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Improved Electroporation Efficiency of Intact *Lactococcus lactis* subsp. *lactis* Cells Grown in Defined Media†

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The impact of growth conditions on electroporation of *Lactococcus lactis* subsp. *lactis* LM0230 (previously designated *Streptococcus lactis* LM0230) was evaluated. Cells grown in M17 broth supplemented with 0.5% glucose (M17-Glu) and two chemically defined synthetic media, FMC and RPMI 1640, all supplemented with 0.24% DL-threonine or 0.5% glycine, were harvested, washed with double-distilled water, diluted, and porated in the presence of 1 μg of pGB301 DNA with a Transfector 100 (BTX, Inc., San Diego, Calif.) or a Gene Pulser (Bio-Rad Laboratories, Richmond, Calif.). Transformants were recovered at consistently higher efficiencies for cells grown in FMC or RPMI 1640 (10^3 to 10^4 transformants per μg of DNA) than for cells grown in M17-Glu (10^1 to 10^2 transformants per μg of DNA). Other parameters influencing electroporation of *L. lactis* cells grown in chemically defined media were growth phase and final concentration of cells, concentration of plasmid DNA, voltage achieved during poration, and expression conditions. A high degree of variability in transformation efficiencies was evident for replicate samples of cells pulsed with either electroporation machine. A trend toward decreased variability was observed for duplicate samples of cells prepared on the same day. In addition, storage studies done with a large batch of cells prepared on the same day indicated that freezing dry cell pellets at −60°C had no deleterious effect on transformation efficiencies over a 30-day period when a new 0.2-cm cuvette was used for porating each sample.

Electroporation has become increasingly popular as a tool for transforming bacterial cells. Although it is much simpler and faster than previous transformation methods using such chemicals as $CaCl_2$ (18) and polyethylene glycol (7), reported electroporation efficiencies have been quite variable for bacteria, ranging from 0 to 10^9 transformants per μg of DNA. Intact bacterial cells at various growth phases and final concentrations have been suspended in a variety of buffers, pulsed at field strengths ranging from 3 to 17 kV/cm and pulse durations of 100 μs to 1 s, and expressed under a variety of time and temperature conditions (3–6, 8, 9, 11, 13, 15, 17, 18, 21). In addition, there are a variety of commercially available electroporation units which allow the investigator to set different parameters, such as the desired voltage, pulse duration, capacitance, and resistance. No data which directly compare results obtained with different electroporation machines and cells grown under identical conditions are currently available.

The objective of this study was to evaluate the impact of growth conditions on electroporation of *Lactococcus lactis* subsp. *lactis* LM0230 (previously designated *Streptococcus lactis* LM0230) grown in M17 broth (20) and two chemically defined synthetic media, FMC (19) and RPMI 1640 (14). The media were also supplemented with 0.24% DL-threonine or 0.5% glycine, agents that weaken the cell wall (12). Use of the FMC chemically defined medium was previously demonstrated to alter the bacterial cell surface of *Streptococcus mutans* and overcome the formation of extracellular enzyme aggregates (16). Parameters influencing transformation of *L. lactis* cells grown in defined media, including growth phase and final concentration of cells, concentration of plasmid DNA, voltage achieved during poration, and expression conditions, were examined. Transformation efficiencies obtained with the same cell preparations were compared following electroporation with a Transfector 100 (BTX, Inc., San Diego, Calif.) and a Gene Pulser (Bio-Rad Laboratories, Richmond, Calif.).

MATERIALS AND METHODS

Bacterial strains and plasmids. *L. lactis* subsp. *lactis* LM0230, a plasmid-free derivative of *L. lactis* C2, was grown in M17 broth (20) supplemented with 0.5% (wt/vol) glucose (M17-Glu). For testing of defined media, LM0230 cells were initially grown in M17-Glu overnight and then subcultured (5%) into FMC medium (19; formulation prepared by KC Biologicals, Lenexa, Kans.) supplemented with 0.95% (wt/vol) beta-glycerophosphate and 2% (wt/vol) glucose or into RPMI 1640 (14; formulation prepared by GIBCO Laboratories, Grand Island, N.Y.) supplemented with 0.3% (wt/vol) glucose. Additional studies were done with LM0230 cells grown in M17-Glu, FMC, or RPMI 1640, each supplemented with 0.24% DL-threonine (M17-Glu-Thr, FMC-Thr, or RPMI 1640-Thr, respectively) or 0.5% glycine (M17-Glu-Gly, FMC-Gly, or RPMI 1640-Gly, respectively). *L. lactis* JK301, harboring the 9.8-kilobase vector pGB301 (2), was propagated in M17-Glu containing 10 μg of erythromycin per ml. All strains were incubated at 32°C.

Isolation of plasmid DNA. Plasmid DNA was isolated from *L. lactis* JK301 by the method of Anderson and McKay (1). Purification of plasmid DNA by CsCl density gradient centrifugation in the presence of ethidium bromide and analysis by agarose gel electrophoresis were performed by the standard methods described by Maniatis et al. (10).

Cell preparation conditions. An overnight culture of *L. lactis* subsp. *lactis* LM0230 was inoculated into 50 ml of M17-Glu broth (1% inoculum), FMC broth (5% inoculum), or RPMI 1640 broth (5% inoculum), each supplemented with 0.24% DL-threonine or 0.5% glycine, and grown to the

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stationary phase. Cells were harvested, washed, and suspended in 1.25 ml of ice-cold double-distilled water (ddH_2O) as described previously (11). In addition, cells grown in RPMI 1640-Thr were harvested at various phases of growth (optical densities at 600 nm [OD_{600}], 0.2, 0.4, 0.7, and 1.2, corresponding to early-log, mid-log, late-log, and stationary phases, respectively), washed, suspended in ddH_2O, and diluted to cell concentrations of 10^{10}, 10^9, 10^8, and 10^7 CFU/ml prior to electroporation.

For storage studies, 1,200 ml of cells was grown in RPMI 1640-Thr, harvested at the stationary phase (OD_{600}, 1.2), and washed with ddH_2O as described previously (11). After the final ddH_2O wash, aliquots of pelleted cells were either air dried for 30 min and frozen as dry pellets or suspended in 10% glycerol prior to storage at −20 or −60°C. Samples stored at −60°C were submerged in liquid nitrogen for 30 s before storage. Prior to electroporation, the dry cell pellets were suspended in 1.25 ml of ddH_2O and the 10% glycerol samples were allowed to thaw. The number of cells in each sample was determined by enumeration on M17-Glu medium. Cells were porated on the day of preparation and following 2, 9, 16, 23, and 30 days of storage.

Electroporation parameters. Purified plasmid DNA (0.01 to 2 μg) was added directly to a semi-micro disposable cuvette (VWR Scientific, Chicago, Ill.) containing 200 μl of chilled cell suspension for use with the Transfector 100 or to a 0.2-cm-gap Gene Pulser disposable cuvette (Bio-Rad) containing 400 μl of chilled cell suspension. The cells were exposed to one high-voltage electric pulse which ranged from 300 to 900 V (corresponding to field strengths [*E*] of 5 to 17 kV/cm) and which was applied for 4.5 to 6.4 ms (theoretical time constant, 5 ms) with the Transfector 100 apparatus as described previously (11). For the Gene Pulser, the voltage was set at 2.5 kV (*E* = 12.5 kV/cm), the capacitor was set at 25 μF, and the pulse controller was set at the 200-Ω parallel resistor, which allowed a theoretical time constant of 5 ms to be generated (actual time constant, 2.5 to 3.9 ms) in a 0.2-cm-gap disposable cuvette. The pulse controller unit connected to the Gene Pulser allowed safe operation of the unit while generating higher field strengths (6 to 12.5 kV/cm) for samples of small volume and high resistance. Both electroporation units were tested at their maximum voltage settings.

Following electroporation, cells were kept on ice for 10 min, diluted 1:2 in M17-Glu broth, and incubated at 30°C for 2 h before being plated. In addition, to study the effects of dilution prior to the expression period, we serially diluted cells 1:2, 1:5, 1:10, and 1:100 in M17-Glu broth before incubating them. Survivors were enumerated on M17-Glu agar following incubation at 32°C for 24 h. Transformants were enumerated on M17-Glu agar containing 2.5 μg of erythromycin per ml following incubation at 32°C for 36 to 48 h. Controls included experimental trials in which either the electric pulse or the plasmid DNA was omitted. Transformants were confirmed by the presence of plasmid DNA following agarose gel electrophoresis.

RESULTS

Effect of medium composition on electroporation efficiency. *L. lactis* subsp. *lactis* LM0230 cells were initially tested in a defined medium, FMC, which had been formulated by KC Biologicals and was available in our laboratory. Stationary-phase (OD_{600}, 1.2) cell suspensions of LM0230 grown in M17-Glu, M17-Glu-Gly, M17-Glu-Thr, FMC, and FMC-Thr were harvested, washed, and pulsed in the presence of 1 μg of pGB301 DNA over a range of field strengths with the Transfector 100. Figure 1 illustrates the number of transformants obtained after delivery of a 5-ms pulse at *E* = 5, 9, 13, and 17 kV/cm. Cells grown in FMC-Thr resulted in transformation efficiencies 5- to 10-fold greater than did cells grown in the other media tested. In addition, greater transformation efficiencies were achievable with higher field strengths (*E* = 13 to 17 kV/cm). LM0230 cells grew poorly in FMC-Gly medium and therefore were not included in this study. Although efficiencies were much higher for cells grown in FMC, this medium is not commercially available and requires extensive preparation; therefore, an alternative commercially available defined medium, RPMI 1640, was evaluated.

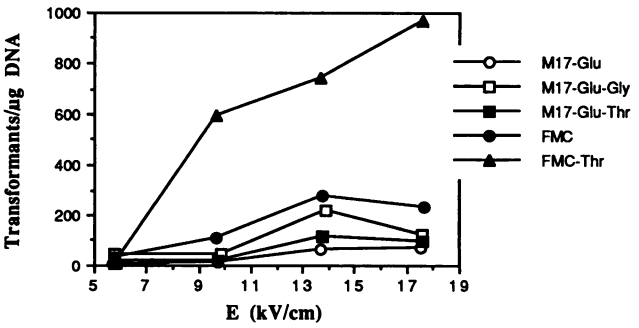

FIG. 1. Cell suspensions of *L. lactis* subsp. *lactis* LM0230 grown in various media (M17-Glu, M17-Glu-Gly, M17-Glu-Thr, FMC, and FMC-Thr) and pulsed over the range of field strengths available on the Transfector 100. The cells were pulsed for 5 ms with 1 μg of pGB301 DNA at each of the voltages tested. Trials were performed in duplicate and then averaged prior to plotting the data.

LM0230 cells were grown in M17-Glu and RPMI 1640 to the stationary phase (OD_{600}, 1.2), harvested, washed, and suspended in ddH_2O to a final concentration of 5×10^{10} CFU/ml. Cells were pulsed at 900 V (*E* = 17 kV/cm) for 5 ms in the presence of 1 μg of pGB301 DNA. In an average of three trials, transformation efficiencies were up to 100-fold higher for cells grown in RPMI 1640 than for cells grown in M17-Glu (data not shown). To determine other parameters that may influence electroporation of *L. lactis* cells grown in defined media, we examined the growth phase and final concentration of cells, voltage achieved during poration, concentration of plasmid DNA, and cell expression conditions.

Effect of other parameters on electroporation efficiency. (i) Culture age, cell concentration, and voltage. Suspensions of LM0230 cells grown in RPMI 1640-Thr to various growth phases (OD_{600}, 0.2, 0.4, 0.7, and 1.2, corresponding to early-log, mid-log, late-log, and stationary phases, respectively) and diluted to final concentrations of 10^{10}, 10^9, 10^8, and 10^7 CFU/ml were electoporated with the Transfector 100. Aliquots were subjected to one 5-ms pulse in the presence of 1 μg of pGB301 DNA at field strengths of 13, 15, and 17 kV/cm. Figure 2 illustrates the transformation efficiencies obtained at each field strength tested. A marked increase in transformation efficiency was demonstrated with stationary-phase cells at high cell concentrations (5×10^{10} CFU/ml) porated at the highest field strength tested (*E* = 17 kV/cm). These conditions agree with previous data for M17-Glu-grown cells (11); however, electroporation with cells grown in defined media resulted in transformation efficiencies approximately 10-fold higher than did electroporation with M17-Glu-grown cells.

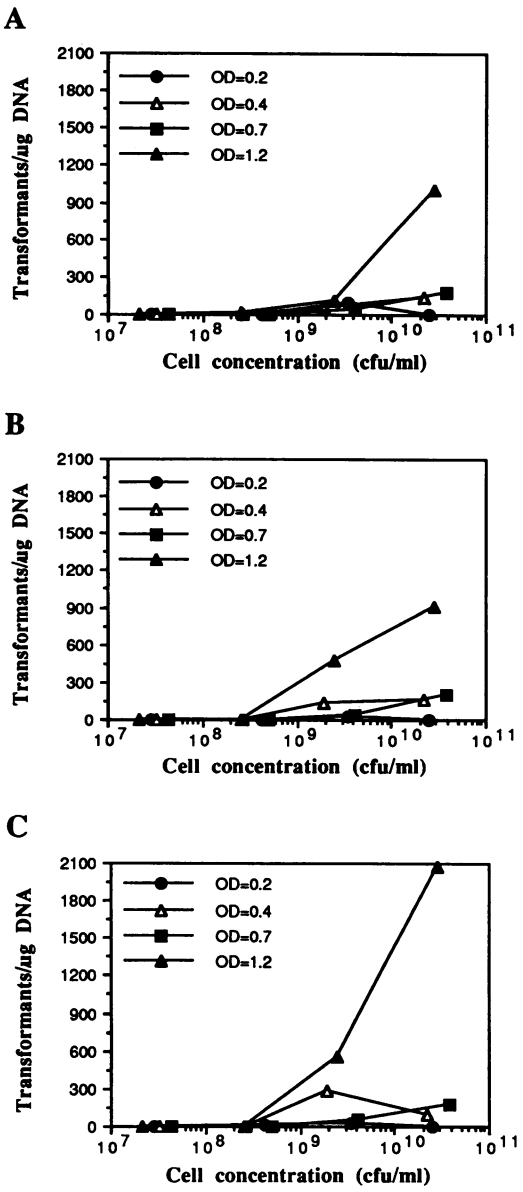

FIG. 2. Electroporation of *L. lactis* subsp. *lactis* LM0230 at various phases of the growth cycle and at various cell concentrations. A 5-ms pulse was delivered at 3 voltages, 700 V (A), 800 V (B), and 900 V (C), which correlated to field strengths of 13, 15, and 17 kV/cm, respectively. Trials were performed once for each cell concentration at each voltage, and 1 μg of pGB301 DNA was used for each trial.

(ii) DNA concentration. The effect of plasmid DNA concentration on the total number of transformants obtained by electroporation with the Transfector 100 was examined. LM0230 cells were grown in FMC-Thr and RPMI 1640 media and porated at 900 V (E = 17 kV/cm) for 5 ms in the presence of various concentrations (0.01, 0.05, 0.1, 0.5, 1, and 2 μg) of purified pGB301 DNA. An increase in transformation efficiency was observed with increasing DNA concentrations (data not shown). The same trend was reported previously with cells grown in M17-Glu (11).

(iii) Dilution prior to expression. LM0230 cells grown in RPMI 1640-Thr to the stationary phase were porated in the presence of 1 μg of pGB301 DNA with the Transfector 100 (900 V, 5 ms) or the Gene Pulser at 2.5 kV (E = 12.5 kV/cm) and 25 μF (pulse controller set at 200 Ω). Following electroporation, cells were kept on ice for 10 min, diluted 1:2, 1:5, 1:10, and 1:100 in M17-Glu broth, and incubated at 30°C for 2 h. In an average of four trials with the Gene Pulser, the transformation efficiency for cells diluted 1:2 was 382 transformants per μg of DNA; this increased 10-fold to an average of 2,000 transformants per μg of DNA when cells were diluted 1:100. Transformant values increased at a substantially higher rate than did survivor values (data not shown). This result is consistent with results obtained in a previous study with cells grown in a rich medium (11).

Comparison of the Transfector 100 and the Gene Pulser. Washed LM0230 cells grown to the stationary phase (OD_{600}, 1.2) in M17-Glu, FMC, or RPMI 1640, each supplemented with 0.5% glycine or 0.24% DL-threonine, were suspended in ddH_2O to a concentration of 5×10^{10} CFU/ml and porated in the presence of 1 μg of pGB301 DNA with the Transfector 100 or the Gene Pulser. Table 1 lists the average transformation efficiencies and 95% confidence levels obtained for replicate samples grown on separate days and porated with each electroporation unit. LM0230 cells grew poorly in the defined media supplemented with 0.5% glycine and therefore were not included in this study. The 95% confidence levels indicated an extremely high degree of day-to-day variability of transformation efficiencies for cells porated with either electroporation unit.

To determine how much variability may be attributed to minor differences in the growth conditions of the cells, we pulsed duplicate samples of cells grown in M17-Glu, M17-Glu-Thr, RPMI 1640, and RPMI 1640-Thr with the Transfector 100 and the Gene Pulser. Table 2 lists the average efficiencies and 95% confidence intervals obtained under these conditions. Since cells were prepared at the same time, trial numbers were kept at a minimum so that cells were not kept on ice too long before poration. Although the sample size was small, there was again a trend toward higher efficiencies for cells grown in defined media. With the exception of two samples porated with the Transfector 100 (cells grown in RPMI 1640 on day 1 and cells grown in M17-Glu on day 3), the 95% confidence intervals decreased for all same-day cell preparations tested, indicating that differences in sample preparation accounted for at least some of the observed variability.

These results indicated that it might be beneficial to prepare a large batch of recipient cells and store aliquots frozen until needed. To determine whether frozen storage would have a negative effect on transformation efficiency, we prepared LM0230 cells under optimum conditions (growth in RPMI 1640 medium to the stationary phase [OD_{600}, 1.2]) and froze them at −20 and −60°C. Cells were stored as dry pellets or suspended in 10% glycerol, sampled at various times over a 30-day period, and porated with the Gene Pulser. Listed in Table 3 are transformation efficiencies obtained with a new curvette for each trial, as well as results obtained with the same cuvette throughout the 30-day period. Although the Bio-Rad cuvettes were designed to be used once, because of the cost and availability it was tempting to use a single cuvette for several experiments prior to discarding it. Freezing dry cell pellets with liquid nitrogen and storing them at −60°C over a 30-day period had no deleterious effects on transformation efficiency when a new cuvette was used for each sample.

TABLE 1. Transformation efficiencies obtained with LM0230 cells grown in various media

Medium	Transfector 100[a]			Gene Pulser[b]		
	No. of replicate trials	Mean no. of transformants/μg of DNA (10^2)[c]	±95% Confidence interval (10^2)	No. of replicate trials	Mean no. of transformants/μg of DNA (10^2)[c]	±95% Confidence interval (10^2)
M17-Glu	7	0.6	0.3	3	4.0	6.5
M17-Glu-Thr	6	2.3	2.2	2	9.2	18.0
FMC	3	1.8	1.8	2	12.1	10.3
FMC-Thr	2	9.7	3.1	2	39.3	1.2
RPMI 1640	4	3.6	4.2	3	19.0	10.7
RPMI 1640-Thr	4	6.5	9.5	5	50.5	28.7

[a] Cells (200 μl) were porated at 900 V (E = 17 kV/cm) for 5 ms in the presence of 1 μg of pGB301.
[b] Cells (400 μl) were porated at 2.5 kV (E = 12.5 kV/cm) and 25 μF; the pulse controller was set at 200 Ω.
[c] Average transformation efficiency for the indicated number of replicate trials.

DISCUSSION

Our initial electroporation studies with *L. lactis* subsp. *lactis* LM0230 demonstrated that the highest transformation efficiencies were obtained when dense suspensions (final concentration, 5×10^{10} CFU/ml) of stationary-phase cells were pulsed at 900 V (E = 17 kV/cm) for 5 ms in the presence of plasmid DNA with a Transfector 100 unit (11). Although these conditions were sufficient to achieve efficiencies of 10^3 transformants per μg of DNA, results on a day-to-day basis were quite variable, ranging from 1 to 10^3 transformants per μg of DNA. We were thus interested in studying other factors influencing electroporation in an attempt to decrease this variability, as well as to further improve transformation efficiency.

In this study, we focused on the impact of growth conditions on electroporation of *L. lactis* subsp. *lactis* LM0230. Cells grown in FMC or RPMI 1640 defined medium consistently exhibited much higher efficiencies than did cells grown in M17-Glu, and these efficiencies increased even more with the addition of 0.24% DL-threonine, an agent known to weaken bacterial cell walls (12). A 10-fold increase in transformation efficiency resulted when cells were grown in FMC-Thr (Fig. 1), and cells grown in RPMI 1640-Thr exhibited up to a 100-fold increase in transformation efficiency.

Transformation efficiencies for RPMI 1640-Thr grown cells were greatest for high concentrations (5×10^{10} CFU/ml) of stationary-phase cells pulsed at 900 V (E = 17 kV/cm) for 5 ms (Fig. 2). Since these results agreed with previously developed electroporation parameters for cells grown in M17-Glu (11), it appeared that the growth of LM0230 in RPMI 1640 defined medium enhanced the number of cells being transformed without altering the conditions used for electroporation.

To our knowledge, no other work comparing electroporation machines with cells grown under identical conditions has been published. With access to a Gene Pulser, we were able to compare this unit with the Transfector 100 unit. The growth of LM0230 in defined media was advantageous in increasing transformation efficiencies in both electroporation units. Average efficiencies calculated over a series of replicate trials for each medium tested demonstrated that transformation efficiencies were 3- to 6-fold higher for cells grown in FMC or RPMI 1640 than for cells grown in M17-Glu with the BTX unit or the Bio-Rad unit, and the addition of 0.24% DL-threonine to the defined media resulted in even greater efficiencies, which were 10- to 16-fold higher than those in M17-Glu-grown cells (Table 1). Both machines appeared to be responsible for some of the variability which was evident by the large 95% confidence intervals (Table 1).

A comparison of the 95% confidence intervals reported in Tables 1 and 2 indicated that at least some of the variability in transformation efficiencies could be explained by differ-

TABLE 2. Comparison of transformation efficiencies obtained with same-day LM0230 cell preparations

Day	Medium	Transfector 100[a]			Gene Pulser[b]		
		No. of replicate trials	Mean no. of transformants/μg of DNA (10^2)[c]	±95% Confidence interval (10^2)	No. of replicate trials	Mean no. of transformants/μg of DNA (10^2)[c]	±95% Confidence interval (10^2)
1	M17-Glu	3	0.1	0.1	2	1.4	0.9
	RPMI 1640	3	8.9	9.9	2	23.8	4.5
	RPMI 1640-Thr	3	4.2	1.9	2	31.2	6.2
2	M17-Glu	3	0.3	0.2	2	0.0	0.0
	M17-Glu-Thr	3	3.1	1.0	2	0.0	0.0
	RPMI 1640	3	0.2	0.0	2	8.1	2.6
	RPMI 1640-Thr	3	1.0	0.7	2	10.6	0.6
3	M17-Glu	5	0.8	1.1	2	10.6	6.4
	M17-Glu-Thr	5	1.1	0.8	2	18.4	10.4
	RPMI 1640	4	0.1	0.2	2	25.1	3.8
	RPMI 1640-Thr	2	0.0	0.0	2	53.9	12.0

[a] Cells were porated at 900 V (E = 17 kV/cm) for 5 ms in the presence of 1 μg of pGB301.
[b] Cells were porated at 2.5 kV (E = 12.5 kV/cm) and 25 μF; the pulse controller was set at 200 Ω.
[c] Average transformation efficiency for the indicated number of replicate trials.

TABLE 3. Effect of storage temperature on transformation efficiency of *L. lactis* subsp. *lactis* LM0230 cells

Suspension buffer	Day	Transformation efficiency (no. of transformants/μg of DNA, 10^2) with:			
		New cuvette[a]		Same cuvette[b]	
		−20°C	−60°C	−20°C	−60°C
ddH_2O	1	38.4	34.8	24.3	6.8
	2	14.6	41.4	11.8	3.8
	9	5.1	32.8	1.9	25.7
	16	0.2	26.6	0.1	10.3
	23	1.2	43.4	1.7	0.2[c]
	30	1.0	53.6	0.4	
10% Glycerol	1	17.8	39.3	2.7	41.4
	2	1.8	16.5	2.6	2.0
	9	2.0	13.1	2.6	5.9
	16	1.1	24.4	1.8	4.1
	23	15.0	21.7	17.6	0.3[c]
	30	2.0	10.6	3.4	

[a] Each trial was performed with a new 0.2-cm Bio-Rad cuvette for each day tested.

[b] Each trial was performed with the same 0.2-cm Bio-Rad cuvette over the 30-day testing period. Cuvettes were washed with 70% ethanol and then rinsed with three ddH_2O washes prior to being reused.

[c] Arcing occurred during these trials, and the cuvettes were not used again.

ences in cell preparation from day to day. However, there are also a number of electroporation system-specific factors which may contribute to differences between the BTX and Bio-Rad units with respect to transformation efficiency and day-to-day variability. These factors include sample chamber volume and design; electrode configuration, composition, and surface area; and reproducibility of pulse delivery (peak voltage, field strength, uniformity of the electric field, pulse duration, and pulse shape). A direct comparison of these parameters would be necessary to account for distinct differences between the two electroporation units.

Since the storage of dry cell pellets at −60°C was not deleterious to transformation efficiencies over a 30-day period (Table 3), it would be beneficial to prepare a large quantity of cells at one time, freeze them in liquid nitrogen, and store them at −60°C until needed for electroporation experiments. This would save time by eliminating cell preparation before each experiment and might help to minimize transformation efficiency variability. This method would be particularly useful for research focusing on optimizing the electrical parameters of the electroporation machine for a certain cell type.

The results clearly indicate that a chemically defined synthetic growth medium, such as FMC or RPMI 1640, with the addition of 0.24% DL-threonine, improves the efficiency of electroporation. Chemically defined media may alter the bacterial cell surface and produce more favorable conditions for electroporation than more complex media. Variability in transformation efficiency is still evident with cells grown in defined medium and may be related somewhat to the variability observed in different electroporation machines, suggesting that the large variability in transformation efficiencies reported in the literature (0 to 10^9 transformants per μg of DNA) may be explained in part by day-to-day cell preparation variability and machine variability. In addition, these results stress the importance of reporting not only the maximum transformation efficiency but also the range of efficiencies obtained for each cell type. These data would aid the investigator attempting to repeat or adapt published electroporation protocols, since they would indicate the range of expected efficiencies. If parameters affecting the variability of transformation efficiency results are understood and controlled, optimization of electroporation conditions for various bacterial strains will be much easier.

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LITERATURE CITED

1. **Anderson, D., and L. L. McKay.** 1983. Simple and rapid method for isolating large plasmid DNA from lactic streptococci. Appl. Environ. Microbiol. **46**:549–552.
2. **Behnke, D., M. S. Gilmore, and J. J. Ferretti.** 1981. Plasmid pGB301, a new multiple resistance streptococcal cloning vehicle and its use in cloning the gentamycin/kanamycin resistance determinant. Mol. Gen. Genet. **182**:414–421.
3. **Calvin, N. M., and P. C. Hanawalt.** 1988. High-efficiency transformation of bacterial cells by electroporation. J. Bacteriol. **170**:2796–2801.
4. **Chassy, B. M., and J. L. Flickinger.** 1987. Transformation of *Lactobacillus casei* by electroporation. FEMS Microbiol. Lett. **44**:173–177.
5. **Fiedler, S., and R. Wirth.** 1988. Transformation of bacteria with plasmid DNA by electroporation. Anal. Biochem. **170**:38–44.
6. **Harlander, S. K.** 1987. Transformation of *Streptococcus lactis* by electroporation, p. 229–233. *In* J. J. Ferretti and R. Curtiss III (ed.), Streptococcal genetics. American Society for Microbiology, Washington, D.C.
7. **Kondo, J. K., and L. L. McKay.** 1984. Plasmid transformation of *Streptococcus lactis* protoplasts: optimization and use in molecular cloning. Appl. Environ. Microbiol. **48**:252–259.
8. **Luchansky, J. B., P. M. Muriana, and T. R. Klaenhammer.** 1988. Application of electroporation for transfer of plasmid DNA to *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Listeria*, *Pediococcus*, *Bacillus*, *Staphylococcus*, *Enterococcus* and *Propionibacterium*. Mol. Microbiol. **2**:637–646.
9. **MacNeil, D. J.** 1987. Introduction of plasmid DNA into *Streptomyces lividans* by electroporation. FEMS Microbiol. Lett. **42**:239–244.
10. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **McIntyre, D., and S. Harlander.** 1989. Genetic transformation of intact *Lactococcus lactis* subsp. *lactis* by high-voltage electroporation. Appl. Environ. Microbiol. **55**:604–610.
12. **Mercenier, A., and B. M. Chassy.** 1988. Strategies for the development of bacterial transformation systems. Biochimie **70**:503–517.
13. **Miller, J. F., W. J. Dower, and L. S. Tompkins.** 1988. High-voltage electroporation of bacteria: genetic transformation of *Campylobacter jejuni* with plasmid DNA. Proc. Natl. Acad. Sci. USA **85**:856–860.
14. **Moore, G. E., R. E. Gerner, and H. A. Franklin.** 1967. Culture of normal human leukocytes. J. Am. Med. Assoc. **199**:519–524.
15. **Powell, I. B., M. G. Achen, A. J. Hillier, and B. E. Davidson.** 1988. A simple and rapid method for genetic transformation of lactic streptococci by electroporation. Appl. Environ. Microbiol. **54**:655–660.
16. **Schachtele, C. F., S. K. Harlander, and G. R. Germaine.** 1976. *Streptococcus mutans* dextransucrase: availability of disaggregated enzyme after growth in a chemically defined medium. Infect. Immun. **13**:1522–1524.
17. **Somkuti, G. A., and D. H. Steinberg.** 1988. Genetic transformation of *Streptococcus thermophilus* by electroporation. Biochimie **70**:579–585.

18. **Taketo, A.** 1988. DNA transfection of *Escherichia coli* by electroporation. Biochim. Biophys. Acta **949**:318–324.
19. **Terleckyj, B., N. P. Willet, and G. D. Shockman.** 1975. Growth of several cariogenic strains of oral streptococci in a chemically defined medium. Infect. Immun. **11**:649–655.
20. **Terzaghi, B. E., and W. E. Sandine.** 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Environ. Microbiol. **29**:807–813.
21. **Van De Guchte, M., J. Van Der Vossen, J. Koh, and G. Venema.** 1989. Construction of a lactococcal expression vector: expression of hen egg white lysozyme in *Lactococcus lactis* subsp. *lactis*. Appl. Environ. Microbiol. **55**:224–228.