Transformation of *Streptococcus lactis* Protoplasts by Plasmid DNA†

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Polyethylene glycol-treated protoplasts prepared from *Streptococcus lactis* LM3302, a lactose-negative (Lac-) derivative of *S. lactis* ML3, were transformed to lactose-fermenting ability by a transductionally shortened plasmid (pLM2103) coding for lactose utilization.

*Streptococcus lactis* is an industrially important microorganism used in dairy fermentations. Strain improvement using recombinant DNA techniques and the study of the genetic organization of group N streptococci have been limited due to the inability of strains to be transformed by plasmid DNA.

Transformation of plasmid DNA in streptococci has been demonstrated for streptococcal groups F (9, 13) and H (2, 3, 9, 14-17), *Streptococcus pneumoniae* (1, 2, 12-23), and *Streptococcus mutans* (20), but these competence-dependent procedures appear to be unsatisfactory for transformation in *S. lactis* (unpublished data). Recently, transformation of polyethylene glycol (PEG)-treated protoplasts with plasmid DNA has been successful in *Staphylococcus aureus* (19), *Bacillus subtilis* (6), *Bacillus megaterium* (5), *Bacillus thuringiensis* (17), *Streptomyces* sp. (4), and *Saccharomyces cerevisiae* (10). This communication describes the PEG-induced transformation of *S. lactis* protoplasts by plasmid DNA.

*S. lactis* LM3302, a lactose-negative (Lac-) derivative of *S. lactis* ML3 (24, 26), was the recipient in all experiments. The plasmid used for transformation (pLM2103) was isolated from *S. lactis* LM0231 (18). Plasmid pLM2103 is a 20-megadalton (Mdal) transductionally shortened plasmid coding for lactose-fermenting ability (7).

Isolation of plasmid DNA was performed by procedures developed by Hansen and Olsen (8) and later modified by Walsh and McKay (26) for *S. lactis*. The crude PEG-concentrated DNA was subjected to CsCl-ethidium bromide density gradient centrifugation (12), and plasmid DNA was dialyzed in TE buffer (10 mM Tris-hydrochloride, 1 mM EDTA, pH 8.0) for 4 days with daily buffer changes. Plasmid DNA was concentrated with sec-butanol and ethanol precipitated. The extensive dialysis and ethanol precipitation steps are required since protoplasts are very sensitive to residual sodium dodecyl sulfate from the plasmid isolation procedure (6). An initial 1,600-ml volume of cells was needed so that a plasmid band could be visualized after CsCl-ethidium bromide density gradient centrifugation.

Protoplast formation in group N streptococci using mutanolysin (27) was performed as described elsewhere (Kondo and McKay, submitted for publication). A 1% inoculum from an overnight M17-lactose (M17-L) broth (25) culture of *S. lactis* LM3302 was transferred to 30 ml of M17-L broth. Cells were harvested after 2 h of growth at 32°C (approximately 2 x 10⁷ colony-forming units per ml), washed with distilled water, and suspended in 4.0 ml of 0.5 M sucrose. A 1.9-ml amount of cell suspension was mixed with 1.9 ml of 0.5 M sucrose in 0.02 M Tris-hydrochloride (pH 7.0), and 0.2 ml of mutanolysin (1 mg/ml) was added. Protoplasts were produced after incubation at 37°C for 20 min.

Transformation was performed essentially as described by Chang and Cohen for *B. subtilis* (6). Protoplasts were harvested by centrifugation at 2,600 x g for 10 min, washed with SMM buffer (0.5 M sucrose, 0.02 M maleate, 0.02 M MgCl₂, pH 6.5), and suspended in 0.5 ml of SMM buffer. Forty microliters of plasmid DNA (0.117 µg/µl) in an equal volume of 2 x SMM was added to protoplasts, followed immediately by the addition of 1.5 ml of 40% PEG. The PEG solution contained 40 g of PEG 8000 (Union Carbide; approximate molecular weight, 6,000) and 50 ml of 2 x SMM in 100 ml. After 2 min, 5.0 ml of SMM was added to dilute the PEG. Protoplasts were recovered by centrifugation at 4,500 x g for 10 min and were suspended in 1 ml of SMM. Transformants were selected by plating directly onto M17-L agar plates containing bromocresol purple (40 mg/liter) as an indicator.

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and 0.5 M sucrose for protoplast stabilization. β-Glycerophosphate as a buffering agent was omitted so acid-producing colonies could be scored. Plates were incubated at 25°C and examined daily for Lac" transformants.

Using independent DNA preparations and transformation trials, Lac" transformants were observed after 5 days at a frequency of about 8.5 transformsants per μg of DNA. Acquisition of pLM2103 by Lac" LM3302 was suggested by the Lac" phenotype of transformants and was confirmed by agarose gel electrophoresis of plasmid DNA isolated from 19 Lac" transformants. All transformants contained a plasmid of approximately 20 Mdal in addition to the normal plasmid complement of LM3302 (Fig. 1). Since no transformants were observed when pancreatic deoxyribonuclease I (20 μg in 5 μl of SMM) was added to transforming DNA 1 h before transformation trials, the mechanism of genetic exchange was dependent upon the uptake of free DNA. Reversion of Lac" protoplasts to Lac" colonies did not occur, and transforming DNA was devoid of viable bacterial cells.

Results of this study indicate that plasmid DNA coding for lactose metabolism is able to transform Lac" S. lactis LM3302 protoplasts to the ability to utilize lactose. This is the first demonstration of plasmid transformation in group N streptococci and may provide the opportunity of improving dairy starter cultures with recombinant DNA techniques. A more extensive characterization of protoplast transformation is now in progress.

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LITERATURE CITED


