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High-Efficiency Transformation of Bacterial Cells by Electroporation

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We have developed a method for efficiently generating transient pores in the outer membranes of *Escherichia coli* K-12 derivatives by using a new type of electroporation apparatus. The pores are large enough and persist long enough to facilitate the equilibration of plasmid molecules between the intracellular and extracellular spaces. The method has been used to transform bacterial cells with an efficiency greater than 10^9 transformants per μg of plasmid. It has also been used to extract intact plasmid from transformed cells with efficiencies comparable to those of the traditional alkaline lysis or CsCl equilibrium density gradient techniques. The technique is simple and rapid, allowing a transformation or the preparation of microgram quantities of plasmid to be accomplished in minutes.

Electroporation refers to the process of subjecting living cells to a rapidly changing, high-strength electric field, thereby producing transient pores in their outer membranes. If pores occur in adjacent cells, this can facilitate fusion of the cells (14). Furthermore, diffusion and exchange of intracellular and extracellular components can take place during the lifespan of the pore (4, 13; G. Chu, H. Hayakawa, and P. Berg, manuscript in preparation). This technique has been used to fuse protoplasts in order to create multinuclear eucaryotic cells (11). More recently, it has been used to introduce DNA into a variety of eucaryotic cell types (9, 10), including mammalian cells resistant to the calcium phosphate coprecipitation method and plant cell protoplasts (5).

The electrical apparatus generally used to porate cells for transformation and gene expression experiments has relied upon the discharge of a capacitor through the cellular suspension to generate the required electric field. The success of this technique has led to the introduction of a variety of commercial devices. In all of these devices, a capacitor of between 2 and 1,000 μF is charged to a voltage of between 200 and 2,000 V and then discharged through the cell suspension by using an electronic or mechanical switch. This results in a voltage pulse having a rise time of less than 10 μs and an exponential decay whose time constant depends upon both the capacitor and the resistance of the cellular suspension. A characteristic of this system is that the maximum voltage is usually limited by the available capacitors to about 2,000 V and the actual time interval the cells are exposed to the maximum voltage is very brief.

Attempts to use this type of apparatus to permeabalize small cells such as bacteria have met with only limited success. Because of their smaller size, bacteria theoretically require a much larger electric field to induce poration than do mammalian cells or the even larger plant cells. Reported transformation efficiencies for electroporated *Escherichia coli* K-12 derivatives range from 10^2 to 10^4 transformants per μg of DNA, values which compare unfavorably with the 10^5 to 10^6 transformants per μg routinely obtained by calcium chloride treatment (7) and the maximum of 10^8 transformants per μg which can be obtained by using certain strains and special cations in the transformation buffer (6, 12).

In order to increase the attainable electric field strength and to increase the time interval of exposure to the highest voltage, we chose a different method for generating the voltage pulse. The apparatus which we have constructed uses a transformer to generate pulses of up to 7,000 V, having a rise time of about 100 μs, a width of about 3 ms, and a sinusoidal fall spanning 4 ms. This apparatus is capable of sequentially applying one to nine unipolar pulses, or 2 to 18 bipolar pulses in succession, at 8-ms intervals.

Using *E. coli* K-12 derivatives and the plasmid pUC18 as a model test system, we have investigated a variety of electroporation conditions to optimize the efficiency of poration-mediated transformation, as well as to understand the underlying molecular mechanisms for the phenomenon.

MATERIALS AND METHODS

Electroporation apparatus: pulse generation circuitry. A block diagram of the pulse generator is shown in Fig. 1. The operation is as follows. Standard line voltage (120 V, 60 Hz) is fed into the primary winding of the variable autotransformer (1), whose output can be varied between 0 and 140 V. The output voltage is buffered by the capacitor bank (2) and applied to the primary winding of the step-up transformer (4) through a solid-state TRIAC switch (3). The TRIAC is triggered by the trigger generator (8) in response to pressing a button (7).

The output of the secondary winding of the pulse transformer is fed into the sample holder (4). The sample holder contains a 100:1 voltage divider and a 1 or 0.1 Ω series resistor for measuring the current flowing through the sample. The output of the voltage divider is fed into a peak reading voltmeter (6), and the voltage developed across the series resistor is fed into another peak reading voltmeter (5) to measure current flow. The peak reading voltmeters must have a response time on the order of 1 ms. Alternatively, a storage oscilloscope may be used to record the actual waveforms.

The trigger generator is synchronized with the line voltage, and the timing of the trigger pulse is set to occur just before the line voltage reaches its peak value. The trigger generator can be set to produce one to nine pulses occurring either on one-half of the line voltage cycle, to generate unipolar pulses, or on both halves of the cycle, thereby producing bipolar pulses.

Our apparatus was initially constructed to be used with sample volumes of up to 1 ml and was therefore designed to supply pulses of up to 7,000 V at a current of up to 2 A. Subsequent experiments (described below) showed that

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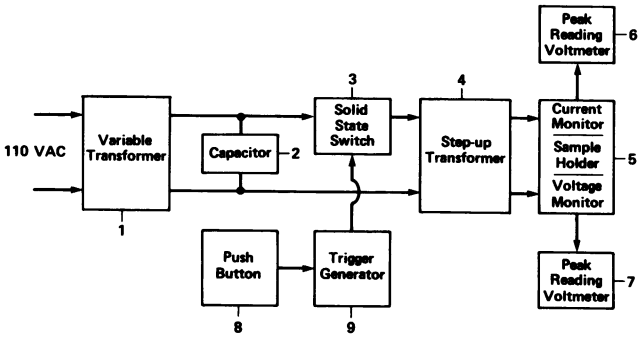

FIG. 1. Block diagram of the circuitry for the electroporation apparatus. VAC, volts (AC).

highly efficient transformation could be achieved using voltages below 2,000 V and currents below 0.2 A.

The waveform produced by the apparatus is essentially one-fourth of a sine wave (Fig. 2). The rise time is determined by the switching time of the TRIAC and the internal capacitance of the pulse transformer. The capacitor shortens the rise time by providing the high current necessary to charge the internal capacitance of the transformer in a very short period of time. The rise time can vary between 10 and 100 μs depending upon the transformer used.

Electroporation apparatus: sample holder. Volumes of 50 μl to 1 ml were processed in electroporation cuvettes obtained from Bio-Rad Laboratories. The cuvettes were used with a specially constructed holder containing a 100:1 voltage divider and a 0.1 Ω series resistor for monitoring voltage and current. Volumes below 50 μl were tested in one of two microcells. The first microcell (microcell 1) consisted of two stainless steel plates separated by an insulator, a piece of G-10 epoxy glass printed circuit board material that was 1/16 in. (1 in. = 2.54 cm) thick (Fig. 3a). The insulator had a groove cut in the top edge which was approximately 2 mm by 4 mm and could hold 12-μl samples. The inside edges of the plates were beveled and polished to inhibit arcing. The assembly was held together by using nylon screws and nylon shoulder washers. Banana plugs were mounted in the assembly so that it could be removed from the pulser apparatus for washing.

Microcell 2 is composed of two 1/4-in. stainless steel rods spaced 1/16 in. apart, with the ends ground flat and polished.

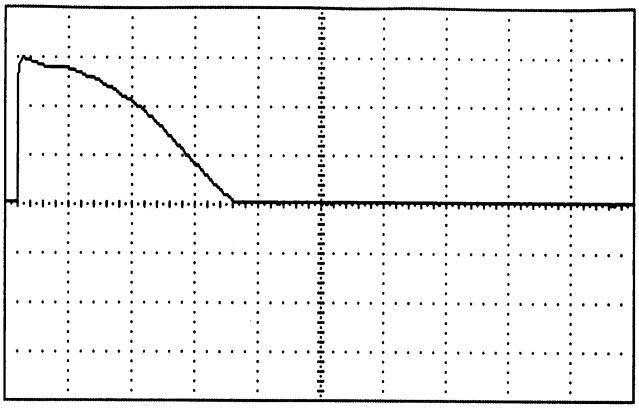

FIG. 2. Typical waveform produced by the electroporation apparatus. Horizontal scale, 1 ms per division. Vertical scale, 5,000 V/cm per division.

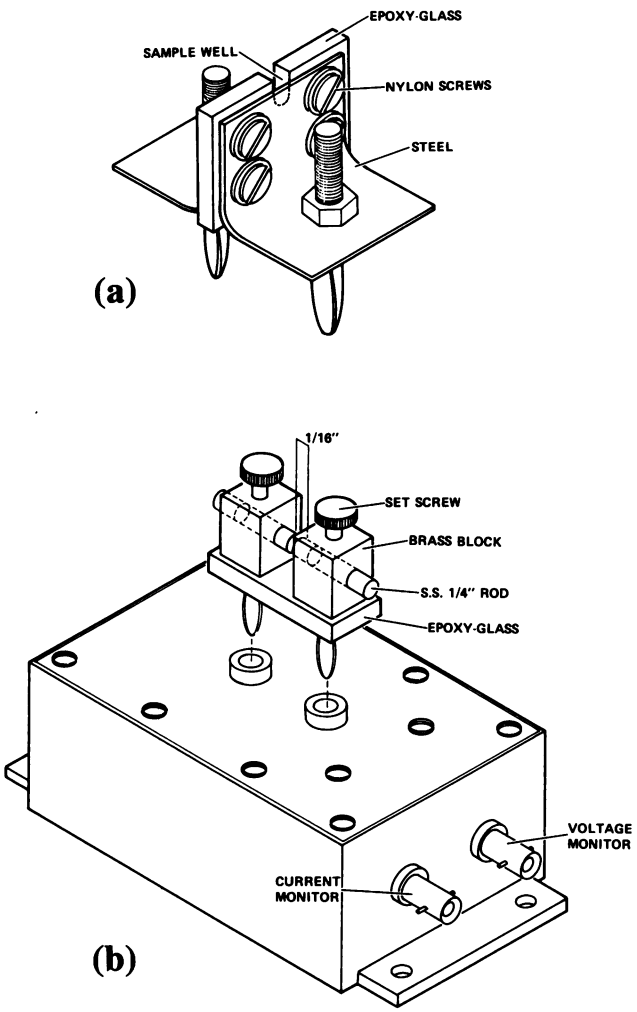

FIG. 3. (a) Construction of microcell 1. (b) Construction of microcell 2. See Methods and Materials for description.

The supports were machined from 3/8-in. square brass stock, and the mounting plate was constructed from 1/8-in. thick G-10 epoxy glass printed circuit board. Samples of cell suspensions of 5 to 50 μl were inserted into the gap by using a micropipette. Once in place, the sample was held in place by surface tension. The orientation of the rods (horizontal or vertical) did not affect the shape of the drop. After poration, the sample could be removed completely with a micropipette. The rods were supported in a holder equipped with banana plugs so that the assembly could be easily plugged into a base containing electrical connections for the pulse generator and measurement instruments (Fig. 3b).

The base (Fig. 4) contained a 100:1 precision voltage divider (R1 and R2) and a 1.0 Ω series resistor (R3) for monitoring current.

Voltage and current were monitored either with a Tektronix dual-trace oscilloscope or with Beckman model 2820 peak-reading voltmeters, which have a response time of about 2 ms, compared to a response time of >1 s for most digital multimeters with a peak-hold feature.

Cells. *E. coli* K-12 derivative MC1061 [F^- *araD139* Δ(*ara leu*)*7697* Δ*lacX74 galU galK hsr hsm*$^+$ *rpsL*] was used for most experiments. L broth (10 g of tryptone and 8 g of NaCl per liter) was used as the growth medium. Survival was

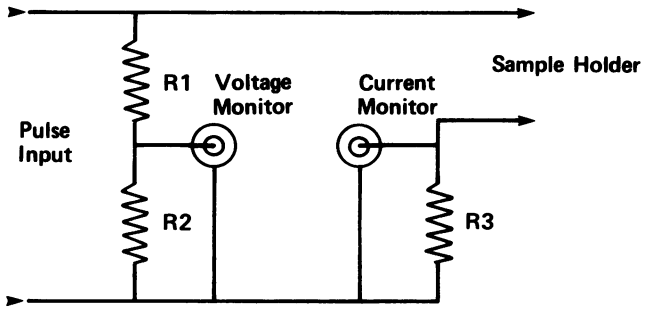

FIG. 4. Schematic diagram of the electroporation base.

measured by plating dilutions on plates made by solidifying L broth with Bacto-Agar (15 g/liter; Difco Laboratories). Transformants were assayed by diluting the porated suspension into L broth and plating 100 μl on L broth plates containing 30 μg of ampicillin per ml.

Harvesting and washing of cells. An overnight culture grown to stationary phase in L broth (37°C) was diluted at least 1:50 into prewarmed medium and grown to a specific optical density at 650 nm (OD_{650}) (usually 1, corresponding to approximately 1.9×10^9 cells per ml). A total of 10 to 25 ml of culture was harvested by pelleting in a Sorvall SS-34 fixed-angle rotor at 5,000 rpm for 5 min. Cells were then washed twice by resuspending them in poration buffer (composition varied between experiments; see text) and pelleting them again. The cells were then resuspended in 1 ml of poration buffer and centrifuged in a microfuge for 2 min. The volume of the pellet was estimated by comparing the size of the pellet to known volumes in the same type of tube, and the cells were resuspended in poration buffer to give a cell concentration (pellet volume/added buffer volume) of between 1:2 and 1:10. A 25-ml culture yielded approximately 70 μl of cells.

Poration buffer. Several different poration buffers were used during the course of our experiments. The compositions were as follows: TB 1, 10% glycerol–0.2 mM K_2HPO_4 (pH 7.5); TB 2, 15% glycerol–0.2 mM K_2HPO_4 (pH 7.5); TB 3, 0.2 mM K_2HPO_4 (pH 7.5).

Plasmid. Plasmid pUC18 was obtained from Bethesda Research Laboratories. Several milligrams of plasmid were prepared by using transformed MC1061 cells. The plasmid was purified by equilibrium sedimentation in CsCl gradients (3).

Electroporation. Plasmid DNA was added to the final cell suspension, which was then vortexed for at least 1 min at maximum speed. When microcell 1 was used, 7 μl of double-distilled H_2O was placed in the microcell and 5 μl of the cell suspension was carefully layered underneath the water. The overlayer greatly reduced the probability of arcing at high voltages. After poration, the entire 12-μl volume was removed and layered on the bottom of a tube containing 1 ml of L broth. The tube was allowed to stand for 10 min (to ensure that all reversible pores were closed before the cells were removed from the poration buffer) and then vortexed. Serial dilutions were then made into L broth; survival was assayed by plating 100 μl of a 1:40,000 dilution on L broth plates, and transformation to ampicillin resistance was assayed by plating 100 μl of 1:1, 1:200, and 1:40,000 dilutions on L broth plates containing 30 μg of ampicillin per ml.

When microcell 2 was used, 12 μl of the cell suspension was inserted between the electrodes by using a micropipette (making sure that the drop was in contact with both electrodes), and the voltage pulse was administered. A 5-μl sample was removed (to keep the number of cells the same as that in the experiments using microcell 1, even though almost all 12 μl can be recovered). The sample was then added to the bottom of a tube containing 1 ml of L broth. Dilutions were as described above for microcell 1.

RESULTS

Initial experiments were carried out by using the Bio-Rad electroporation cuvettes. After three washes in 10% glycerol, cells were suspended in 10% glycerol–0.2 mM K_2HPO_4 at a 1:50 ratio of pellet volume to final volume. Typically, transformation efficiencies of about 10^6 transformants per μg of plasmid DNA were attained under these conditions (data not shown). However, when the cell pellet volume was increased to 10% of the final volume, transformation efficiencies rose to 10^7 transformants per μg. To reduce arcing within the cuvette, we used an underlayer of 100 μl of 20% glycerol and an overlayer of 600 μl of high-purity H_2O with a sample size of 200 μl. This technique allowed us to use higher voltages, raising the efficiency to 2×10^7 transformants per μg of DNA.

The geometry of the Bio-Rad cuvettes required a minimum sample volume of about 200 μl and fixed the electrode spacing at 4 mm. This required very high voltage and high current pulses. We therefore constructed microcell 1, which allowed sample volumes of less than 12 μl and reduced the maximum required pulse voltage from 6,000 to less than 2,000 V. The results of the first experiment using microcell 1 are shown in Fig. 5. The maximum transformation efficiency attained was 1.8×10^7 transformants per μg of plasmid DNA at a field strength of 14,300 V/cm. The maximum voltage was limited by arcing within the cuvette.

Figure 5 also shows the corrected transformation efficiency (CTE), which is equal to the transformation efficiency

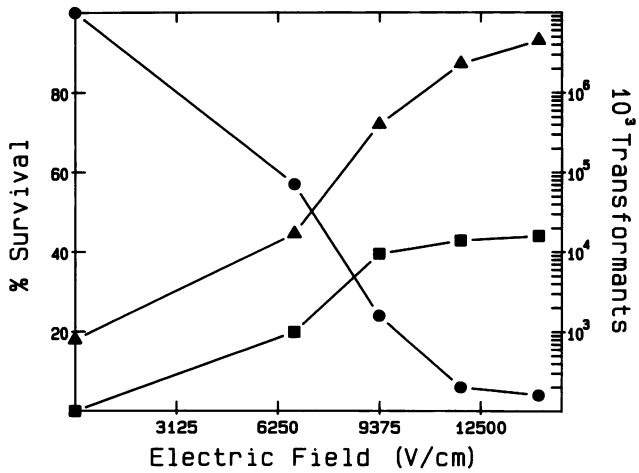

FIG. 5. Transformation experiment using microcell 1. MC1061 cells were grown to an OD_{650} of approximately 1.5 (1.9×10^9 cells per ml) and harvested and washed, as described in Materials and Methods, by using TB 1. The final cell concentration was 1:50. pUC18 DNA was added to a concentration of 0.3 μg/ml. Poration was carried out at pulse voltages spaced at 3,000-V/cm increments, as described in Materials and Methods. Survival (●) and transformation were determined as described in Materials and Methods. Transformation is expressed as transformants per μg (■) or as CTE (▲).

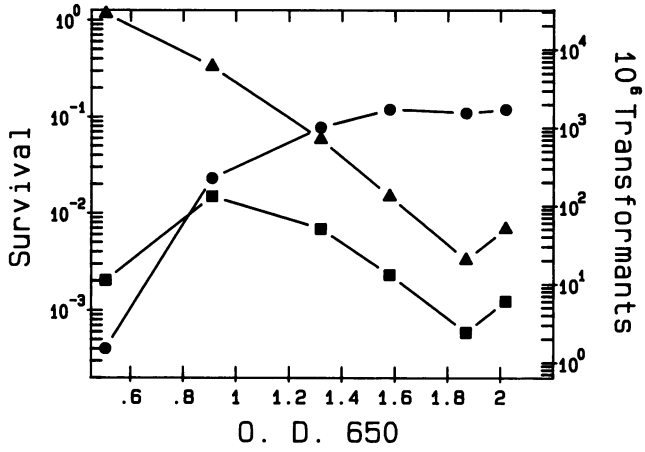

FIG. 6. Effect of culture growth stage. MC1061 cells were grown as described in Materials and Methods, and portions of the culture were harvested, washed, and transformed at 40, 80, 120, and 230 min after an OD_{650} of 0.5 was attained. Transformation was carried out by using 0.3 μg of pUC18 per ml, TB 1, a cell concentration of 1:50, and a field strength of 13,000 V/cm. The survival (●), transformation efficiency (■), and CTE (▲) are plotted as a function of the culture density, expressed as OD_{650}.

divided by the survival. This number corresponds to the maximum number of transformants that might have been obtained if all cells had survived. This quantity was used to compare the degree to which different conditions were capable of porating cells independent of survival levels in a given experiment. It also provides a way of comparing the measured efficiencies with theoretical maximum values.

The maximum CTE observed was 4.5×10^9 transformants per μg of plasmid. If the poration process allowed diffusion of plasmid molecules to proceed to equilibrium, then the fraction of plasmid molecules inside bacterial cells after closure of the pores should be proportional to the fraction of the suspension volume occupied by cells. The cells comprise roughly 10% of the total volume, so the theoretical maximum CTE is 3.5×10^{10}. The measured value of an order of magnitude lower might be due to incomplete poration of the cell population, lack of uniform equilibration, or degradation or lack of expression of the plasmids.

Effect of growth stage. Bacterial cultures in either very early or very late log phase routinely gave transformation frequencies below 10^7 transformants per μg. By adjusting the stage at which a culture was harvested, the transformation efficiency could be improved by nearly 3 orders of magnitude. The optimal time to harvest a culture varied between cell lines and with growth conditions such as growth medium and temperature, but if these were kept constant, variation between experiments was typically less than a factor of 2. Figure 6 shows the effect of varying the OD at harvest from the optimal value of 1 for MC1061 cells grown in L broth. The CTE in exponentially growing cultures was extremely high, approaching the theoretical maximum to within a factor of 2. If cell killing in these cultures is due to poration, this result suggests that (i) poration is extremely efficient and (ii) virtually every plasmid that gets into a surviving bacterial cell will be expressed.

Effect of multiple pulses on cells in stationary phase. We tested the possibility that the resistance of stationary-phase cultures to transformation and killing could be overcome by administering multiple sequential pulses. An overnight culture of MC1061 cells (OD_{650}, 2.6; about 2.6×10^9 cells per ml) was pelleted and washed as described in Materials and Methods. Transformation was carried out with 1 μg of pUC18 DNA per ml in 0.2 mM K_2HPO_4 and 15% glycerol at a field strength of 13,000 V/cm. Multiple pulses of the same polarity were administered at 1/60-s intervals. Multiple pulses of alternating polarity were administered in 1/120-s intervals. Transformation and survival were assayed as described in Materials and Methods. The data (not shown) showed that the single pulse was optimal for both transformation frequency and CTE.

Effect of carrier DNA. We tried adding nonhomologous DNA to the transformation buffer to see whether it would improve the transformation efficiency by competitively inhibiting the possible degradation of the plasmid by nucleases. A stationary-phase culture was used, and various amounts of sonicated calf thymus DNA were added to poration suspensions containing 0.1 μg of pUC18 DNA per ml. Poration was carried out at 13,000 V/cm. The results (not shown) indicated that additional DNA did not improve the transformation frequency.

Effect of plasmid DNA concentration. If plasmids enter cells by diffusion and have a very high probability of expression (see above), then the efficiency of transformation should decrease as the concentration of plasmid exceeds one molecule per cell volume. The results of an experiment using different amounts of plasmid are shown in Fig. 7.

The values predicted on the basis of random diffusion were consistently lower than those measured. This result suggests that plasmid molecules are concentrated in or around the bacteria. This might be due to plasmid molecules adhering to the surfaces of cells prior to poration or to molecules being somehow attracted to cells during the poration. When cells are mixed with radioactively labeled plasmid, only a small percentage of the label remains bound

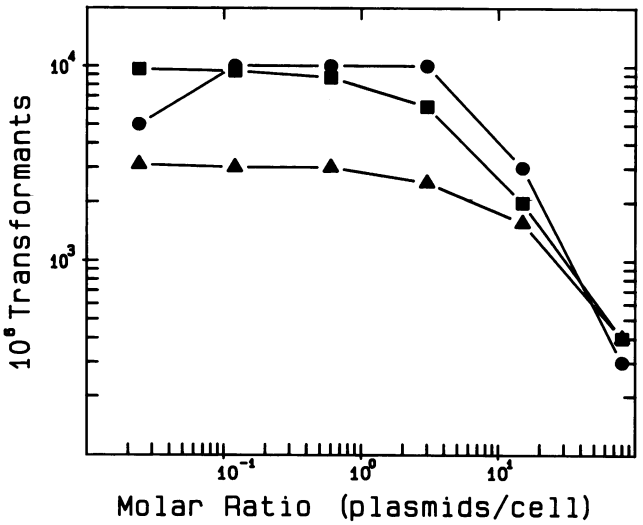

FIG. 7. Transformation efficiency as a function of plasmid concentration. MC1061 cells were grown to an OD_{650} of 1.0, harvested, and washed as described in the text. Transformation was carried out by using 3 ng to 10 μg of pUC18 per ml, TB 1, a cell concentration of 1:50, and a field strength of 13,000 V/cm; no calf thymus DNA was added. CTE (●) is shown as a function of the number of plasmid molecules per cell in the suspension. Symbols: ▲, the theoretical maximum efficiency if plasmids enter cells only by diffusion; ■, the theoretical maximum efficiency if plasmids within a volume three times that of the cell are electrostatically attracted toward the cell (see text).

to the cells after two washes, suggesting that binding does not play a major role.

A possible basis for the second mechanism is simple electrostatic attraction between the cells and the plasmid molecules. During the application of the pulse, both cells and plasmid molecules would acquire an induced dipolar charge. Cells and plasmids would experience mutual attraction, thus increasing the effective plasmid concentration in the neighborhood of each cell.

Figure 7 also shows that the efficiencies predicted if each cell concentrates plasmids in a volume equal to roughly three times its volume agree well with the measured values.

Effect of cell density. The result described above suggested that increasing the cell concentration in the poration suspension should increase the transformation efficiency. MC1061 cells were grown to an OD_{650} of 1 and pelleted and washed as described in Materials and Methods. The final wash suspension was divided into successively smaller volumes. The pellets were taken up in the same volume of poration buffer to give a ratio of pellet volume to buffer volume of 1:1, 1;2, 1:4, 1:8, and 1:16. pUC18 was added to each sample at approximately 0.1 μg/ml, and poration was carried out at field strengths of 9,000, 11,000, and 13,000 V/cm.

The results (not shown) indicated two trends. As the cell concentration was increased at a given field strength, the CTE went up but at the same time survival went down. The net result is that at higher cell densities, the optimal field strength drops and the maximum CTE rises. The maximum efficiency occurred at a field strength of 10,000 V/cm and a cell density (pellet volume/added buffer) of 1:2.

A possible explanation for the lower optimal field strength at high cell density is that the effective field strength increases with cell concentration. This might occur because the ionic strength inside the bacteria is much higher than that outside; the potential gradient across the volume of the bacteria (as distinct from that developed across its membranes) would therefore be small. This would have the effect of increasing the field strength between bacteria, which is formally equivalent to moving the electrodes closer together.

Effect of buffer composition. The 15% glycerol concentration used in the experiments described above was chosen as a value likely to minimize osmotic shock in porated cells. The K_2HPO_4 concentration for the buffer was the highest ionic strength that did not cause excessive resistive heating of the suspension.

The effect of glycerol concentration was investigated by porating cells at field strengths between 3,000 and 13,000 V/cm in 0, 15, and 30% glycerol solutions. The K_2HPO_4 concentration was 0.2 mM, cell concentration was 1:2, and pUC18 was used at 0.1 μg/ml. The results (not shown) indicated that at the highest field strengths, a 30% glycerol concentration improved survival by a factor of 3 but reduced transformation by 80%. Lowering the glycerol concentration from 15 to 0% did not result in a significant change in either survival or transformation.

To investigate the effect of varying buffer concentrations, the experiment described above was repeated without glycerol and with K_2HPO_4 buffer concentrations of 0, 0.2, or 1 mM. Survival was not significantly affected (data not shown), but transformation efficiency was marginally higher in the 0 mM solution (Fig. 8).

Enhancement by pantoyl lactone. We tested the transformation technique on two additional cell lines, HB101 and JM109. In preliminary experiments, HB101 cells gave efficiencies similar to those of the MC1061 cell line. JM109 cells, however, yielded considerably lower survivals and transformation efficiencies. Microscopic examination of JM109 cultures several hours after poration revealed large numbers of multicellular filaments. Some *E. coli* derivatives fail to form macrocolonies following exposure to stresses such as ionizing radiation or short wavelength UV light because they cannot carry out septation and therefore form multinucleate filaments (8). UV sensitivity in such cell lines is typically an order of magnitude greater than that of normal cells, as judged by the slope of the survival curves (1). It has been reported that growth at 43°C or growth on plates containing pantoyl lactone after irradiation can restore near normal survival in such cultures (2).

In order to determine whether we could use either of these conditions to suppress filamentation and thereby improve the transformation efficiency of JM109 cells, we performed a standard electroporation and grew the porated cells at 37 or 43°C or on plates containing pantoyl lactone at 37°C (data not shown). Both pantoyl lactone and growth at 43°C enhanced survival following poration. Growth at 43°C marginally reduced the transformation efficiency, but pantoyl lactone increased the transformation efficiency by almost threefold.

Recovery of plasmid by poration. We tested the possibility that plasmid molecules could be released from transformed cells after poration. A 10-ml overnight culture was prepared from a transformed clone obtained in one of the earlier experiments. The culture was harvested, washed three times in ddH_2O, and suspended in water at a cell density of 1:2 (the cell pellet comprised one-third of the final volume). Samples (12 μl) were porated at several different voltages, and multiple pulses were applied to some samples. After poration, a 5-μl sample was placed in 100 μl of water and vortexed briefly. Cells and debris were removed by centrifugation, and a portion of the supernatant was analyzed on an agarose minigel. The results (Table 1) showed that plasmid was efficiently released from the cells. Comparison of the band intensities with the band intensity of a known amount of plasmid run in a parallel lane of the same gel indicated that

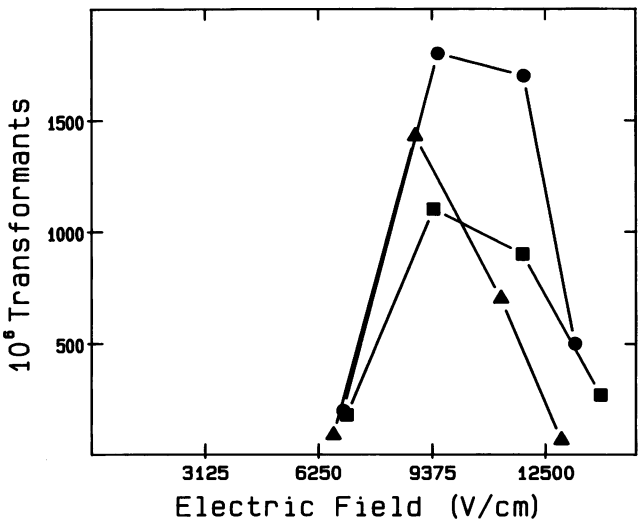

FIG. 8. Effect of buffer concentration on transformation. Cells were prepared as described in the legend to Fig. 7. Washes were carried out in TB1, but the K_2HPO_4 concentration in the final suspension was either 0 (●), 0.2 (▲), or 1 mM (■). No glycerol was used, and the cell concentration was 1:2. pUC18 (0.1 μg/ml) was added to each sample, and porations carried out field strengths between 5,000 and 13,000 V/cm. Transformation (shown) and survival (not shown) were assayed as described in the text.

TABLE 1. Extraction of plasmid by electroporation

Pulse	Field strength[a]	Amt recovered[b]		
		Plasmid DNA	Cellular DNA	Cellular RNA
1+	13,788	1.7	5.3	0.25
2+	13,738	3.5	1	4.5
1+, 1−	13,801	3.4	1.9	7.2
1+	15,174	2.7	3.2	9.0
4+	15,174	3.1	3.9	12

[a] Field strength is expressed as volts per centimeter.
[b] The amount of DNA or RNA is expressed as the relative intensity of the band.

about 5 μg of plasmid could be recovered from a 2-ml culture by this method, close to the yield obtained by using an alkaline minipreporation method on the same cells. The poration conditions for each sample in the gel are shown in Table 1. Even the lowest voltage pulse released most of the plasmid molecules. Additional pulses increased the amount of plasmid DNA released but increased the amounts of cellular DNA and RNA released by an even larger factor. Just as with other methods of preparing plasmid, the yield could often be improved by chloramphenicol amplification (data not shown).

DISCUSSION

We have developed a new method for electroporating bacterial cells. Using this apparatus, we have performed field-mediated transformation of *E. coli* K-12 at unprecedented efficiencies, approaching 5×10^9 transformants per μg of plasmid DNA. The optimal conditions for the method are simple, involving only several washes of the cells in deionized water. The same method can be used to extract plasmid DNA from transformed cells, providing an efficient and faster alternative to more traditional methods for preparing small quantities of plasmid for analysis.

While optimizing the conditions used for transformation, we found the following results. (i) Transformation efficiency and cell survival are dependent upon growth stage of the bacterial culture prior to poration. In exponentially growing cultures, individual cells are very sensitive to killing by poration but those that survive are transformed at an extremely high rate. As a culture begins to approach the stationary phase, survival increases much faster than transformation efficiency decreases. Survival eventually plateaus, while transformation efficiency continues to decrease, generating a peak transformation efficiency for MC1061 cells at a culture OD_{650} of 1.

(ii) The mechanism of cell killing may be different from that responsible for transformation. The definition we have used for cell killing is simply that the cells do not form macrocolonies when plated on appropriate agar plates. We initially assumed that death was due to the formation of nonreversible pores in the cell membrane, which allowed essential cytoplasmic components to leak out. However, microscopic examination of cultures at various times following poration showed the formation of cellular filaments. It is possible that the lack of macrocolonies is due more to the onset of filamentous growth than to irreversible pore formation. This hypothesis is supported by the finding that growth on plates containing pantoyl lactone increases the transformation efficiency of JM109 cells, a procedure known to inhibit filamentation. We are currently attempting to determine what percentage of a population actually enters filamentous growth following poration and to optimize conditions for preventing or reversing it.

(iii) Electroporation can be used to recover plasmid molecules from cells while releasing relatively small quantities of cellular RNA and DNA. This suggests that the plasmid is not strongly bound within the bacterium. It also indicates that many other cellular components, including most of the RNA, are confined in some way that is not disrupted by the poration procedure.

We have not yet investigated the effects of the size and state (i.e., form I, II, or III) of the transforming DNA on transforming efficiency. Experiments using different size molecules will be important to determine the effective size of the pores.

Because the state of the cell culture immediately prior to harvesting for poration is critical, we are also exploring the possibility that cells at the appropriate stage might be frozen and then thawed for use. If this procedure can be made to work, a complete transformation might be carried out in less than 30 min by using our technique.

ACKNOWLEDGMENTS

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