JOURNAL OF BACTERIOLOGY, Mar. 1984, p. 833–838
0021-9193/84/030833-06$02.00/0

Vol. 157, No. 3

Conjugal Transfer and Characterization of Bacteriocin Plasmids in Group N (Lactic Acid) Streptococci

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Received 26 September 1983/Accepted 29 November 1983

Thirteen bacteriocin-producing strains of group N (lactic acid) streptococci were screened for their potential to transfer this property by conjugation to *Streptococcus lactis* subsp. *diacetylactis* Bu2-60. Bacteriocin production in three strains was plasmid encoded as shown by conjugal transfer and by analysis of cured, bacteriocin-negative derivatives of the donor strains and the transconjugants. With *Streptococcus cremoris* strains 9B4 and 4G6 and *S. lactis* subsp. *diacetylactis* 6F7 as donors, bacteriocin-producing transconjugants were isolated with frequencies ranging from ca. 2×10^{-2} to 2×10^{-1} per recipient cell. Bacteriocin-producing transconjugants had acquired a 39.6-megadalton plasmid from the donor strains 9B4 and 4G6, and a 75-megadalton plasmid from the donor strain 6F7. As shown by restriction endonuclease analysis, the plasmids from strains 9B4 and 4G6 were almost identical. The plasmid from strain 6F7 yielded some additional fragments not present in the two other plasmids. In hybridization experiments any of the three plasmids strongly hybridized with each other and with some other bacteriocin but nontransmissible plasmids from other *S. cremoris* strains. Homology was also detected to a variety of cryptic plasmids in lactic acid streptococci.

The ability to produce bacteriocins is widely distributed among gram-positive bacteria (20). Production of bacteriocins has been observed in several immunological groups of streptococci, including group N (lactic acid) streptococci (1, 8, 11). Production of some bacteriocins is known to be plasmid associated, and the transmissibility of such plasmids has been described in many bacterial genera (9).

Plasmids are very common in lactic acid streptococci (4, 10, 12, 15). There is evidence that plasmids are involved in the ability of lactic acid streptococci to metabolize several carbohydrates, to utilize citrate, and to produce proteinases and bacteriocins (for a review, see reference 13). The function of many of the plasmids, however, is unknown.

Conjugative systems have been described recently in group N streptococci which function in the transfer of the drug resistance plasmid pAMβ1 from *Streptococcus faecalis* into lactic acid streptococci and among different lactic acid streptococci (6; H. Neve and A. Geis, unpublished data) and of plasmids which determine the ability to metabolize lactose (7, 13, 14, 18, 22). Conjugation-like transfer of a factor coding for diplococcin, a bacteriocin excreted by several *Streptococcus cremoris* strains, has been observed (3). The ability of the transconjugants to produce bacteriocin could not be correlated with the transfer of a particular plasmid. Very recently, the transfer of lactose-fermenting and bacteriocin-producing abilities from *Streptococcus lactis* subsp. *diacetylactis* to *S. lactis* by conjugal mating has been reported (17). The bacteriocin production was claimed to be linked to an 88-megadalton (Mdal) plasmid.

This paper deals with the transfer of bacteriocin factors from strains of *S. lactis*, *S. lactis* subsp. *diacetylactis*, and *S. cremoris* to a plasmid-free *S. lactis* subsp. *diacetylactis* recipient strain and the analysis of the transferred plasmids.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. All streptococcal strains used in this study have been isolated from starter cultures (8). The organisms were maintained frozen in litmus milk in liquid nitrogen and grown at 30°C in lactic acid broth (5). Lactic acid agar supplemented with 0.005% bromcresol purple–1% lactose was used as the indicator agar for lactose metabolism. The strains used and their relevant properties are listed in Table 1.

Bacteriocin production and assays. The methods employed for screening and quantitative bacteriocin determination have been described previously (8).

Conjugation experiments. Donor and recipient strains were grown in lactic acid broth at 30°C to the late-exponential phase of growth (ca. 1×10^9 to 2×10^9 CFU/ml). Routinely, 0.1 ml of donor and 1 ml of recipient culture were mixed in a total volume of 5 ml of lactic acid broth. The mixture was collected on a sterile membrane filter (Schleicher & Schüll; RC 55, 0.45-μm pore size). The filters were washed with 2 ml of buffered saline and incubated right-side up for 4 and 18 h at 30°C on lactic acid agar. Control cultures of recipient and donor strains alone were treated in the same manner. After incubation the bacteria were washed from the filters by rigorous shaking in 1.5 ml of buffered saline. Dilutions were spread onto the appropriate media. Transconjugants and recipient cells were selected on lactic acid agar supplemented with streptomycin (0.5 mg/ml) and rifampin (0.1 mg/ml). After growth at 30°C for 1 to 2 days and replica plating, the plates were overlaid with soft agar containing indicator bacteria. Bacteriocin positive (Bac^+) transconjugants were identified by a surrounding zone of growth inhibition of the indicator bacteria. Lactose-fermenting (Lac^+) transconjugants were selected on lactose indicator agar containing antibiotics.

DNase sensitivity test. Sensitivity of transfer to DNaseI was tested as follows. Donor cultures were treated with

TABLE 1. Strains of lactic acid streptococci

Strain	Relevant phenotype[a]	Bacteriocin type[b]	Plasmids (Mdal)
S. cremoris			
AC1	Lac$^+$ Bac$^+$	I	44, 36.5, 25.6, 18.2, 11.4, 4.85, 3.81, 1.85, 1.32[c]
3A6	Lac$^+$ Bac$^+$	I	54, 48, 43, 34, 28, 20, 12, 3
9B4	Lac$^+$ Bac$^+$	I	39.6, 20, 12, 10.5, 2.6, 1.8
4G6	Lac$^+$ Bac$^+$	I	39.6, 20, 12, 10.5, 2.7, 1.8
1A1	Lac$^+$ Bac$^+$	II	35, 28, 11, 4.4, 3.4, 1.4
3E9	Lac$^+$ Bac$^+$	II	44, 36, 23, 17, 11, 4.9, 3.8, 1.9, 1.3
4E9	Lac$^+$ Bac$^+$	III	44, 36, 23, 17, 11, 5.3, 4.9, 3.8, 1.9, 1.3
3C6	Lac$^+$ Bac$^+$	IV	27, 13, 1.3
S. lactis			
5D8	Lac$^+$ Bac$^+$	VI	53, 45, 29, 17, 9.8, 3
6F3	Lac$^-$ Bac$^+$	VI	19, 1.3
6F5	Lac$^+$ Bac$^+$	VI	None
7C1	Lac$^+$ Bac$^+$	VII	75, 33, 31, 19
S. lactis subsp. *diacetylactis*			
6F7	Lac$^-$ Bac$^+$	VIII	75[c], 48, 13, 5.2, 4.7
Bu2-60	Lac$^-$ Bac$^-$ Str^r Rif^r		None, plasmid cured
C2-202	Lac$^-$ Bac$^-$ Str^r Rif^r		Streptomycin- and rifampin-resistant indicator strain, sensitive to bacteriocins of type I to VIII
Transconjugants			
Bu2-61	Lac$^-$ Bac$^+$		39.6[c] from mating Bu2-60 × 9B4
Bu2-62	Lac$^-$ Bac$^+$		39.6 and 1.8 from mating Bu2-60 × 9B4
Bu2-63	Lac$^-$ Bac$^+$		39.6 from mating Bu2-60 × 4G6
Bu2-64	Lac$^-$ Bac$^+$		75 from mating Bu2-60 × 6F7
Bu2-65	Lac$^-$ Bac$^+$		75 and 4.7 from mating Bu2-60 × 6F7
Bu2-67	Lac$^-$ Bac$^+$		None from mating Bu2-60 × 6F3

[a] Lac$^+$, Metabolizes lactose; Lac$^-$, does not metabolize lactose; Bac$^+$, produces bacteriocin; Bac$^-$, does not produce bacteriocin; Str^r, resistant to 0.5 mg of streptomycin per ml; Rif^r, resistant to 0.1 mg of rifampin per ml.

[b] See reference 8.

[c] The sizes of these plasmids have been determined by electron microscopy and were used as size references.

DNase (100 μg/ml) and 1 mM $MgSO_4$ for 60 min at 37°C before mixing with the recipient strain. DNaseI and $MgSO_4$ were also included in the agar medium of the plates.

Chloroform sensitivity of transfer. Chloroform (2% [vol/vol]) was thoroughly mixed with donor cells, and the mixture was incubated for 60 min at 37°C. After complete removal of the solvent, the chloroform-treated cells were used for conjugation experiments.

Plasmid isolation and purification. For the analysis of the plasmid content, the rapid screening method described by LeBlanc and Lee (12) was used with some modifications. Cells were grown in 1.5 ml of lactic acid broth supplemented with 20 mM D,L-threonine to the stationary phase of growth. Washed cells were suspended in 0.1 ml of 50 mM Tris-hydrochloride (pH 8)–25% sucrose, followed by the addition of 0.1 ml of lysozyme (10 mg/ml) and RNase (50 μg/ml). After 20 min of incubation at 37°C, 50 μl of 0.25 M EDTA (pH 8) was added, and lysis was achieved by the addition of 50 μl of 20% sodium dodecyl sulfate. The lysate was passed through an Eppendorf tip (four times), and the DNA was denatured by the addition of 50 μl of 1 N NaOH. The lysate was neutralized with 70 μl of 2 M Tris-hydrochloride, pH 7. High-molecular-weight DNA was precipitated by the addition of 0.1 ml of 5 M NaCl at 4°C for 2 h. The supernatant fraction was collected by centrifugation at 12,000 × *g* at 4°C and extracted with an equal volume of NaCl-saturated phenol. The aqueous phase was then extracted with 1 volume of chloroform-isoamyl alcohol (24:1), and the DNA was concentrated by ethanol precipitation. For preparative plasmid isolation, cells were grown in lysis medium (10) overnight at 30°C, washed twice, and concentrated 50-fold in 50 mM Tris-hydrochloride (pH 8.0)–25% sucrose. Lysozyme (5 mg/ml) and RNase (10 μg/ml) were added, and the cells were incubated at 37°C for 20 min. After the addition of EDTA to a final concentration of 60 mM, the cells were lysed by sodium dodecyl sulfate (4% final concentration). The lysate was enriched for plasmid DNA essentially by the method of Currier and Nester (2). Plasmids were finally purified in a cesium chloride (1.6 g/cm^3)–ethidium bromide (0.5 mg/ml) gradient by centrifugation in a vertical rotor at 180,000 × *g* for 20 h.

Restriction endonuclease analysis. Purified plasmid DNA was dialyzed against TES buffer (30 mM Tris-hydrochloride, 50 mM NaCl, 5 mM EDTA [pH 8.0]) and concentrated by ethanol precipitation. Restriction endonucleases were used according to the instructions of the manufacturers. Molecular weights of fragments were calculated from their relative mobilities in agarose gels with phage λ-*Hin*dIII standard fragments.

Gel electrophoresis. Electrophoresis of DNA was carried out in vertical agarose gels. For intact plasmids, agarose concentration of 0.6% (agarose type II; Sigma Chemical Co.) was used. Gels were run at 10 V/cm at ca. 10°C in TBE buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA).

Restriction fragments were separated on 1% agarose gels in TBE buffer. Gels were stained with ethidium bromide (5 μg/ml) and photographed under UV transillumination.

Nick translation of plasmid DNA. Whole plasmid DNA was labeled in vitro by nick translation with ^{35}S-labeled dATP according to the instructions of the enzyme supplier (BRL, Neu Isenburg).

DNA blotting and hybridization. Plasmid DNA or restriction fragments were transferred from agarose gels to nitrocellulose sheets by the method of Southern (19). To facilitate the transfer of large plasmids, DNA was depurinated by soaking the gel twice for 10 min at 20°C in 0.25 M HCl (21). The DNA was denatured by soaking twice for 15 min at 20°C in 0.5 M NaOH–1 M NaCl. After neutralization in 0.5 M Tris-hydrochloride (pH 7.4)–3 M NaCl, the DNA was transferred to nitrocellulose in 10× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate). The dried and baked (80°C, 4 h) blot was preincubated for 1 h at 65°C in 6× SSC containing 0.6 mg of Ficoll 400 per ml, 0.6 mg of bovine serum albumin per ml, 0.6 mg of polyvinylpyrrolidone per ml, 0.2% sodium dodecyl sulfate, and 10 mM dithiothreitol. ^{35}S-labeled denatured plasmid DNA (ca. 5×10^6 cpm) was hybridized with blotted DNA in 50 ml of preincubation buffer for 16 h at 65°C in sealed plastic bags. In some experiments the hydridization buffer contained unlabeled sheared salmon testes DNA (10 μg/ml). The nitrocellulose sheets were washed three times in 2× SSC and one time in 0.1× SSC, dried, and transferred onto X-ray films.

RESULTS

The first 13 strains listed in Table 1 were mated with the plasmid-free strain Bu2-60 as the recipient on membrane filters. Strain Bu2-60 is streptomycin and rifampin resistant and sensitive to the bacteriocins from lactic acid streptococci mentioned in this study. The times of mating contact were 4 and 18 h.

Bac$^+$ transconjugants could be detected in matings of 4 of the 13 donor strains, namely *S. cremoris* strains 9B4 and 4G6, *S. lactis* 6F3, and *S. lactis* subsp. *diacetylactis* 6F7 (Table 2). The transfer frequencies per donor cells were significantly lower than the frequencies per recipient cells. This was due to counterselection against the recipient strains by the bacteriocins produced by the donor strains during the filter mating (Table 2). No Bac$^+$ transconjugants were found after 4 h of mating contact. No Lac$^+$ transconjugants were detected.

No Bac$^+$ transconjugants were detected in mating experiments with chloroform-treated donor cells. The recipient strain grew well after mating with the solvent-treated donor bacteria, indicating that residual chloroform did not affect bacterial growth. In other control experiments, donor cultures were treated with DNaseI before mating. DNaseI was also included in the agar plates to avoid transformation during the incubation of the filters. No differences in the transfer frequencies in matings with untreated and DNaseI-treated donor cultures were observed.

When tested for lysis with a set of 17 bacteriophages, all transconjugants showed the same lysis pattern as the recipient strain Bu2-60.

Plasmid analysis. Plasmid DNA was prepared by the rapid screening method and analyzed by agarose gel electrophoresis. The size of the plasmids was determined by comparison of their electrophoretic mobilities with plasmids of known molecular weight and by direct measurement of the length by electron microscopy. Each of the transconjugants obtained in Bu2-60 × 9B4 matings carried a 39.6-Mdal (p9B4-6) plasmid. Two of the Bac$^+$ isolates contained an additional 1.8-Mdal plasmid. A 39.6-Mdal plasmid was also found in the Bac$^+$ transconjugants isolated after Bu2-60 × 4G6 matings (p4G6-6). No additonal plasmid was detected. All of the Bac$^+$ isolates obtained after mating of strain Bu2-60 with strain 6F7 carried a large 75-Mdal plasmid (p6F7-5). An additional 4.7-Mdal plasmid was found in two transconjugants (Fig. 1). No plasmid DNA could be detected in any of the Bac$^+$ transconjugants obtained from matings of strain Bu2-60 with 6F3 as the donor (data not shown).

Curing experiments. Lactic streptococci could be efficiently cured of plasmids by incubation for 24 h and more at elevated but sublethal temperatures. This temperature depended on the individual strain and was between 40 and 42°C. The efficiency of curing for the bacteriocin marker was about 13, 6, and 8% of the donor strains 9B4, 4G6, and 6F7, respectively, and 3, 2, and 9% for corresponding transconjugants after 24 h at 40°C. All of the Bac$^-$ isolates of strains 9B4 and 4G6 and the transconjugants obtained in matings with these strains had lost a 39.6-Mdal plasmid. Bac$^-$ derivatives of strain 6F7 and of Bu2-60 × 6F7 transconjugants had lost a 75-Mdal plasmid. No Bac$^-$ derivates could be obtained even after prolonged incubation at elevated

TABLE 2. Frequencies of conjugal transfer of Bac$^+$ (bacteriocin) markers[a]

Donor strain	Bac$^+$ transconjugants per recipient[b]	Bac$^+$ transconjugants per donor	Plasmids in Bac$^+$ transconjugants (Mdal)
9B4	2×10^{-2} (9)	6.6×10^{-4}	39.6, 39.6 + 1.8
4G6	3×10^{-2} (6)	8.0×10^{-4}	39.6
6F3	8×10^{-3} (3)	3.5×10^{-7}	None
6F7	2×10^{-1} (3)	2.0×10^{-7}	75, 75 + 4.7

[a] Transfer frequencies are given as the average obtained in the indicated number of experiments. *S. lactis* subsp. *diacetylactis* Bu2-60 was the recipient in all mating experiments.

[b] Number in parentheses is the number of experiments.

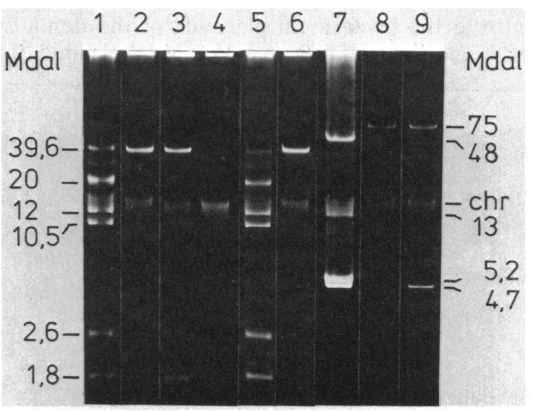

FIG. 1. Gel electrophoresis pattern of DNA isolated from Bac$^+$ donor strains *S. cremoris* 9B4 (1) and 4G6 (5) and *S. lactis* subsp. *diacetylactis* 6F7 (7), the Bac$^-$ plasmid-free recipient strain Bu2-60 (4), and some transconjugants. Transconjugants Bu2-61 (2) and Bu2-62 (3) were obtained from filter matings of strain 9B4 and Bu2-60 and acquired a 39.6-Mdal plasmid (p9B4-6). Transconjugant Bu2-62 showed an additional 1.8-Mdal plasmid. Transconjugant Bu2-63 (6) isolated from matings of strains 4G6 and Bu2-60 contained a 39.6-Mdal plasmid (p4G6-6). The transconjugants Bu2-64 (8) and Bu2-65 (9) obtained from matings of strains 6F7 and Bu2-60 acquired a 75-Mdal plamid (p6F7-5). Bu2-65 showed an additional 4.7-Mdal plasmid. Gel electrophoresis was performed in 0.6% agarose gels as described in the text.

TABLE 3. Curing efficiencies for the bacteriocin markers[a]

Strain	Curing efficiency (%)	Plasmid lost
9B4	13	39.6
Bu2-61	3	39.6
4G6	6	39.6
Bu2-63	2	39.6
6F7	8	75
Bu2-64	9	75
6F3	0	—[b]
Bu2-67	0	—

[a] Average of at least three experiments after 24 h at 40°C.
[b] Plasmid free.

temperature from strain 6F3 and its Bac$^+$ transconjugants (Table 3). After curing of the ability to produce bacteriocin, all Bac$^-$ isolates had also lost the resistance or immunity to the bacteriocin of the parent strain.

Restriction endonuclease and hybridization analysis of plasmids isolated from Bac$^+$ transconjugants. Plasmids p9B4-6, p4G6-6, and p6F7-5 were isolated from Bac$^+$ transconjugants Bu2-61, Bu2-63, and Bu2-64 and purified. Analysis of plasmid DNA was done by agarose gel electrophoresis after digestion with the restriction endonucleases *Bgl*II, *Eco*RI, *Hpa*II, *Pvu*II, and *Xba*I. Plasmids p9B4-6 and p4G6-6 showed identical restriction fragment patterns in all digests except that of *Xba*I. The small fragment obtained in these digests differed in size. All fragments of the 39.6-Mdal plasmids p9B4-6 and p4G6-6 were also found in the corresponding digests of the 75-Mdal plasmid p6F7-5. In addition to these common fragments several additional bands were observed in p6F7-5 (Fig. 2). It seems that plasmid p6F7-5 contains DNA homologous to plasmids p4G6-6 and p9B4-6. This interpretation was confirmed when ^{35}S-labeled plasmids were hybridized to a nitrocellulose blot containing DNA of Bac$^+$ transconjugants. All three plasmids strongly hybridized to each other. Radioactive-labeled DNA from plasmids p4G6-6, p9B4-6, and p6F7-5 was also hybridized efficiently to the bacteriocin plasmids of the donor strains 4G6, 9B4, and 6F7 and also to a 36.5-Mdal plasmid of strain AC1 and a 43-Mdal plasmid of strain 3A6 (Table 4 and Fig. 3). These two strains produce a bacteriocin of type I-like strains 9B4 and 4G6 but do not transfer this ability in filter matings. These observations and the simultaneous loss of these plasmids in cured Bac$^-$ isolates of strains AC1 and 3A6

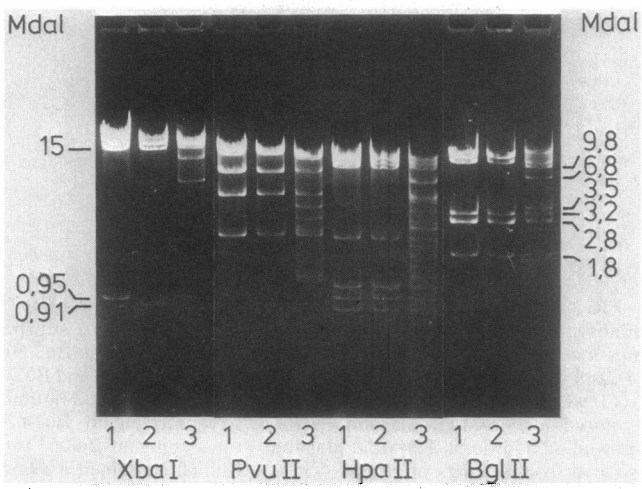

FIG. 2. Restriction endonuclease fingerprints of plasmids p9B4-6 (lane 1), p4G6-6 (lane 2), and p6F7-5 (lane 3) isolated from the transconjugants Bu2-61, Bu2-63, and Bu2-64, respectively, and digested with the endonucleases *Xba*I, *Pvu*II, *Hpa*II, and *Bgl*II. The fragments were separated electrophoretically in a 1% agarose gel.

TABLE 4. Homology of the plasmids p9B4-6, p4G6-6, and p6F7-5 with plasmids from bacteriocin-producing lactic acid streptococci

Source of plasmids	Bacteriocin produced[a]	Plasmids (Mdal) hybridizing with plasmids:	
		p9B4-6 and p4G6-6	p6F7-5
AC1	I	36.5 11.4 4.85	44 36.5 25.6 18.2 11.4 4.85
3A6	I	43 28 12	43 28 12
9B4	I	39.6 10.5	39.6 20 10.5 2.6
4G6	I	39.6 10.5	39.6 20 10.5 2.7
1A1	II	35 11	35 28 11
3E9	II	36 11 4.9	44 36 23 17 11 4.9
4E9	III	36 11 4.9	44 36 23 17 11 4.9
3C6	IV	27 13	27 13
5D8	VI	29 17	29 17
6F3	VI	19	19
6F5	VI	None	None
7C1	VII	None 19	31
6F7	VIII	75 48	75 48 5.2 4.7

[a] See reference 8.

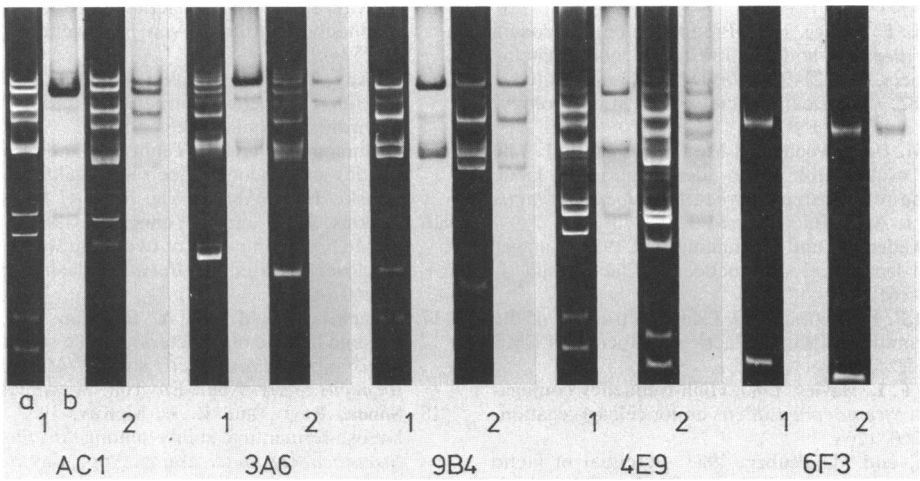

FIG. 3. DNA-DNA hybridization of plasmid DNA from Bac$^+$ lactic acid streptococci with ^{35}S-labeled plasmids p9B4-6 (lane 1) and p6F7-5 (lane 2). Plasmid patterns (ethidium bromide stained) in 0.6% agarose gels (a) are compared with the corresponding autoradiograms (b). Hybridization experiments with ^{35}S-labeled plasmid p4G6-6 DNA gave results identical to p9B4-6.

suggests that these plasmids are bacteriocin but nontransmissible plasmids.

Weak hybridization of plasmids p9B4-6 and p4G6-6 to several other plasmids could be observed. This was not due to nonspecific hybridization since only a few but not all plasmids showed hybridization. In strain AC1, for instance, only three of nine plasmids showed hybridization with p4G6-6 and p9B4-6. With p6F7-5 DNA as a radioactive probe, hybridization to an even larger number of plasmids was obtained. Strong hybridization could be observed to the bacteriocin plasmids of strains AC1, 3A6, 4G6, and 9B4, which produce bacteriocin of type I, and also to a large number of plasmids which did not show any homology to plasmids p4G6-6 and p9B4-6 (Table 4 and Fig. 3). No hybridization was obtained with control DNA from *S. faecalis* DS5 and several bacteriophages.

DISCUSSION

Results from conjugation experiments and the analysis of cured Bac$^-$ derivatives of both donor strains and transconjugants clearly showed that bacteriocin production in *S. cermoris* strains 4G6 and 9B4 and *S. lactis* subsp. *diacetylactis* 6F7 is plasmid coded. Since the recipient strain used in the mating experiments is a plasmid-cured derivative of *S. lactis* subsp. *diacetylactis* Bu2, which does not produce any antibacterial activity, the results suggest that the structural genes for bacteriocin production are located on the transferred plasmid.

Hybridization experiments and analysis of plasmid-cured Bac$^-$ derivatives demonstrated the presence of nontransmissible bacteriocin plasmids of slightly different sizes in other Bac$^+$ strains of *S. cremoris*. The bacteriocins produced by these strains showed very similar chemical properties and inhibitory spectra after partial purification (8).

Restriction endonuclease analysis of the bacteriocin plasmids showed that the plasmids p4G6-6 and p9B4-6 were almost identical. All fragments of these plasmids and several additional DNA bands were observed on agarose gels of digests of the 75-Mdal plasmid p6F7-5. This result and the strong hybridization of these plasmids to each other as well as the close similarity of the bacteriocins encoded by these plasmids implies that the genes for these bacteriocins are of common origin.

S. lactis 6F3 transferred a bacteriocin "factor" during conjugal mating. However, no plasmid DNA could be detected in any of the transconjugants. In curing experiments at elevated temperature, no Bac$^-$ derivatives either from the donor strain or the transconjugants were obtained. This is in agreement with results reported by Davey and Pearce (3). They isolated plasmid-free derivatives from nisin-producing *S. lactis* H1 which retained the ability to produce nisin. These authors propose that the nisin genes of *S. lactis* may be located on the chromosome. We cannot, however, definitively exclude the presence of an unstable plasmid in strain 6F3 and the corresponding transconjugants unless the chromosomal location of the gene(s) for the antibiotic substance has been proved by hybridization analysis.

The bacteriocin plasmids isolated from Bac$^+$ transconjugants not only hybridized to related bacteriocin plasmids but also to some others (Table 4). Since there was only hybridization to some but not all plasmids of a particular strain, hybridization was not due to a nonspecific process but must reflect the presence of homologous DNA sequences in these different plasmids.

Intramolecular recombination at homologous sequences dissociates plasmid pAMα1, a tetracycline resistance plasmid of *S. faecalis*, into two circular molecules, each containing a single homologous sequence (16). Intermolecular recombination at homologous sequences may generate multiple-copy plasmids. Further studies of the homologous regions of different plasmids in lactic acid streptococci may, therefore, contribute significantly to our understanding of the relatedness of these plasmids and shed some light on the mechanism by which the large number of different plasmids has evolved in these bacteria.

ACKNOWLEDGMENT

This work was supported by grant Te 46/7-1 from the Deutsche Forschungsgemeinschaft.

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