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Plasmid Associated with Diplococcin Production in *Streptococcus cremoris*

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The ability to produce diplococcin (Dip^+) was transferred by conjugation from *Streptococcus cremoris* 346 to two plasmid-free *S. cremoris* recipients at a high frequency (10^{-1} per donor). Dip^+ transconjugants from each mating gained a 54-megadalton plasmid. Spontaneous loss of this plasmid restored the Dip^- phenotype.

Diplococcin is a bacteriocin produced by some strains of *Streptococcus cremoris*, and its purification and properties have been reported (5). Diplococcin-producing strains (Dip^+) were cured of this property by growth at elevated temperature, and the Dip^+ character was transferred by conjugation to plasmid-containing *S. cremoris* recipients. The Dip^+ phenotype, however, could not be correlated with any particular plasmid (4), possibly due to the multiplasmid nature of the recipients used in these experiments. To provide a clear background in which to observe any plasmid transfer by conjugation, two plasmid-free strains of *S. cremoris* were isolated. Conjugal transfer of Dip^+ was reexamined, and evidence is presented for plasmid linkage of diplococcin production.

The strain *S. cremoris* 346, which has been characterized as a diplococcin producer (5), was used as the donor in conjugation experiments. Unrelated wild-type strains of *S. cremoris* 4358 (containing eight plasmids) and 4365 (containing nine plasmids) were subjected to six cycles of growth at elevated temperatures. Lac^- derivatives were isolated on SALT medium (2). The stress temperatures and Lac^- frequencies were 36°C and 2.8% for strain 4358 and 37°C and 3% for strain 4365.

Both plasmid-free strains were non-bacteriocin producing and sensitive to diplococcin; they were used as recipients in conjugation experiments. Spontaneous antibiotic-resistant mutants of these strains were isolated on M17 agar (10) plates containing streptomycin (250 μg/ml). Donor (0.5-ml) and recipient (5-ml) cultures were mixed and collected on 0.45-μm membrane filters (Millipore Corp.). Filters were incubated for 16 h at 30°C on M17-glucose agar plates. Dilutions of the filter populations were plated on M17-lactose agar and streptomycin (250 μg/ml), and incubation was continued at 22°C for 16 h. Donor and recipient controls alone were treated in a similar manner. Transconjugants were detected by soft agar overlay of the colonies with streptomycin-resistant *S. cremoris* $480B_1$ and further incubations for 16 to 18 h at 22°C (3). Cleared lysates for plasmid DNA examination were obtained by the lysis procedure of Anderson and McKay (1) and were examined by agarose gel electrophoresis as previously described (2).

The transfer frequency of diplococcin for both recipients was high, ca. 10^{-1} transconjugants per donor. This is in contrast to a frequency of ca. 10^{-5} for transfer of diplococcin to other group N streptococci (4). On control plates, no donor streptomycin-resistant mutants ($<10^{-9}$ per recipient) were isolated. A total of 15 colonies from strain 4358 and 4365 transconjugants examined contained a 54-megadalton (Md) plasmid and produced diplococcin (Fig. 1). None of the transconjugants examined were lactose positive, nor was any other transferred plasmid detected. Transconjugants were immune to diplococcin, suggesting that this plasmid may also carry the genes for immunity. From each strain, spontaneous Dip^- colonies were isolated at a frequency of 0.1 to 0.2%. Plasmid analysis revealed the loss of the 54-Md plasmid. There is increasing evidence for involvement of plasmids in bacteriocin production by lactic streptococci. Scherwitz et al. (9) indicated that the ability of a *Streptococcus lactis* subsp. *diacetylactis* strain to produce a bacteriocin is linked to an 88-Md conjugative plasmid. A recent report describes bacteriocin-producing transconjugants that have acquired a 39.6-Md plasmid from two *S. cremoris* strains and a 75-Md plasmid from *S. lactis* subsp. *diacetylactis* (8). Evidence for plasmid involvement of nisin-producing and sucrose-fermenting ability in *S. lactis* has also been demonstrated (6, 7). The results presented here show that diplococcin, the bacteriocin of the industrially important *S. cremoris*, is linked to a 54-Md conjugative plasmid in *S. cremoris* 346.

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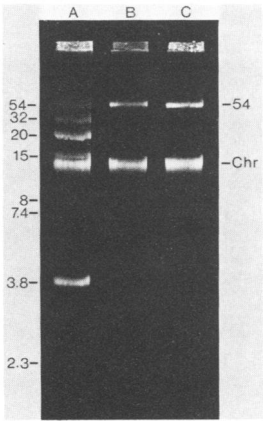

FIG. 1. Agarose gel electrophoresis patterns of plasmid DNA isolated from the donor strain *S. cremoris* 346 (A) and two transconjugants, 4358 Dip^+ (B) and 4365 Dip^+ (C). Molecular masses (megadaltons) of plasmids were determined from known standards. Chr, Chromosomal.

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