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Genetic Evidence for Plasmid-Linked Lactose Metabolism in *Streptococcus lactis* subsp. *diacetylactis*†

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It has been previously observed that loss of plasmid pGK4101 occurred concomitantly with loss of lactose-fermenting ability in *Streptococcus lactis* subsp. *diacetylactis* 18-16. Transfer of this 41-megadalton plasmid to LM0230, a lactose-negative (Lac^-) strain of *S. lactis*, required cell-to-cell contact and resulted in a conversion of LM0230 to the Lac^+ phenotype. This confirms the linkage of lactose-fermenting ability to the 41-megadalton plasmid in *S. lactis* subsp. *diacetylactis* and, in addition, demonstrates transfer by a process resembling conjugation in the group N streptococci.

Plasmid deoxyribonucleic acid (DNA) appears to play a significant role in the metabolism of group N streptococci. Investigations in this and other laboratories have shown that lactose-fermenting ability, proteinase activity, and the ability to ferment citrate are unstable and associated with extrachromosomal elements (4, 7, 9, 10, 12). The instability of these genes is of major interest in the food industry in that the metabolic activities which they control are essential in the production of many fermented diary products. To date, the sole method of transferring a plasmid-associated characteristic in the group N streptococci has been by transduction (9, 11). Stabilized transductants of *Streptococcus lactis* which have been isolated appear to have lactose-fermenting genes integrated into the chromosome (8). However, transduction is dependent upon the presence of a temperate phage in the donor strain as well as the ability of the phage to transfer the desired genetic material to the recipient. Conjugal transfer, to our knowledge, has not been reported in the group N streptococci. However, this type of plasmid-mediated transfer has been found to occur in other species of streptococci (1-3, 6). Transfer of antibiotic-resistance plasmids and plasmids which determine the synthesis of hemolysin and bacteriocins has been demonstrated in *S. faecalis* (1-3, 6), with many of these plasmids being self-transferable (1, 2). In this communication we report a similar conjugation system involving transfer of plasmid pGK4101 from *S. diacetylactis* strain 18-16 to *S. lactis LM0230.*

The donor strain used in this study was *S. diacetylactis* 18-16, which has been shown to possess six plasmid species (4). Phenotypic data, in addition to physical evidence, indicated that the genes responsible for the ability to ferment lactose are linked to the largest (41-megadalton [Mdal]) plasmid in this strain. The recipient strain, *S. lactis* LM0230, was isolated previously and is a lactose-negative (Lac^-) strain cured of any detectable plasmid DNA (9). A streptomycin-resistant mutant of LM0230 was used in conjugation experiments to allow for recipient selection. Conjugation was performed as follows. Two percent inocula of fresh, overnight broth cultures of *S. diacetylactis* 18-16 and *S. lactis* LM0230 were made into M17-lactose and M17-glucose broth, respectively. After growth at 32°C for 5 h, donor and recipient cells were mixed in a ratio of approximately 1:1. A 0.2-ml amount of these mixtures was then spread onto 5% milk agar plates containing 1% glucose. After 20 h of incubation at 32°C, cells were scraped from the surface of the plates and spread onto lactose indicator agar containing 600 μg of streptomycin sulfate per ml. After 48 h at 32°C, Lac^+ transconjugants were scored. Controls included LM0230 and *S. diacetylactis* 18-16 alone. No reversion of *S. lactis* LM0230 to a Lac^+ phenotype was observed. Spotting with DRC3 phage, which is lytic toward *S. diacetylactis* 18-16, was utilized to distinguish between Lac^+ transconjugants of LM0230 and *S. diacetylactis* 18-16 clones which were resistant to streptomycin. Concentrations of donor and recipient cells in mating mixtures were determined on lactose agar plates.

The frequency of transfer of lactose-fermenting ability from *S. diacetylactis* 18-16 to *S. lactis* LM0230 was extremely low (about 10^{-9} transconjugants per donor). Out of 99 Lac^+ clones obtained from eight separate mating experi-

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ments, 33 were susceptible to lysis by DRC3 phage, thus suggesting that they were streptomycin-resistant revertants of *S. diacetylactis* 18-16. The remaining 66 isolates were assumed to be LM0230 which had acquired the ability to ferment lactose. Control experiments were conducted to determine the nature of the transfer. When culture filtrates of *S. diacetylactis* 18-16 were used in mating mixtures with LM0230, no transfer of lactose-fermenting ability occurred. In addition, *S. diacetylactis* 18-16 was examined for prophage induction by ultraviolet irradiation and mitomycin C. No inducible prophage was demonstrated. This suggested that bacteriophage was not involved in the transfer of the genes associated with lactose utilization. The effect of deoxyribonuclease on the transfer frequency was also examined. Both *S. diacetylactis* 18-16 and LM0230 were incubated with 100 mg of deoxyribonuclease per ml before conjugation on milk agar plates which were also flooded with 100 mg of deoxyribonuclease. No reduction in the frequency of transfer of lactose-fermenting ability was observed. Therefore, the transfer did not appear to be due to the release of transforming DNA from strain 18-16. These control experiments suggested that the transfer of the lactose-fermenting determinants required cell-to-cell contact and appeared to be the result of conjugation. Because lactose-fermenting ability was postulated to be linked to a 41-Mdal plasmid in *S. diacetylactis* 18-16, Lac^+ transconjugants of LM0230 were examined for the presence of this plasmid. Ten lactose-positive clones of LM0230 were purified and subsequently grown and lysed by the method of Klaenhammer et al. for detection and characterization of plasmid DNA (5). The agarose gel electrophoretic pattern obtained for one such conjugant is shown in Fig. 1. In addition, plasmid profiles of the donor and recipient strains, as well as a Lac^- mutant of *S. diacetylactis* 18-16, are included. The gel patterns of all 10 strains examined indicated that Lac^+ LM0230 clones obtained from conjugation contained plasmid DNA corresponding to pGK4101.

Currently, the rate of conjugal transfer from *S. diacetylactis* 18-16 to LM0230 is extremely low. Nevertheless, this appears to be the first instance where conjugal transfer of plasmid DNA has been demonstrated in the group N streptococci. In addition, this confirms the linkage of lactose-fermenting ability to plasmid pGK4101 in *S. diacetylactis* 18-16. Because this 41 Mdal plasmid appears to be self-transmissible, studies are currently being conducted to determine whether the plasmid is capable of mobilization of other plasmids and chromosomal genes.

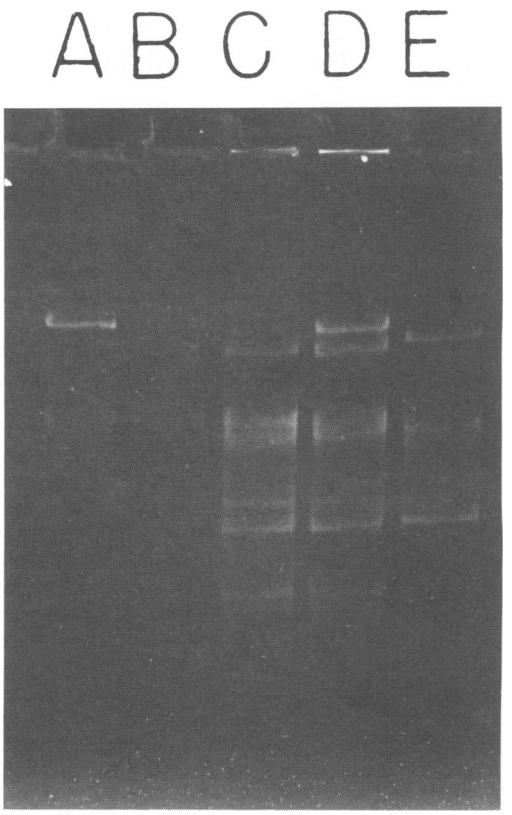

FIG. 1. *Agarose gel electrophoretic patterns of plasmid DNA isolated from ethanol-precipitated cleared lysates. (A) Lac^+ transconjugant of LM0230, demonstrating presence of pGK4101. Diffuse bands seen in all wells are composed of chromosomal fragments. (B) S. lactis strain LM0230 (recipient). (C) S. diacetylactis GK1, a Lac^- mutant of strain 18-16 which is missing plasmid pGK4101. (D) S. diacetylactis 18-16 containing plasmids (top to bottom) pGK4101 (41 Mdal), pGK2801 (28 Mdal), pGK0641 (6.4 Mdal), pGK0551 (5.5 Mdal), pGK0341 (3.4 Mdal), and pGK0301 (3.0 Mdal); (E) Escherichia coli reference mobility markers RP4 (34 Mdal) and RSF1010 (5.5 Mdal).*

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