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Genetic Transformation of Intact *Lactococcus lactis* subsp. *lactis* by High-Voltage Electroporation†

DEBORAH A. McINTYRE AND SUSAN K. HARLANDER*

Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55108

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To apply recombinant DNA techniques for genetic manipulation of the industrially important lactococci, an efficient and reliable high-frequency transformation system must be available. High-voltage electric pulses have been demonstrated to enhance uptake of DNA into protoplasts and intact cells of numerous gram-negative and gram-positive microorganisms. The objective of this study was to develop a system for electroporating intact cells of *Lactococcus lactis* subsp. *lactis* LM0230 (previously designated *Streptococcus lactis* LM0230) with a commercially available electroporation unit (BTX Transfector 100; BTX, Inc., San Diego, Calif.). Parameters which influenced the efficiency of transformation included growth phase and final concentration of cells, ionic strength of the suspending medium, concentration of plasmid DNA, and the amplitude and duration of the pulse. Washed suspensions of intact cells suspended in deionized distilled water were subjected to one high-voltage electric pulse varying in voltage (300 to 900 V corresponding to field strengths of 5 to 17 kV/cm) and duration (100 μs to 1 s). Transformation efficiencies of 10^3 transformants per μg of DNA were obtained when dense suspensions (final concentration, 5×10^{10} CFU/ml) of stationary-phase cells were subjected to one pulse with a peak voltage of 900 V (field strength, 17 kV/cm) and a pulse duration of 5 ms in the presence of plasmid DNA. Dilution of porated cells in broth medium followed by an expression period of 2 h at 30°C was beneficial in enhancing transformation efficiencies. Plasmids ranging in size from 9.8 to 30.0 kilobase pairs could be transformed by this procedure.

Electroporation involves the application of high-voltage electric field pulses of short duration to induce the formation of transient pores in the membranes of cells. Under appropriate conditions, the diffusion and exchange of intracellular and extracellular components can take place during the lifespan of the pore (7, 32), and macromolecules such as DNA present in the suspending medium may enter into the treated cells. This technique has been shown to be extremely effective in enhancing the transfer of genetic information into mammalian cells (17, 20, 22, 24, 28), plant protoplasts (18, 19, 25, 31), and nonprotoplasted (intact) yeast cells (10).

In addition to eucaryotic cells, electroporation has been used to enhance transformation in bacterial systems. Shivarova et al. (26) were the first to demonstrate this application using *Bacillus cereus* protoplasts. Shortly thereafter, protoplasts of *Streptomyces lividans* (13), *Enterococcus faecalis* (6), and *Lactococcus lactis* subsp. *lactis* (previously designated *Streptococcus lactis*) (21) were also transformed by electroporation. However, this method offered little advantage over standard protoplast transformation protocols which require optimization of numerous strain-dependent parameters for efficient transformation and regeneration. If applicable to intact bacterial cells, electroporation, as a physical method for enhancing uptake of DNA, would offer a viable alternative to transformation methods employing such chemicals as $CaCl_2$ and polyethylene glycol.

Our laboratory was the first to demonstrate electroporation of intact cells of *L. lactis* subsp. *lactis* LM0230. Washed, untreated cells of *L. lactis* LM0230 were subjected to a single 30-μs pulse (field strength, 6 to 8 kV/cm) delivered during centrifugation of cell suspensions (9). Transformation efficiencies of 10^4 transformants per μg of DNA were obtained, which was in the range of efficiencies obtained by polyethylene glycol-induced protoplast transformation (11, 12) or polyethylene glycol-induced transformation of intact cells (23). Subsequently, numerous investigators have demonstrated electroporation of a host of intact gram-negative and gram-positive organisms. *Escherichia coli* and *Pseudomonas putida* have been transformed at efficiencies of 10^4 to 10^5 transformants per μg of plasmid DNA by electroporation (6), while efficiencies of 10^6 transformants per μg DNA were obtained with *Campylobacter jejuni* (16). In addition, Calvin and Hanawalt (3) reported efficiencies exceeding 10^9 transformants per μg of DNA with *E. coli* using an electroporation unit constructed in their laboratory. Recent reports have also confirmed the effectiveness of electroporation with intact gram-positive cells, including *Lactobacillus casei* (4; P. M. Muriana, J. B. Luchansky, and T. R. Klaenhammer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, H64, p. 155), *Streptococcus thermophilus* (27), 10 different strains of *L. lactis* and *L. lactis* subsp. *cremoris* (21), and strains from other genera including *Enterococcus*, *Bacillus*, and *Staphylococcus* (Muriana et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). All the gram-positive organisms were transformed, with highest efficiencies ranging from 10^3 to 10^5 transformants per μg of DNA.

Although electroporation has been successfully demonstrated for each of these strains, transformation efficiencies and optimum electroporation conditions appear to vary from strain to strain. In addition, successful electroporation is highly dependent on the duration, field strength, and shape of the pulse delivered by different electroporation machines. In this communication, we present studies on transformation of intact cells of *L. lactis* LM0230 and evaluate the use of a new instrument, the BTX Transfector 100 system (BTX, Inc., San Diego, Calif.), which has the ability to deliver higher field strengths than other commercially available units. Parameters which influence the efficiency of transfor-

* Corresponding author.

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TABLE 1. Bacterial strains used in this study

Strain	Plasmid designation (kb)	Relevant phenotype[a]
Lactococcus lactis subsp. *lactis* LM0230	None	Lac^- Em^s Tet^s
Lactococcus lactis LM0232	pLM2001 (30)	Lac^+
Lactococcus lactis JK301	pGB301 (9.8)	Em^r
Escherichia coli SA3	pSA3 (9.8)	Em^r
Escherichia coli CG120	pAM120 (21.4)	Tet^r

[a] Relevant phenotype: Lac^+, lactose fermenting; Lac^-, lactose nonfermenting; Em^s, erythromycin sensitive; Em^r, erythromycin resistant; Tet^s, tetracycline sensitive; Tet^r, tetracycline resistant.

mation, including growth phase and cell concentration, ionic strength of the suspending medium, concentration and size of plasmid DNA, the amplitude and duration of the electric pulse, and cell expression conditions, are discussed.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. *L. lactis* LM0230 and LM0232 were grown in M17 broth (30) supplemented with 0.5% (wt/vol) glucose (M17-Glu) or 0.5% lactose (M17-Lac), respectively. *L. lactis* JK301 harboring the 9.8-kilobase-pair (kb) vector pGB301 (2) was propagated in M17-Glu containing 10 μg of erythromycin per ml. Lactococcal strains were incubated at 32°C. *E. coli* SA3 harboring the shuttle plasmid vector pSA3 (5) was grown at 37°C in Luria-Bertani broth (14) containing 10 μg of erythromycin per ml. *E. coli* CG120 containing the chimeric plasmid pAM120 (8) was grown at 37°C in Luria-Bertani broth containing 4 μg of tetracycline per ml.

Isolation of plasmid DNA. Plasmid DNA was isolated from *L. lactis* JK301 and LM0232 by the method described by Anderson and McKay (1) and from *E. coli* SA3 and CG120 by the alkali lysis procedure (14). Purification of plasmid DNA by CsCl density gradient centrifugation in the presence of ethidium bromide and analysis by agarose gel electrophoresis were done by standard methods described by Maniatis et al. (14).

DNA transformation by electroporation. An overnight culture of *L. lactis* LM0230 (1 ml) was inoculated into 50 ml M17-Glu broth and grown to various growth phases (optical density at 600 nm = 0.2, 0.45, 0.7, and 1.2, corresponding to early-log, mid-log, late-log, and stationary phases, respectively). Cells were harvested by centrifugation (5,500 × *g*, 10 min, 4°C) and washed twice in 10 ml of ice-cold deionized distilled H_2O (ddH_2O). The cell suspension was centrifuged for 10 min (12,000 × *g*, 4°C) after the first ddH_2O wash and for 15 min after the second ddH_2O wash. Pellets were then suspended in 1 ml of ddH_2O and transferred to a 1.5-ml microcentrifuge tube. After centrifugation at full speed in an Eppendorf model 5414 centrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.) for 3 min, cells were suspended in 1.25 ml of ice-cold ddH_2O (approximately 5×10^{10} CFU/ml) and held on ice for not longer than 2 h.

Purified plasmid DNA (0.01 to 4 μg) was added per 300 μl of cell suspension. DNA and cells were mixed thoroughly by vortexing at slow speed for 15 s and held on ice for 10 min prior to electroporation. A 300-μl portion of the DNA-cell mixture was transferred to a semi-micro disposable cuvette (VWR Scientific Inc., Chicago, Ill.) and exposed to one or more high-voltage electric pulses with the BTX Transfector 100 system described below. Following electroporation, cells were held on ice for 10 min. For expression experiments, porated cells were serially diluted in M17-Glu broth (1:5, 1:10, and 1:100) and incubated at 30°C for 2 h before plating. Survivors were enumerated on M17-Glu agar following incubation at 32°C for 24 h. Plasmid pGB301 and pSA3 transformants were enumerated on M17-Glu agar containing 2.5 μg of erythromycin per ml, pLM2001 transformants were differentiated on M17-Lac plates containing bromcresol purple as a pH indicator, and pAM120 transformants were selected on M17-Glu plates containing 4 μg of tetracycline per ml, following incubation at 32°C for 36 to 48 h. Controls included experimental trials in which either the electric pulse or plasmid DNA was omitted. Transformants were confirmed by the presence of plasmid DNA following agarose gel electrophoresis.

Electroporation apparatus. High-voltage pulses were applied with a BTX Transfector 100 apparatus containing a 400-μF capacitor capable of generating field strengths (*E*) of up to 17 kV/cm. The electrode head assembly consisted of two flat parallel metal electrodes separated by a 0.5-mm gap which were inserted into a semi-micro disposable cuvette containing the DNA-cell suspension. After a pulse duration ranging from 100 μs to 1 s and an output voltage of 0 to 900 V (E = 0 to 17 kV/cm) were selected, the pulse was generated by the BTX Transfector 100 and delivered to the cell-DNA suspension between the electrodes. The maximum voltage attainable with the BTX Transfector 100 unit was 900 V. To increase the field strength beyond 17 kV/cm, a chamber assembly with a narrower gap distance would be required.

Analysis of the pulse generated by the BTX Transfector 100 was possible by coupling the unit in parallel with the BTX Optimizor. A typical pulse resulted in an exponential decay of the electric potential applied by the capacitor discharge system. The length of this pulse was defined as the duration between the steep front of the pulse and the time it took for the pulse to decay in amplitude to $1/e$, which was typically one-third of the maximum voltage applied. Following each pulse delivery, the actual field strength (kilovolts per centimeter), peak voltage (volts), pulse length (microseconds, milliseconds, or seconds), and resistivity (ohm-centimeters) of the cell suspension were recorded on the Optimizor. The change in resistivity of a sample was recorded both before and after pulse delivery.

RESULTS

Optimization of electroporation procedure for *L. lactis*. To study the parameters affecting transformation by electroporation, we selected strain LM0230, a plasmid-free derivative of *L. lactis* C2, as the recipient in these studies.

Culture age and concentration. Washed cell suspensions at various stages of growth (optical density at 600 nm = 0.2, 0.45, 0.7, and 1.2, corresponding to early-log, mid-log, late-log, and stationary phases, respectively) were diluted to cell concentrations of 10^{10}, 10^9, 10^8, and 10^7 CFU/ml and then electroporated to study the effect of these parameters on transformation efficiency. Aliquots were subjected to one 5-ms pulse in the presence of 1 μg of pGB301 plasmid DNA at field strengths of 13, 15, and 17 kV/cm. Cells survived exposure to high field strengths with less than a 10-fold decrease in cell number (data not shown). Figure 1 illustrates the transformation efficiencies obtained at each field strength tested. A marked increase in efficiency was noted with stationary-phase cells at high cell concentrations (5×10^{10}

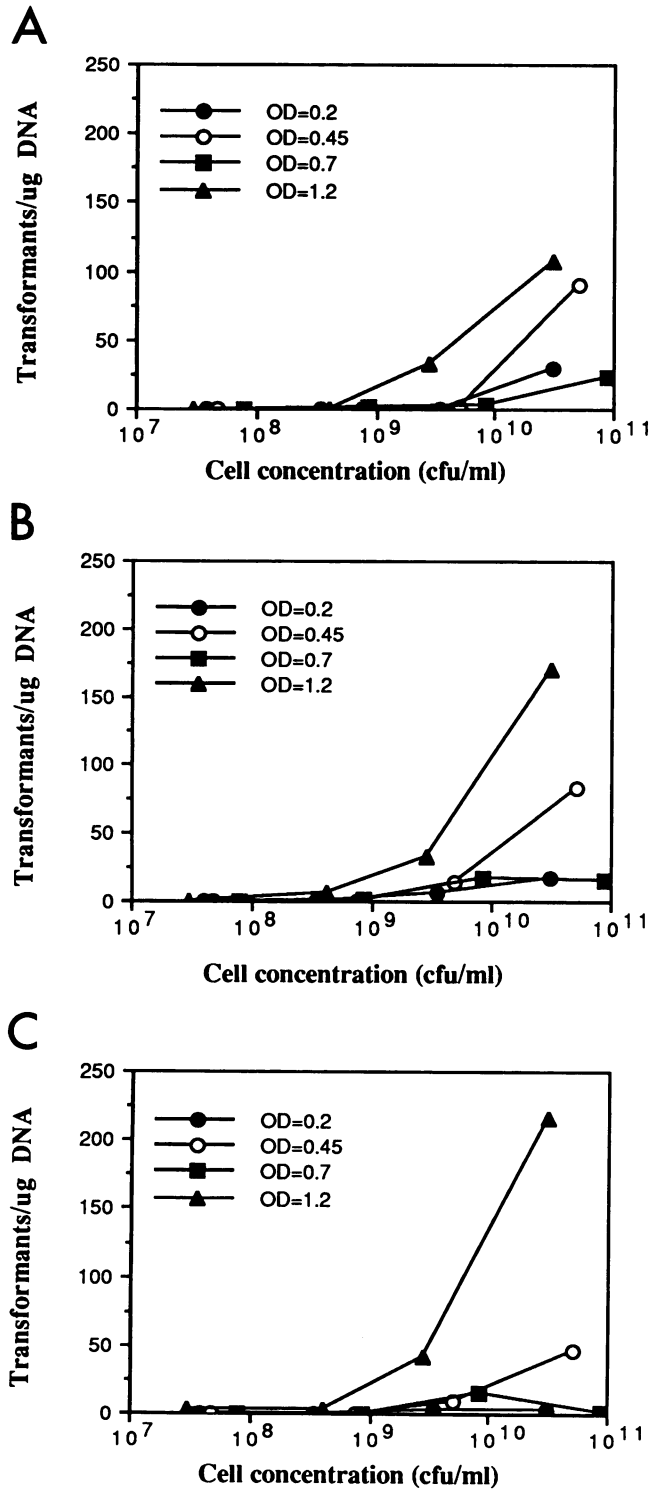

FIG. 1. Electroporation of *L. lactis* LM0230 at various phases of the growth cycle and at various cell concentrations. A 5-ms pulse was delivered at 3 voltages: 700 V (A), 800 V (B), and 900 V (C), which correlated with field strengths of 13, 15, and 17 kV/cm, respectively. Trials were performed once for each cell concentration at each voltage, and 1 μg of pGB301 DNA was used for each trial.

TABLE 2. Effect of ionic strength of suspending medium on transformation efficiency

Medium[a]	Conductivity (mS/cm)	Field strength (kV/cm)	Pulse length (ms)	Transformation efficiency (transformants/μg of pGB301 DNA)
EPB	2.04	6.64	3.74	14
		9.68	3.21	12
		13.44	1.25	9
		17.28	0.60	18
EPM	1.43	6.88	4.64	17
		9.68	4.30	29
		13.68	4.02	17
		17.44	4.12	3
EB	0.50	7.68	5.10	27
		10.00	5.53	26
		13.60	5.27	24
		17.36	4.47	8
ddH_2O	0.07	6.80	5.84	27
		9.76	5.84	12
		13.68	5.90	83
		17.60	5.53	396

[a] EPB, 0.5 M sucrose, 1 mM $MgCl_2$, 7 mM K_2HPO_4-KH_2PO_4 (pH 7.4); EPM, 5 mM K_2HPO_4-KH_2PO_4, 1 mM $MgCl_2 \cdot 6H_2O$, 0.3 M raffinose (pH 7.4); EB, 7 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4), 272 mM sucrose, 1 mM $MgCl_2$.

CFU/ml); however, transformation efficiency did not appear to reach a peak value, indicating that higher efficiencies may be attainable with higher field strengths. Unless otherwise indicated, subsequent electroporation experiments were conducted with high cell concentrations subjected to one 5-ms pulse at 17 kV/cm.

Ionic strength of suspending medium. The effect of the ionic strength of the suspending medium on pulse length and transformation efficiency was compared by using several resuspending buffers and ddH_2O. The composition of each buffer and resulting transformation efficiencies are listed in Table 2. *L. lactis* LM0230 suspensions were porated in the presence of 1 μg of pGB301 DNA at output voltages of 300 to 900 V (E = 6 to 17 kV/cm). Although the duration was 5 ms, the actual pulse length delivered depended on the ionic strength of the suspending buffer. Resistivity readings were measured directly from the BTX Optimizor (ohm-centimeters) and converted to units of conductivity (millisiemens per centimeter).

DNA concentration. The effect of plasmid DNA concentration on the total number of transformants obtained by electroporation was examined. Various concentrations (0.01, 0.05, 0.1, 0.5, 1, 2, and 4 μg) of purified pGB301 plasmid DNA were added to LM0230 cell suspensions, and cells were electroporated under standard conditions. In an average of two trials, the DNA dose-response curve was linear between 10 ng and 1.0 μg of plasmid DNA (data not shown); concentrations higher than 1 μg increased the transformation efficiency only minimally.

Plasmid size. Table 3 lists the efficiencies obtained when purified plasmid DNA of various sizes was introduced into LM0230 by electroporation. Each trial was performed with 1 μg of DNA under standard conditions. Results indicate that plasmids as large as 30 kb can be introduced by electroporation with no loss of efficiency.

Pulse duration and voltage. Cell suspensions of LM0230 were pulsed throughout the range of pulse durations available on the BTX Transfector 100 (100 μs, 500 μs, 1 ms, 5 ms, 10 ms, 50 ms, 100 ms, 500 ms, and 1 s) and through a range of field strengths (5, 9, 13, and 17 kV/cm) in the presence of

TABLE 3. Effect of plasmid size on transformation efficiency of *L. lactis* LM0230[a]

Plasmid (kb)[b]	Transformants/μg of DNA[c]
pGB301 (9.8)	1.1×10^1–1.0×10^3
pSA3 (9.8)	7.0×10^2
pAM120 (21.4)	4.2×10^2
pLM2001 (30.0)	1.1×10^3

[a] Stationary-phase cells were used (approximately 5×10^{10} CFU/ml).
[b] DNA was added as 1 μg/300-μl cell suspension.
[c] Electroporation was done at 900 V and 5-ms pulse length. Efficiencies for pGB301 are shown as a range over 13 trials. Results for other plasmids represent one trial.

1 μg of plasmid DNA. Figure 2 illustrates the number of transformants obtained at set pulse durations of 1, 5, 10, and 50 ms, respectively, and the actual pulse length delivered over the range of field strengths. One microgram of DNA was used for each sample. Other pulse durations tested were 100 μs, 500 μs, 100 ms, 500 ms, and 1 s. For the shorter set pulse durations (100 and 500 μs), few transformants were obtained; for the longer set pulse durations (100 ms, 500 ms, and 1 s), the actual pulse length delivered was extremely variable (data not shown).

Expression. *L. lactis* LM0230 cells were diluted (1:5, 1:10, and 1:100) in M17-Glu broth following electroporation and incubated at 21 or 30°C for various intervals (15 min, 1 h, or 2 h) to determine the effect on transformation efficiency. To obtain consistent results, a minimum regeneration time of 2 h was required. Table 4 shows the number of survivors and transformants obtained before (initial) and after (final) the 2-h regeneration. The ratio of final/initial values allowed the comparison of the relative increase in survivor and transformant numbers. The survivors increased 3 to 4 times the initial value when diluted 1:100, whereas the transformant numbers increased 30 to 50 times the initial value, indicating that for certain studies it may be desirable to regenerate porated cells under nonselective conditions prior to plating onto selective media.

TABLE 4. Effect of 2-h expression period on *L. lactis* survivors and transformants[a]

Temp (°C)	Dilution	Survivors (CFU/ml)			Transformants (CFU)/μg of DNA		
		Initial (10^{10})	Final (10^{10})	Final/initial	Initial	Final	Final/initial
21	1:5	2.2	3.7	1.7	24	135	5.6
	1:10	2.2	5.1	2.3	24	270	11.3
	1:100	2.2	8.0	3.6	24	750	31.3
30	1:5	2.5	3.6	1.5	21	233	11.1
	1:10	2.5	1.9	0.8	21	435	20.7
	1:100	2.5	10.0	4.2	21	1,050	50.0

[a] Cells were porated in the presence of 1 μg of pGB301 DNA at 900 V (E = 17 kV/cm) and 5-ms pulse length and expressed in M17-Glu medium.

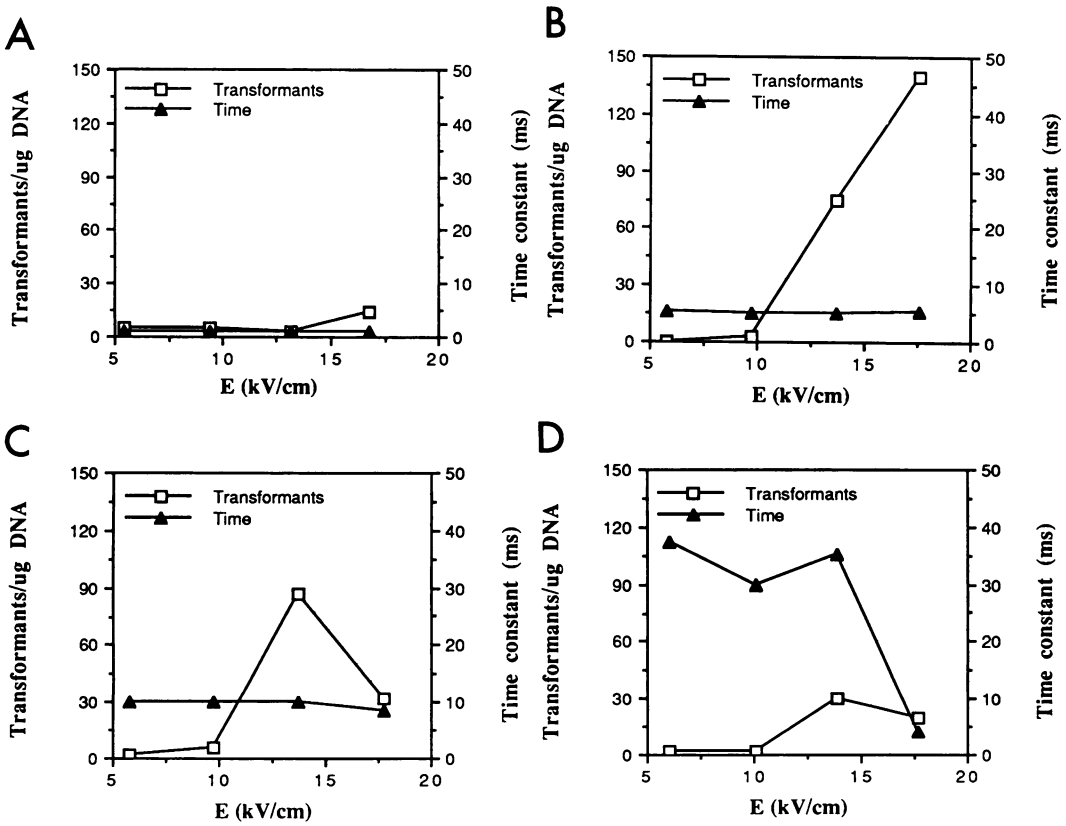

FIG. 2. Cell suspensions of *L. lactis* LM0230 pulsed through the range of durations and field strengths available on the BTX Transfector 100. Panels A to D illustrate the number of transformants obtained at set pulse durations of 1, 5, 10, and 50 ms, respectively, and illustrate the actual pulse length (▲) delivered over the range of field strengths (5, 9, 13, and 17 kV/cm). Trials were performed once for each of the pulse duration-voltage combinations, and 1 μg of pGB301 DNA was used for each trial.

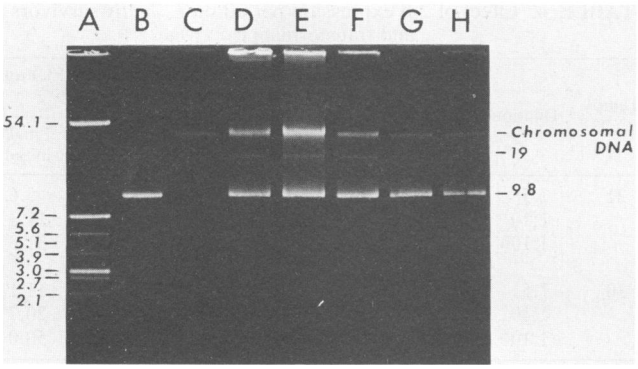

FIG. 3. Agarose gel electrophoresis of plasmid DNA isolated from transformant clones. Lane A, DNA from *E. coli* V517; lane B, pGB301 plasmid DNA isolated from *L. lactis* and purified by CsCl density gradient centrifugation (monomer weight, 9.8 kb; dimer weight, 19 kb); lane C, DNA from *L. lactis* LM0230 recipient; lanes D to H, DNA present in lysates enriched for plasmid DNA from five randomly selected erythromycin-resistant clones. Electrophoresis conditions were 0.6% agarose gel in TAE buffer (0.04 M Tris acetate, 0.002 M EDTA, pH 8.0) for 2 h at 60 V. Numbers on left show plasmid sizes in kilobases.

Confirmation of plasmid transfer by electroporation. Following incubation for 36 to 48 h, representative transformant colonies were subcultured in the appropriate broth media, tested for catalase and Gram-stain reactions, and subjected to small-volume lysis and agarose gel electrophoresis to confirm the presence of the appropriate plasmid (Fig. 3).

DISCUSSION

An important prerequisite for applying genetic techniques for improvement of the industrially important lactic acid bacteria is an efficient and reliable high-frequency transformation system. Electroporation offers a relatively simple, rapid, and reliable alternative to currently available protoplast transformation procedures. However, to apply electroporation, a number of electrical and biological parameters must be optimized for each instrument and strain to be porated.

Variations in growth phase and cell concentration between strains, as well as the ionic strength of the electroporation medium, all appear to influence the success of electroporation. Among the most important parameters for bacteria, however, are the field strength, pulse duration, and shape of the pulse. The BTX Transfector 100 electroporation system was used to optimize conditions for transformation of intact cells of *L. lactis* subsp. *lactis* LM0230. Transformation efficiencies between 1.1×10^1 and 1.0×10^3 transformants per μg of DNA were obtained when dense suspensions of ddH_2O-washed stationary-phase cells were subjected to one 900-V pulse for 5 ms in the presence of 1 μg of plasmid DNA. Consistently higher efficiencies could be obtained if porated cells were allowed to regenerate for 2 h at 30°C.

The conditions used in this study differed somewhat from procedures which have been successfully employed in other systems. With mid-log-phase cells at a density of 2×10^8 CFU/ml, strains of *L. casei* were porated at 5 kV/cm, resulting in efficiencies ranging from 0 to 8.5×10^4 transformants per μg of DNA (4). Strains of *S. thermophilus* were porated at field strengths of 3 to 4 kV/cm, and efficiencies of 0 to 5×10^3 transformants per μg DNA were obtained (27). Powell et al. (21) obtained from 8×10^0 to 5×10^5 transformants per μg of DNA with *L. lactis* strains porated at 6.25 kV/cm; however, to achieve efficiencies of 10^4 to 10^5 transformants per μg of DNA, partial protoplasting of cells with lysozyme was required prior to electroporation.

With the BTX Transfector 100 system, transformation efficiencies decreased dramatically if cells were diluted prior to electroporation or if early- or late-log-phase cells were used (Fig. 1). Other investigators have recommended the use of early- or mid-log-phase cells and have utilized cell concentrations in the range of 2×10^8 to 5×10^9 CFU/ml (4, 16, 21; Muriana et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1988). Strain-dependent variations in the optimal phase of growth for electroporation of *E. coli* have been noted (3), and this may also apply to gram-positive bacteria.

The medium used for washing and suspending cells before and during poration also appeared to influence electroporation efficiency (Table 2). Cells suspended in ddH_2O were more readily porated than cells suspended in EPB (21), EPM (27), or EB (4). Although the pulse duration was set at 5 ms for each trial, the actual pulse length delivered depended on the conductivity of the suspending solution. The BTX Transfector 100 contains a bank of timing resistors which can be switched into the circuit by the pulse duration selector. At a given pulse duration, if the chamber resistance (i.e., the resistance of the electrode and suspending solution) is greater than the timing resistance, then the instrument setting determines pulse length. However, when the chamber resistance is less than the timing resistance, the resistance of the chamber determines the longest pulse length which can be delivered at a set voltage. Use of the highest ionic strength and most conductive solutions (EPB and EPM) resulted in reduced pulse duration (as low as 0.60 ms for EPB-suspended cells subjected to a 900-V pulse and 4.02 ms for EPM-suspended cells at 700 V) and transformation efficiencies. The highest transformation efficiency was obtained when the least conductive suspending solution, distilled water, was used. Results were also more reproducible with ddH_2O, since the ions present in the other suspending media limited the duration of the pulse.

The concentration of purified plasmid DNA influenced the number of transformants obtained. Transformation efficiencies increased linearly with the addition of between 10 ng and 1.0 μg of pGB301 DNA; however, a minimal increase in the number of transformants was observed when greater than 1 μg of DNA per trial was used. Miller et al. (16) and Taketo (29) reported linear DNA dose-response curves over a wide range of DNA concentrations (Miller et al. [16], 0.02 to 10 μg/ml; Taketo [29], 500 pg/ml to 5 μg/ml). In contrast, Powell et al. (21) tested DNA concentrations ranging from 0.01 to 5.0 μg and demonstrated that higher transformation efficiencies were obtained when lysozyme-treated *L. lactis* LM0230 cells were porated in the presence of smaller amounts of DNA. Similar results have been obtained with *E. coli* (3).

Plasmid size did not appear to be a limitation since pLM2001 (30 kb) and pAM120 (21.4 kb) were able to transform *L. lactis* LM0230 at efficiencies equivalent to that obtained with the smaller plasmids pGB301 and pSA3 (Table 3). Powell et al. (21) also reported no observable difference in transformation efficiencies of *L. lactis* LM0230 with plasmids of 4.4, 7.4, and 26.5 kb. On the other hand, transformation efficiencies with *S. thermophilus* were shown to decline with an increase in plasmid size (27).

A number of investigators have utilized a regeneration-expression period following poration to allow for cell recovery and expression of antibiotic resistance genes. Following

electroporation, *L. casei* was diluted and incubated for 1 h at 37°C (4). Powell et al. (21) diluted porated cells of *L. lactis* and incubated them for 1 h at 30°C, while *S. thermophilus* was diluted and allowed to incubate for up to 24 h at 4°C before plating (27). Our results indicate that *L. lactis* cells are remarkedly resistant to damage by high-voltage electric pulses, but incubation of porated cells at 30°C for 2 h did show an increase in transformant numbers which was markedly higher when cells were diluted 1:100 prior to incubation. The increase in survivor values during this period may be due to recovery of the injured cells or to growth and cell division or to both. Transformant values increased at a substantially higher rate, indicating that an expression period may be beneficial in recovering porated cells; however, it should be noted that amplification of cells during this period may result in an increase in the number of transformants obtained.

There are a number of advantages to the BTX Transfector 100 electroporation system. The electrode is relatively easy to sterilize and is cost efficient since it is reusable. The optional BTX Optimizor is an extremely valuable tool for developing an electroporation protocol as it allows documentation of the important electrical parameters, including the shape of the discharged pulse, peak voltage, field strength, and time constant of the pulse actually delivered to the cell suspension. In addition, the Optimizor records resistivity of the cell suspension before and after the pulse which indirectly monitors the biological state of the cell (i.e., degree of damage to cell) as indicated by the lowered resistivity readings owing to the leakage of ions. Some commercially available instruments, particularly those utilizing mechanical relays, may generate electric pulses which vary significantly in maximal voltage and pulse shape from experiment to experiment, yielding variable results. The Optimizor allows one to monitor the consistency and reproducibility of the pulse. High peak voltages and field strengths can be generated with the high internal capacitance of the BTX unit, and pulse durations can be set over a broad range, which may be advantageous in developing systems for cells recalcitrant to other transformation procedures. One drawback of routinely using the machine at maximum field strengths is that pitting of the electrodes can occur after repeated arcing at high voltages, and this appeared to decrease transformation efficiencies. Another disadvantage in all the commercially available electroporation chamber designs to date is the requirement for relatively large volumes (0.3 to 0.8 ml) of cells. Calvin and Hanawalt (3) were able to use much smaller sample volumes (10 to 12 μl), which would provide a distinct advantage for cloning studies, as much less DNA is required.

In a recent review of bacterial transformation systems, Mercenier and Chassy (15) indicated that field strength and pulse duration are the most important parameters to consider in developing bacterial transformation systems and that electroporation units currently available cannot generate a sufficiently high field strength to achieve maximal efficiency in many bacterial strains. Several investigators have successfully porated intact (4, 16, 27) or partially protoplasted (21) microorganisms using field strengths of 6.25 kV/cm or less and have obtained transformation efficiencies equivalent to those obtained in this study in which field strengths as high as 17 kV/cm were employed; however, our results indicate that with the BTX unit, achievement of even higher field strengths may be required to achieve higher transformation efficiencies in *L. lactis*. In all cases to date, the electrical apparatus used to porate cells involves the discharge of a capacitor and the generation of a voltage pulse having a rapid rise time and an exponential decay whose time constant depends on both the capacitor and the resistance of the cell suspension. Calvin and Hanawalt (3) have designed an apparatus which uses a transformer to generate pulses of up to 7,000 V, having a peak rise time of about 100 μs, a width of about 3 ms, and a sinusoidal fall spanning 4 ms. Using this system, these investigators have achieved the highest *E. coli* transformation efficiencies reported to date, 10^9 transformants per μg of DNA. Preliminary results indicate that this system is also more effective in transforming *L. lactis* (unpublished data). The shape of the pulse may therefore be as critical as the peak voltage or field strength.

The technique of electroporation is an extremely simple and efficient method for the transfer of genetic information in bacteria. In the future, as we gain a better understanding of the mechanism of electroporation and as protocols and electroporation equipment are developed and refined, it is projected that this technique will have broad application for genetic improvement of many industrially important microorganisms recalcitrant to current biochemical gene transfer methods.

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