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Conjugative R Plasmids in Group C and G Streptococci

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Two streptococcal isolates of groups C and G harbored conjugative R plasmids with molecular weights of 17 × 10^6 (pIP646) and 20 × 10^6 (pIP920). These plasmids carried genetic markers for resistance to macrolides and related drugs, as well as to chloramphenicol (pIP920), and have very similar *Hin*dIII restriction enzyme patterns.

Beta-hemolytic streptococci (Lancefield groups A, B, C, and G) are the most common streptococcal pathogens in humans. They are usually very susceptible to penicillins, macrolides and related drugs, tetracyclines, and chloramphenicol. The appearance of antibiotic-resistant strains of these streptococci has previously been reported for macrolides—erythromycin, spiramycin; lincosamides—lincomycin, clindamycin; and streptogramin B (MLS) (6, 7). The incidence of resistance to macrolides and related drugs by groups C and G has been reported (R. Minck, personal communication) to be very low (1 to 2%), as noted for group B streptococci (1, 2, 21). The resistance of streptococci to tetracycline is very prevalent in various countries. In our laboratory the percentage of resistance of beta-hemolytic streptococci to this antibiotic is very high: 60% for groups A and C, and 75% for groups B and G (unpublished data).

A number of previous investigations have demonstrated that MLS resistance in different streptococcal strains is plasmid borne, with molecular weights of plasmid DNA ranging from 17 × 10^6 to 20 × 10^6 (4, 5, 11, 12, 18). Conjugative transfer (16) of these R plasmids has been reported for groups A (19), B (11, 14), and D (13, 15, 23). A genetic exchange by conjugation between multiple antibiotic-resistant donor and recipient streptococci in the absence of extrachromosomal elements has been reported in *Streptococcus pneumoniae* (3, 22), as well as in groups A, B, F, and G (T. Horodniceanu, L. Bougueleret, G. Bieth, submitted for publication).

Group C and G streptococci are etiological agents of upper respiratory tract and skin infections. Evidence for R plasmids in group C and G streptococci has not yet been reported. In this study we describe the genetic and molecular properties of two R plasmids carried by two strains of group C and G after their conjugative transfer into streptococcal recipients.

Bacteria and plasmids used in this study are listed in Table 1. Wild-type beta-hemolytic streptococci were identified by Fuller serological test (9). Media, growth conditions, drugs used for selection of donor resistance markers and to counterselect bacterial donors, mating experiments (carried out on membrane filters), and curing procedures have been described previously (12-14). Plasmid DNA isolation from streptococcal transconjugants was performed as described previously (3). In addition, group C and G wild-type strains were treated before lysis with 2 μg of trypsin per ml (Sigma Chemical Co., St. Louis, Mo.) for 2 h at 37°C. Crude and cleared lysates were obtained as described elsewhere (20). Plasmid DNA was isolated from cleared lysates by centrifugation in cesium chloride-ethidium bromide density gradients, dialyzed against 10 mM Tris-hydrochloride–1 mM EDTA (pH 8), and stored at 4°C. Gel electrophoresis for plasmid DNA was performed as described (3). Molecular weights of plasmid DNAs were calculated from the relative mobilities in agarose of standard DNA from plasmids RP4 (34 × 10^6), RSA (23 × 10^6), and ColE1::Amp (7.7 × 10^6). Plasmid DNA of pIP501 (12) (20 × 10^6) was used as a reference streptococcal control. Restriction endonuclease analysis with *Hin*dIII (New England Biolabs, Beverly, Mass.) was used according to the manufacturer's instructions. The cleavage fragments were analyzed by electrophoresis on horizontal 1% agarose gels and run at 100 V for 5 h. Molecular weights of fragments were calculated from relative mobilities in comparison with linear λ DNA standard fragments.

The results of the frequency of transfer of resistance markers carried by the wild-type strains are shown in Table 2. Selection was done for erythromycin and tetracycline. Transconjugants were obtained by selection on erythromycin from strain C87 at a high frequency after 1 or 2 h of mating contact, and from G49 at a low frequency after 18 h of contact. When tetracycline was the selective agent, no transconjugants

were detectable from either the wild-type or BM6502 strain. The transconjugants obtained were crossed with appropriate recipients (BM133 or BM134); these matings were designated as retransfers. The frequency of retransfer was high for BM5101 and BM5102 donors, but no detectable transconjugants were obtained from BM5107 (Table 2). Analysis for unselected markers of transconjugants obtained in transfer matings (at least 100 clones for each selection) revealed that strains C87 and G49 transferred only MLS resistance and Cm MLS resistance, respectively, as linked resistance markers. The wild-type strains, as well as their transconjugants, were tested for spontaneous loss of resistance markers and by curing with acridine orange and ethidium bromide. We found a high efficiency (73%) of curing for C87 (for MLS resistance) and a low efficiency (1 to 4%) for transconjugant clones. No loss of the Tc marker was obtained from wild-type strains.

TABLE 1. *Bacterial donor and recipient strains*

Strain designation	Serogroup	Resistance marker(s)[a]	Origin or reference
Donor			
C87	C	MLS Tc	Strasbourg 1976 (skin)
BM6502	C	Tc	C87 cured for MLS (this study)
G49	G	Cm MLS Tc	Strasburg 1979 (nose)
BM5201[b] (pIP501)	D	Cm MLS Fus^r Rif^r	14
Recipient			
JH2-2	D	Fus^r Rif^r	15
BM133	D	Str^r	14
BM132	B	Fus^r Rif^r	14
BM134	B	Str^r	14

[a] Drug resistance markers: Cm, chloramphenicol; Tc, tetracycline; MLS, macrolides (erythromycin, spiramycin)-lincosamides (lincomycin, clindamycin)-streptogramin B; Fus^r, fusidic acid; Rif^r, rifampin; Str^r, streptomycin.

[b] pIP501 is a plasmid first isolated from a group B *Streptococcus* (12); BM5201 is JH2-2 harboring pIP501 (14).

The presence of plasmid DNA in wild-type, transconjugant, and BM6502 strains was examined by dye-buoyant density centrifugation. Except for BM6502, all strains contained bands of satellite DNA which were further analyzed by agarose gel electrophoresis before and after digestion by restriction endonuclease *Hin*dIII (Fig. 1). Molecular weights of plasmid DNA molecules, determined by adding the band sizes after cleavage with *Hin*dIII, were found to be 17.9×10^6, 20.4×10^6, and 19.7×10^6 for pIP646 (carried by C87), pIP920 (carried by G49), and the control pIP501 (carried by JH2-2), respectively. The restriction endonuclease fragment patterns of pIP646 and pIP920 were very similar to that obtained with pIP501 (Fig. 1): a large number of digestion fragments, "b, c, f, g, h, i, k, l, m, n," were common to all three plasmids, fragment a (molecular weight, 4×10^6) was in pIP501 and pIP920, j (molecular weight, 8.2×10^5) was in pIP646 and pIP501; pIP646 and pIP920 additionally had fragments d and e, respectively.

The results presented in this study demonstrate that MLS resistance (with or without Cm linked marker) in these group C and G streptococci is carried by plasmids, which are transferred by conjugation into different streptococcal recipients; in addition to the results reported here, pIP646 and pIP501 are also transferred into group A, C, or G recipients (data not shown). The retransfer of pIP646 occurs at a high frequency; in contrast, the retransfer of pIP920 was not obtained although physical evidence of this plasmid DNA was consistently demonstrated in both the wild-type strain and in the new host (JH2-2). This result suggests that a deletion of transfer genes might have occurred in pIP920, thus rendering it nonconju-

TABLE 2. *Frequency of conjugative transfer of R plasmids from wild-type strains and transconjugants*

Donor × recipient	Mating time (h)	Selective marker[a]	No. of donors (CFU/ml)[a]	No. of transconjugants (CFU/ml)	Transfer frequency/donor cell	Strain designation of transconjugants	Phenotype of transconjugants	Plasmid designation (mol wt, 10^6)
Transfer expt								
C87 × JH2-2	1	Em	2.5×10^7	7×10^6	2.8×10^{-1}	BM5101	MLS Fus^r Rif^r	pIP646 (17.5)
C87 × JH2-2	18	Tc	1.2×10^9	<1	$<8 \times 10^{-10}$			
C87 × BM132	2	Em	2.4×10^8	4.8×10^6	2×10^{-2}	BM5102	MLS Fus^r Rif^r	pIP646 (17.5)
BM6502 × JH2-2	18	Tc	1.5×10^9	<1	$<6 \times 10^{-10}$			
G49 × JH2-2	18	Em	2.7×10^9	30	1.1×10^{-8}	BM5107	Cm MLS Fus^r Rif^r	pIP920 (20)
G49 × JH2-2	18	Tc	2.7×10^9	<1	$<3 \times 10^{-10}$			
Retransfer expt								
BM5101 × BM133	2	Em	1×10^9	2×10^5	2×10^{-4}	BM5111	MLS Str^r	pIP646
BM5102 × BM134	2	Em	4×10^8	1.6×10^7	4×10^{-2}	BM5112	MLS Str^r	pIP646
BM5107 × BM133	18	Em	4×10^9	<1	$<2.5 \times 10^{-10}$			

[a] Em, Erythromycin; CFU, colony-forming unit; for other abbreviations, see Table 1, footnote *a*.

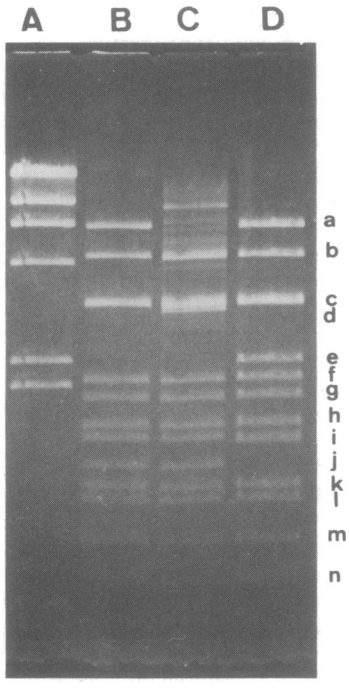

FIG. 1. *Agarose gel electrophoresis of three plasmid DNAs cleaved by HindIII. Lane A: λ HindIII fragments, 15 × 10^6, 6.4 × 10^6, 4.3 × 10^6, 2.9 × ^{6}, 1.6 × 10^6, 1.4 × 10^6, and 0.32 × 10^6; lane B, pIP501; lane C, pIP646; lane D, pIP920. Fragments c of lanes B, C, and D are doublets. Note that fragments which are above the first fragment (b) of pIP646 are due to incomplete digestion that occurred in this experiment.*

gative. Six other wild-type streptococci of groups C (one strain) and G (five strains) were analyzed for conjugative transfer of their resistance markers (Tc and MLS) and for the presence of plasmid DNA: neither detectable conjugative transfer nor evidence of extrachromosomal elements could be demonstrated (unpublished data). The possibility that the resistance markers in these strains are chromosome borne as transposable elements could not be excluded.

Although wild-type group C and G strains are also resistant to tetracycline, this marker is not detectable in transconjugants. Moreover, strain BM6502 does not contain bands of satellite DNA. These results suggest that in these streptococci, wild-type strains carry both an MLS plasmid and a chromosome-borne Tc marker. We reported the same type of results with group B streptococci (14).

MLS resistance has been demonstrated to be plasmid borne in many streptococcal strains (4, 5, 12, 18). These plasmids have a high molecular similarity as demonstrated by enzyme restriction fingerprints (11, this study) and by DNA:DNA homology (8, 24). El-Solh (personal communication) recently reported that hybridization of pIP646 with other MLS plasmids isolated in France, as well as in other countries, is very high (>90%). On the other hand, the broad host range of certain MLS plasmids, such as pAMβ1 (4), pIP501 (12), and pIP646 (this study), was demonstrated by the conjugative transfer of these plasmids into several streptococcal (3, 11, 14, 17, 18, this study) and lactobacilli (10) recipients, and by their stable maintenance in the new hosts. These results support the hypothesis that very similar, if not identical, plasmids may be disseminated among different species of streptococci (8).

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