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Plasmid Linkage of a Bacteriocin-Like Substance in *Streptococcus lactis* subsp. *diacetylactis* Strain WM_4: Transferability to *Streptococcus lactis*†

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Streptococcus lactis subsp. *diacetylactis* strain WM_4 transferred lactose-fermenting and bacteriocin-producing (Bac^+) abilities to *S. lactis* LM2301, a lactose-negative, streptomycin-resistant (Lac^- Str^r), plasmid-cured derivative of *S. lactis* C2. Three types of transconjugants were obtained: Lac^+ Bac^+, Lac^+ Bac^-, and Lac^- Bac^+. *S. diacetylactis* WM_4 possessed plasmids of 88, 33, 30, 5.5, 4.8, and 3.8 megadaltons (Mdal). In Lac^+ Bac^+ transconjugants, lactose-fermenting ability was linked to the 33-Mdal plasmid and bacteriocin-producing ability to the 88-Mdal plasmid. Curing the 33-Mdal plasmid from Lac^+ Bac^+ transconjugants resulted in loss of lactose-fermenting ability but not bacteriocin-producing ability (Lac^- Bac^+). These strains retained the 88-Mdal plasmid. Curing of both plasmids resulted in a Lac^- Bac^- phenotype. The Lac^+ Bac^- transconjugant phenotype was associated with a recombinant plasmid of 55 or 65 Mdal. When these transconjugants were used as donors in subsequent matings, the frequency of Lac transfer was about 2.0×10^{-2} per recipient plated, whereas when Lac^+ Bac^+ transconjugants served as donors, the frequency of Lac transfer was about 2.0×10^{-5} per recipient plated. Also, Lac^- Bac^+ transconjugants were found to contain the 88-Mdal plasmid. The data indicate that the ability of WM_4 to produce bacteriocin is linked to an 88-Mdal conjugative plasmid and that lactose-fermenting ability resides on a 33-Mdal plasmid.

Certain strains of *Streptococcus lactis* subsp. *diacetylactis* are known to produce substances inhibitory to pathogenic and spoilage bacteria as well as to other members of the group N streptococci. Vedamuthu et al. (25) showed that a creaming mixture prepared with *S. diacetylactis* extended the shelf life of cottage cheese up to 3 weeks, and Daly et al. (4, 5) showed that *S. diacetylactis* in growth-association studies was capable of inhibiting the associative pathogen or spoilage organism. Strain dominance exhibited by certain *S. diacetylactis* strains in mixed-strain starter cultures was shown by Collins (2) to be due to production of antibiotic-like substances. Some of the factors that contribute to these antagonistic properties of *S. diacetylactis*, as well as to those of *S. lactis* and *Streptococcus cremoris*, include organic acids, hydrogen peroxide, and low-molecular-weight peptides (1). The antibiotics nisin and diplococcin are produced by certain strains of *S. lactis* and *S. cremoris*, respectively (7, 14). More recently, it has been shown by Kozak et al. (17) that bacteriocins (Bacs) are produced by *S. diacetylactis* and *S. lactis*, and the authors suggested that the antibacterial activity of these Bacs may contribute to the antagonistic properties of some lactic streptococci. They also indicated that the inhibitory potential of these Bacs should be considered when preparing multiple-strain dairy starter cultures.

The results presented in this communication indicate that the ability of *S. diacetylactis* WM_4 to produce a bacteriocin-like substance can be transferred to *S. lactis* through conjugal matings and is associated with an 88-megadalton (Mdal) plasmid.

MATERIALS AND METHODS

Bacterial strains. All streptococcal cultures used in this study were maintained in our stock culture collection and were transferred biweekly at 32°C in M17 broth (24) containing 0.5% glucose or 0.5% lactose. The strains and relevant properties are listed in Table 1. *S. lactis* subsp. *diacetylactis* strain WM_4 was originally obtained as *S. lactis* WM_4 from the New Zealand Dairy Research Institute in 1974 via W. E. Sandine, but since it has the ability to produce Westerfeld-positive material from citrate (unpublished data), it has

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TABLE 1. Strains of *S. lactis* and *S. diacetylactis* used in this study

Strain	Relevant phenotype	Plasmid composition (Mdal)	Comment
S. lactis subsp. *diacetylactis* strain WM_4	Lac^+ Bac^+	88, 33, 30, 5.5, 4.8, 3.8	Parent culture
S. lactis LM2301	Lac^- Bac^-	None	Spontaneous Str^r mutant of LM0230
S. lactis LM2304	Lac^- Bac^-	None	UV-induced Mal^- Neo^r mutant of LM0230
S. lactis WW_4	Lac^+ Bac^+	88, 33	Transconjugant of WM_4 × LM2301
S. lactis PWN1	Lac^+ Bac^+	88, 33, 3.9	Transconjugant of WM_4 × LM2301
S. lactis PWN3	Lac^+ Bac^+	88, 33, 4.8	Transconjugant of WM_4 × LM2301
S. lactis NP1	Lac^- Bac^+	88, 4.8	Transconjugant of WM_4 × LM2301
S. lactis NP2	Lac^- Bac^+	88	Transconjugant of WM_4 × LM2301
S. lactis WN2	Lac^+ Bac^-	55, 3.9	Transconjugant of WM_4 × LM2301
S. lactis WN8	Lac^+ Bac^-	65	Transconjugant of WM_4 × LM2301
S. lactis CS1	Lac^- Bac^+	88	Ethidium bromide-induced mutant of WW_4
S. lactis CS3	Lac^- Bac^-	None	Novobiocin-induced mutant of WW_4
S. lactis WP1	Lac^+ Bac^+	88, 33	Transconjugant of WW_4 × LM2304
S. lactis WN1	Lac^+ Bac^-	62, 33	Transconjugant of WW_4 × LM2304
S. lactis NN1	Lac^+ Bac^-	65	Transconjugant of WN8 × LM2304
S. lactis NN2	Lac^+ Bac^-	55	Transconjugant of WN2 × LM2304
S. lactis DL16	Nis^+	Not examined	

been designated an *S. diacetylactis* strain. *S. lactis* DL16, which produces nisin (Nis^+), was kindly supplied by D. LeBlanc of the National Institutes of Health. The recipient strain LM2304, (lactose negative [Lac^-], maltose negative [Mal^-], neomycin sulfate resistant [Neo^r]) was obtained by UV irradiation of LM0230 followed by selection of a Mal^- mutant. A Mal^- isolate was transferred in Elliker broth (9) containing increasing concentrations of neomycin sulfate until growth was obtained at 2,000 μg of neomycin sulfate per ml. The recipient strain LM2301 (Lac^- Str^r) has been described previously (26).

Screening for production of Bac. The method of Kékessy and Piguet (15) was used for detecting Bac production. One loopful of a 16-h M17-lactose broth culture incubated at 32°C was spot inoculated onto the surface of an Elliker agar plate and incubated at 32°C for 16 to 18 h. The agar was detached from the edges of the petri dish with a sterile spatula. The covered plate was then inverted and tapped sharply on a hard surface so that the agar fell into the lid. Four strains of *S. diacetylactis* (18-16, DRC-3, 11007, and DRC-1), five strains of *S. cremoris* (E8, EB9, ML1, TR, and Z8), and five strains of *S. lactis* (C2, C10, ML3, ML8, and SK1) were tested for sensitivity to Bac. The strains were grown in M17-lactose broth at 32°C for 18 h. A suspension of cells (0.5 ml) was added to a tube containing 7 ml of 45°C-tempered Elliker medium which contained 0.7% agar, mixed, and poured over the surface of the inverted agar. Plates were incubated at 26 or 32°C for 16 to 20 h and examined for zones of inhibition.

Conjugal matings. Conjugal matings between *S. diacetylactis* WM_4 (Lac^+ Str^s) and *S. lactis* LM2301 (Lac^- Str^r) were conducted as previously described (22). Lac^+ Str^r transconjugants were selected on lactose-indicator agar (23) containing 600 μg of streptomycin per ml. In addition, individual Lac^+ Str^r and Lac^- Str^r colonies were selected and tested for Bac-producing ability. Lac^+ Str^r Bac^+ and Lac^+ Str^r Bac^- transconjugants were also used as donors in matings with LM2304, a Lac^- Mal^- Neo^r derivative of LM0230. Lac^+ Neo^r recombinants were selected on lactose-indicator agar containing 600 μg of neomycin sulfate per ml and were confirmed as Mal^- by streaking on indicator agar containing maltose as the sole carbohydrate source.

Phage-typing tests. Transconjugants of the mating between WM_4 and LM2301 were tested for lysis by C2 phage. A 1% inoculum of LM2301 was made into M17-glucose broth and incubated at 32°C for 2 h, at which time a 10% inoculum of C2 phage was introduced.

Incubation continued for an additional 4 h to achieve lysis. The supernatant fraction was collected after centrifugation at 7,500 × *g* and then filter sterilized. The entire procedure was repeated with the fresh phage lysate in a 3.5-h culture. The new lysate was used for phage sensitivity testing. For each strain tested, 0.1 ml of overnight culture was plated onto Elliker agar and allowed to dry. The plates were then spotted with C2 phage lysate. After incubation at 32°C for 16 to 18 h, the plates were examined for zones of clearing.

Curing trials. Two purified Lac$^+$ Str^r Bac$^+$ transconjugants were grown in M17-lactose broth. A 1% inoculum was made into Elliker broth and into Elliker broth containing 6 μg of acriflavin per ml, 30 μg of acridine orange per ml, or 6 μg of ethidium bromide per ml. Each of the transconjugants was also treated with 75 s of UV irradiation as described previously by McKay and Baldwin (21), followed by a 1% inoculum into Elliker broth. All tubes were incubated at 32°C for 16 h. The UV-treated cultures were diluted with 0.85% sodium chloride and plated on lactose-indicator agar for detection of Lac$^-$ variants. An inoculum (1%) from each of the other tubes was transferred into fresh broth with respective curing agents and incubated at 32°C, and the process was repeated. The cultures were then diluted, plated on the lactose-indicator agar, and incubated at 32°C for 40 h. Lac$^-$ isolates were selected and tested for Bac-producing ability as described above. One Lac$^-$ isolate from each treatment was also examined for plasmid DNA by agarose gel electrophoresis.

One Lac$^+$ Str^r Bac$^+$ transconjugant was grown in the presence of novobiocin as described previously by McHugh and Swartz (20). A 1% inoculum was grown in M17-lactose broth containing 50 μg of novobiocin per ml. Tubes were incubated at 32°C for 24 h. The culture was then diluted and plated onto Elliker agar. After incubation for 16 to 18 h at 32°C, the agar was flipped into the lid and overlaid with soft Elliker agar (0.7% agar) containing 0.7% of the indicator strain *S. diacetylactis* 18-16. Incubation continued for 16 to 18 h. Individual colonies were examined for zones of inhibition. Suspect Bac$^-$ colonies were retested for Bac-producing ability and for lactose-fermenting ability. Those lacking the ability to produce Bac were examined for plasmid DNA.

Plasmid isolation. The technique used to obtain plasmid DNA for examination by agarose gel electrophoresis was based on the Currier and Nester procedure (3) as modified by Anderson and McKay (manuscript in preparation). Agarose gel electrophoresis was performed on 10- to 15-μl samples of DNA from cleared lysates as previously described (16).

Bac inactivation studies. Bac$^+$ and Bac$^-$ strains were plated onto Elliker agar and incubated at 32°C for 16 to 18 h. The agar was then flipped into the lid. Plates were subjected to one of three treatments. To test for heat inactivation, we placed the plates at 80°C for 10 to 60 min. To determine whether the inhibitory substance was susceptible to proteolytic enzymes, we added 0.4 ml of an enzyme (1 mg/ml in 0.1 M sodium phosphate buffer, pH 7.0) to the flipped agar. The enzymes used were pronase (Calbiochem Behring Corp., La Jolla, Calif.), trypsin (Sigma Chemical Co., St. Louis, Mo.), and alpha-chymotrypsin (Sigma Chemical Co.). The susceptibility of nisin produced by *S. lactis* DL16 to the proteolytic enzymes was also examined as described above. Plates also were exposed to chloroform vapors for 15 min at 26°C, followed by 30 min of aeration at 37°C. After treatment, all plates were overlaid with soft Elliker agar seeded with *S. diacetylactis* 18-16. After incubation for 16 to 18 h at 32°C, the plates were examined for zones of inhibition.

RESULTS

Detection of Bac production by *S. diacetylactis* WM_4 and its transconjugants. Solid surface matings of Lac$^+$ Str^s *S. diacetylactis* WM_4 with Lac$^-$ Str^r LM2301 yielded two types of Lac$^+$ Str^r transconjugants on lactose-indicator agar: Lac$^+$ colonies growing on a background of confluent Lac$^-$ growth and Lac$^+$ colonies surrounded by a clear zone in the Lac$^-$ background. This latter colony type, which produced an inhibitory substance, was designated Bac$^+$. Representative transconjugants were purified, grown on Elliker agar, and screened for production of Bac. Faint zones of inhibition formed in the agar overlay seeded with *S. lactis* C2 above the Bac$^+$ transconjugants and WM_4 colonies, but not above Bac$^-$ transconjugants or LM2301 colonies. To find a better indicator strain, various group N streptococci were examined for sensitivity to Bac. *S. cremoris* EB9 and two strains of *S. diacetylactis* (11007 and DRC-1) were not sensitive. Two *S. diacetylactis* strains and all five of the examined *S. lactis* strains ranged from slightly (DRC-3, ML3, C2, and SK1) to highly (18-16, C10, and ML8) sensitive, and four *S. cremoris* strains (E8, ML1, TR, and Z8) were highly sensitive. *S. diacetylactis* 18-16 was chosen as the indicator strain for all subsequent Bac studies. Fig. 1 shows the response of individual colonies of the Lac$^+$ Bac$^+$ donor WM_4, the Lac$^-$ Bac$^-$ recipient LM2301, and the two types of Lac$^+$ transconjugants represented by WN2 (Lac$^+$ Bac$^-$) and WW2 (Lac$^+$ Bac$^+$) when screened for production of Bac, using 18-16 as the indicator strain.

Plasmid analysis. Plasmid isolation of *S. diacetylactis* WM_4 by a modification of the method of Currier and Nester (3) combined with agarose gel electrophoresis provided evidence for resident plasmids of about 88, 33, 30, 5.5, 4.8, and 3.8 Mdal (Fig. 2). To examine the plasmid profiles of the transconjugants resulting from WM_4 × LM2301 matings, we selected 10 suspect Lac$^+$ Bac$^+$ and 10 Lac$^+$ Bac$^-$ colonies from conjugation plates. When these isolates were examined for Bac production, using 18-16 as the indicator organism, 3 were found to be Lac$^+$ Bac$^-$, and 17 were Lac$^+$ Bac$^+$. Of 800 Lac$^-$ Str^r colonies tested from the conjugation, 6 were Bac$^+$. The Lac$^+$ Bac$^+$ transconjugants possessed differing plasmid profiles: some contained plasmids of approximately 88 and 33 Mdal (WW4); others possessed three plasmids of

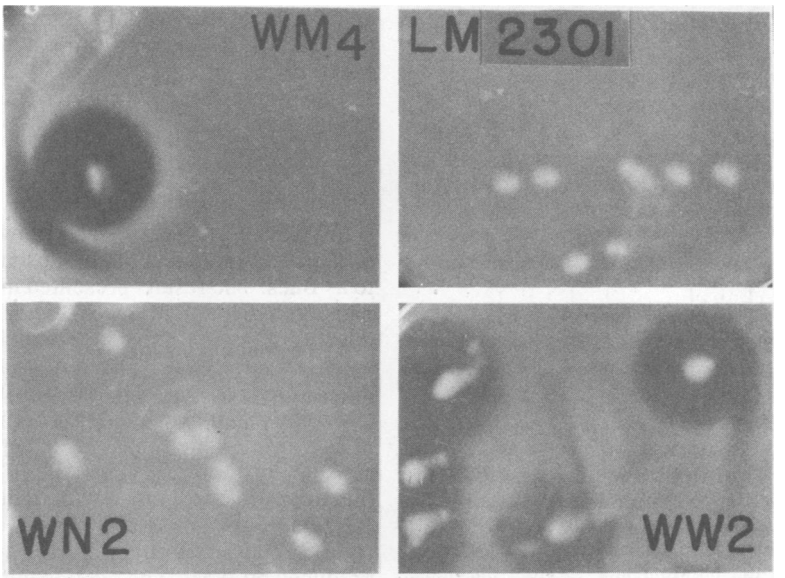

FIG. 1. Growth or inhibition of the indicator strain *S. lactis* subsp. *diacetylactis* strain 18-16 when incorporated into agar and overlaid on individual colonies of Lac$^+$ Bac$^+$ Str^s WM$_4$ (donor), Lac$^-$ Bac$^-$ Str^r LM2301 (recipient), and transconjugants Lac$^+$ Bac$^-$ Str^r WN2 and Lac$^+$ Bac$^+$ Str^r WW2.

about 88, 33, and 4.8 Mdal (PWN3); and one contained plasmids of 88, 33, and 3.9 Mdal (PWN1). Two Lac$^+$ Bac$^-$ transconjugants contained a plasmid of approximately 65 Mdal (WN8); the third Lac$^+$ Bac$^-$ recombinant possessed plasmids of about 55 and 3.9 Mdal (WN2). Of the six Lac$^-$ Bac$^+$ transconjugants, five contained a single plasmid of approximately 88 Mdal (NP2) and one possessed plasmids of about 88 and 4.8 Mdal (NP1) (Fig. 3).

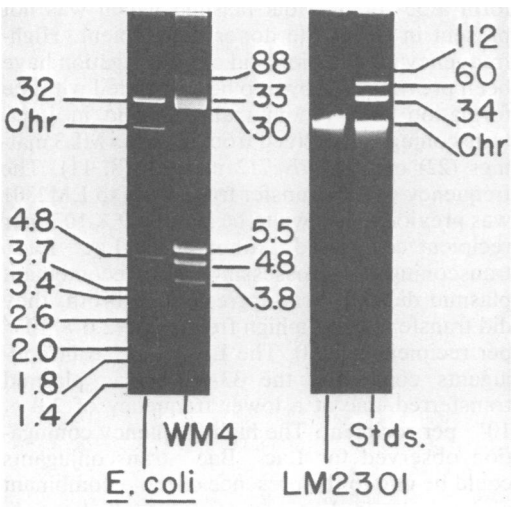

FIG. 2. Agarose gel electrophoresis of plasmid DNA from *S. diacetylactis* WM$_4$ (Lac$^+$ Bac$^+$) and *S. lactis* LM2301 (Lac$^-$ Bac$^-$). *Escherichia coli* V517 (plasmids listed on far left), *E. coli* DT41 (112 Mdal), *E. coli* J5 (34 Mdal), and *Salmonella typhimurium* LT2 (60 Mdal) were used as molecular mass standards as previously described (16).

Curing studies. We attempted to cure two Lac$^+$ Bac$^+$ transconjugants of plasmid DNA by consecutive broth transfers and by broth transfers in the presence of acriflavin, acridine orange, or ethidium bromide. Cells were also treated with UV irradiation to effect loss of plasmid DNA. With acridine orange, no Lac$^-$ derivatives were isolated from either transconjugant. For the spontaneous, acriflavin, ethidium bromide, and UV treatments, 1 to 50 Lac$^-$ derivatives from each transconjugant were tested for Bac-producing ability; all were Bac$^+$. We then examined one Lac$^-$ Bac$^+$ variant from each treatment for plasmid DNA and found that the 33-Mdal plasmid present in Lac$^+$ Bac$^+$ transconjugants was absent in the Lac$^-$ Bac$^+$ derivatives (CS1). The 88-Mdal plasmid was retained. Treatment with novobiocin resulted in one colony with a Bac$^-$ phenotype (CS3). When tested for lactose-fermenting ability, this variant was found to be Lac$^-$. Plasmid analysis revealed the loss of both the 33- and the 88-Mdal plasmids in this derivative (Fig. 3).

Phage testing. WM$_4$ and transconjugants from the mating of WM$_4$ and LM2301 were tested for sensitivity to C2 phage. Phage propagated through LM2301 was spotted onto a spread plate of WM$_4$ or one of its transconjugants. After

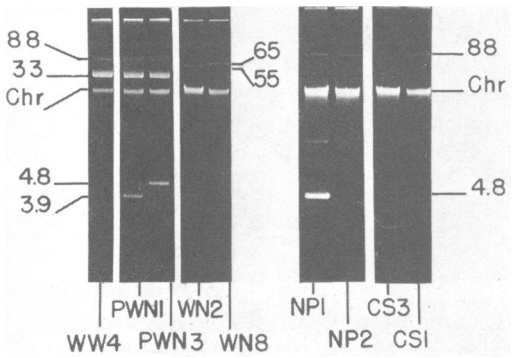

FIG. 3. Agarose gel electrophoresis of plasmid DNA detected in transconjugants obtained from *S. diacetylactis* WM_4 × *S. lactis* LM2301 matings and from derivatives of WW4, a Lac^+ Bac^+ transconjugant. Three types of transconjugants are presented: Lac^+ Bac^+ (WW4, PWN1, PWN3), Lac^+ Bac^-(WN2, WN8), and Lac^- Bac^+ (NP1, NP2). CS1 (Lac^- Bac^+) and CS3 (Lac^- Bac^-) are derivatives of WW4 missing the 33-Mdal or the 88-Mdal and 33-Mdal plasmids, respectively. Molecular masses of plasmids were determined as illustrated in Fig. 2.

overnight incubation, zones of clearing were evident on all of the transconjugants, but not on WM_4.

Bac inactivation. Bac was tested for inactivation by heat, pronase, trypsin, alpha-chymotrypsin, or chloroform. Zones of inhibition were present above Bac^+ strains, regardless of heat treatment or exposure to chloroform. However, no zones of inhibition were evident above Bac^+ cells on plates to which pronase, trypsin, or alpha-chymotrypsin had been added. The nisin produced by *S. lactis* DL16 exhibited sensitivity only to alpha-chymotrypsin.

Conjugation with Lac^+ Bac^- or Lac^+ Bac^+ transconjugants as donors. Transconjugants of both Lac^+ Bac^+ and Lac^+ Bac^- phenotypes were used as donors in matings with *S. lactis* LM2304. Lac^+ Bac^+ transconjugants containing the 88- and 33-Mdal plasmids transferred lactose-fermenting ability at an average frequency of 2.0×10^{-5} per recipient plated. Of 192 Lac^+ transconjugants examined, 176 were Lac^+ Bac^+ and 16 were Lac^+ Bac^-. When examined by agarose gel electrophoresis for plasmid DNA, Lac^+ Bac^+ recombinants contained plasmids of 88 and 33 Mdal (WP1). One Lac^+ Bac^- transconjugant was examined and found to possess plasmids of about 62 and 33 Mdal (WN1).

Lac^+ Bac^- transconjugants containing a 65-Mdal plasmid or a 55-Mdal and a 3.9-Mdal plasmid transferred lactose-fermenting ability at a higher frequency of 2.0×10^{-2} per recipient plated. One hundred Lac^+ recombinants were examined from each of two Lac^+ Bac^- donors; all 200 were Bac^-. Matings between LM2304 and Lac^+ Bac^- recombinants containing a 65-Mdal or a 55-Mdal and a 3.9-Mdal plasmid resulted in transconjugants containing the 65-Mdal (NN1) or the 55-Mdal (NN2) plasmid.

DISCUSSION

Many bacterial species are known to produce proteinaceous antibacterial substances referred to as Bacs. The results presented in this communication indicate that *S. diacetylactis* WM_4 produced a Bac-like substance which was active against a variety of *S. lactis*, *S. cremoris*, and *S. diacetylactis* strains. Treatment with pronase or trypsin resulted in inactivation of this substance, unlike nisin which has been reported to be resistant to these enzymes (6). It is interesting that the antibiotic diplococcin produced by some *S. cremoris* strains is also sensitive to pronase, trypsin, and alpha-chymotrypsin (6), as is Bac produced by WM_4. The relationship between the two inhibitory substances is not known. The ability to produce Bac could be transferred from *S. diacetylactis* WM_4 to *S. lactis* through conjugal matings, and the genetic results indicated that Bac-producing ability is mediated by a plasmid of 88 Mdal. In addition, lactose-fermenting ability was conjugally transferred from *S. diacetylactis* WM_4 to *S. lactis*. Our results suggest that this trait is located on a 33-Mdal plasmid.

In some cases, it appeared that the 33-Mdal plasmid was recombining with other DNA to form a 55- or 65-Mdal plasmid which was not present in either the donor or recipient. High-frequency conjugation and cell aggregation have been previously shown to be associated with the formation of a recombinant plasmid in Lac^+ transconjugants derived from *S. lactis* ML3 matings (22) or *S. lactis* 712 matings (8, 11). The frequency of Lac transfer from WM_4 to LM2301 was previously shown to be about 8.9×10^{-7} per recipient cell plated. Although the Lac^+ Bac^- transconjugants possessing the recombinant plasmid did not form aggregates in broth, they did transfer Lac at a high frequency (2.0×10^{-2} per recipient plated). The Lac^+ Bac^+ transconjugants containing the 33-Mdal Lac plasmid transferred Lac at a lower frequency of 2.0×10^{-5} per recipient. The high-frequency conjugation observed for Lac^+ Bac^- transconjugants could be due to the presence of the recombinant plasmid, as in Lac^+ transconjugants from *S. lactis* ML3 (26). Alternatively, the high conjugation frequency of Lac^+ Bac^- as compared with Lac^+ Bac^+ transconjugants could be due to the absence of inhibitor production by the former type of cell and hence greater survival of recipient cells.

The inhibitors produced by *S. diacetylactis* have received considerable research and industrial attention. Although antagonistic behavior of this organism can be due to a variety of factors, Kozak et al. (17) have demonstrated the production of Bacs by *S. diacetylactis* and *S. lactis*, and Geis et al. (12) have demonstrated Bac production in *S. cremoris*. Both investigators stated that Bacs could account for the incompatibility of starter cultures. In mixed-strain starter cultures, Bac-producing ability could lead to strain dominance. When WW4 (a Lac^+ Bac^+ transconjugant of WM_4) was inoculated into milk with *S. diacetylactis* 18-16 and consecutively transferred at 21°C, WW4 dominated the population after two to three subcultures, whereas WN2 (a Lac^+ Bac^- transconjugant) and 18-16 exhibited equal cell numbers after two to three transfers when subcultured together (unpublished observations). This result indicates that the potential for dominance had been transferred from *S. diacetylactis* WM_4 to *S. lactis* LM2301.

Recently, other workers have examined the potential of lactic streptococci to produce Bacs (13, 17) and have tried to link production of inhibitory substances by these bacteria to plasmid DNA. Kozak et al. (18) reported the appearance of spontaneous nisin-negative (Nis^-) derivatives from Nis^+ strains. The frequency of Nis^- variants increased when the strains were grown in the presence of ethidium bromide or proflavin, or at elevated temperatures, indicative of involvement of plasmid DNA. However, attempts to correlate nisin production to plasmid DNA were not conclusive (10). Davey and Pearce (6) have also been unable to link nisin production to plasmid DNA. They suggested that the *nis* genes of *S. lactis* could be chromosomal or plasmid mediated. In *S. lactis* 11454, LeBlanc et al. (19) reported an association of the Nis^+ and sucrose-fermenting phenotype with a 28-Mdal plasmid.

Davey and Pearce (6) have attempted to link diplococcin production in several *S. cremoris* strains to plasmid DNA. Growing diplococcin producers (Dip^+) at elevated temperatures resulted in the occurrence of diplococcin-negative (Dip^-) isolates at a frequency of 0.1 to 0.2%. No reversion of Dip^- isolates to the Dip^+ parental phenotype was noted. In addition, the ability to produce diplococcin was transferable by conjugation, and Dip^+ transconjugants were able to transfer this ability to other strains within the group N streptococci. These observations suggest the involvement of plasmid DNA, but the authors were unable to link diplococcin production to a plasmid.

Initially, our results with Bac in WM_4 closely resembled those observations reported above for Nis and Dip (K. A. Baldwin and L. L. McKay, Am. Dairy Sci. Assoc. Annu. Meet. Divisional Abstr. 1982, DR16, p. 51). We could find no evidence that the Bac genes were plasmid mediated. However, the recent development of an improved plasmid DNA isolation procedure applicable to the lactic streptococci (D. G. Anderson and L. L. McKay, manuscript in preparation) has enabled us to link this characteristic to an 88-Mdal plasmid. The question arises as to whether the inability of other workers to link plasmid DNA to Nis, Dip, or Bac is also related to the lysis technique used for plasmid isolation. This appears likely since the phenotypic data for the traits as well as conjugal transfer frequency for Dip certainly indicate plasmid involvement.

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