients having acquired antibiotic resistance.*

was seen when selection was made for Em resistance transfer (data not shown).

Eighteen transconjugants selected for transfer of resistance to Cm or Em were examined for transfer of other resistances by replica plating. It was found that there was always cotransfer of resistance to Cm or Em, accompanied by an increase in resistance to Lm and Sm. MICs of antibiotics for the recipient, JH2-2/187, are shown in Table 3. The mechanism of transfer was not further examined.

Neutral sucrose gradient analysis of CCC DNA isolated from 18 antibiotic-resistant recipient colonies always showed the presence of plasmids 2 and 3. Co transfer of plasmid 1 was detected in 1 out of 18 strains, whereas plasmid 4 was cotransferred in 4 of the clones examined.

DISCUSSION

Resistance to antimicrobial agents is frequently encountered among enterococci. At the Institute of Medical Microbiology in Zurich, more than 70% of the enterococcal strains isolated from patient materials in 1976 exhibited resistance to the tetracyclines, and up to 30% of the cultures exhibited resistance to Cm and Em (15). Many of the strains showed resistance to 2 or more antimicrobial agents. One explanation for the frequency of multiple-antibiotic resistance in enterococci could be the occurrence of plasmids carrying resistance determinants and a mechanism of transfer of such plasmids between enterococcal strains. Recent investigations have shown that resistance plasmids exist in enterococci (4, 6, 21, 27) and that there is a mechanism of transfer of such plasmids similar to conjugation (14).

TABLE 3. *MICs of donor strain HK187, recipient strain JH2-2, and recipient strain having acquired resistance JH2-2/187*

Strain	MIC of antibiotic (μg/ml)			
	Sm	Lm	Cm	Em
JH2-2	312.5	12.5	12.5	0.38
HK187	10,000	1,000	100	100
JH2-2/187	10.000	1,000	100	100

Our results with the multiple-antibiotic-resistant strain HK187 isolated in Zurich confirm these observations. Spontaneous instability of resistance to Em and Cm as well as the increase of instability by intercalating dyes suggest the extrachromosomal location of the resistances. Isolation of plasmid DNA and analysis of the plasmid fraction in a neutral sucrose gradient showed that HK187 contains four plasmids of different molecular sizes. Plasmid 3 was missing in all antibiotic-susceptible variants tested, suggesting the location of the resistance determinants on plasmid 3. The molecular weight of this resistance plasmid was determined by comparing its sedimentation rate with a reference DNA and by measuring plasmid DNA contour length. With both methods, a molecular weight of about 26×10^6 was found.

Loss of resistance to Cm and Em was accompanied by an alteration in the MICs of Lm and Em; the decrease was from 10,000 to 625 μg/ml for Sm and from 1,000 to 50 μg/ml for Lm. We suggest that genes determining high-level resistance to Sm and Lm are also carried by plasmid 3. The residual resistance to these antibiotics in variants that have lost plasmid 3 can be explained by the "natural" resistance of enterococci to the two antimicrobial agents (see also data of strain JH2-2 in Table 3).

Among more than 8,000 colonies examined for loss of resistance, 1,927 were found to have lost resistance to Cm and Em concomitantly (see Table 1). One colony (S1), however, had lost only resistance to Em and exhibited a diminution in resistance to Lm ($Em^s/Cm^r/Sm^r/Lm^s$). Another colony (S2) had exclusively lost resist-

ance to Cm ($Em^r/Cm^s/Sm^r/Lm^r$).

In strain S2, plasmid 3 had decreased in molecular weight by about 3×10^6. The simplest explanation would be that a piece of DNA encoding the Cm resistance marker had been deleted from the plasmid. The plasmid DNA of strain S1, however, showed no change in the molecular weight of plasmid 3 or any other plasmid. We have no data to explain this, but we assume that a mutation to susceptibility in the gene governing resistance to Em and Lm has occurred in this strain.

Very little is known concerning the transfer mechanism of resistance markers in streptococci. Nevertheless, we tried to transfer resistance to Cm and Em to a suitable plasmid-free receptor. All recipients simultaneously acquired resistance to Cm and Em as well as high resistance to Sm and Lm. Analysis of plasmid DNA of resistant recipient strains showed that they always contained plasmids 3 (26×10^6 daltons) and 2 (31×10^6 daltons). Whether plasmid 2 is present by chance in the 18 colonies so far tested or is a helper in transfer is not known and has to be further investigated.

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