Improved method for electroporation of *Staphylococcus aureus*

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1. SUMMARY

We have developed a significantly improved method for the electroporation of plasmid DNA into *Staphylococcus aureus*. The highest transformation efficiency achieved with this procedure was $4.0 \times 10^8$ transformants per µg of plasmid pSK265 DNA. This represents a 530-fold improvement over the previously reported optimum efficiency of $7.5 \times 10^5$ transformants per µg of plasmid DNA after electroporation of *S. aureus* cells [9]. Identical results were obtained when electrocompetent cells, which had been stored frozen at $-80^\circ$C, were used. The improved efficiency is due primarily to the use of a modified medium (designated as B2 medium) and secondarily to the use of 0.1-cm cuvettes. Several other plasmids (p1258, pMH109, and pSK270) were also electrotransformed into competent cells using our procedure, and for each plasmid, the transformation efficiency was significantly reduced compared to that observed when pSK265 DNA was used. With respect to plasmid p1258, the transformation efficiency was 3500-fold higher than that reported previously for transformation of this plasmid into *S. aureus* RN4220 [9].

The optimized electroporation procedure was less successful in transforming other staphylococci. Electrocompetent cells of *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228 produced $5.5 \times 10^5$ and $5 \times 10^3$ transformants per µg of pSK265 DNA, respectively.

2. INTRODUCTION

Electrotransformation produces transiently permeabilized cells. Permeability occurs when bacterial cells are exposed to an electrical field, which produces channels in the cell membrane through which DNA can pass [16]. Transformation efficiencies are usually higher in Gram-negative bacteria than Gram-positive bacteria [4–6, 9, 11]. For example, more than $10^{10}$ transformants per µg of DNA have been produced after electroporation of *Escherichia coli* [5] compared with a maximum of $7.5 \times 10^5$ transformants per µg of
DNA produced after electroporation of \textit{S. aureus} cells [9]. The reason(s) for the difference in transformation efficiencies is not known, but is assumed to be due to the differences in the structure of the Gram-positive cell wall and the Gram-negative cell envelope.

There are several parameters that can be varied in an electroporation experiment. These variable parameters include the following: (i) the growth medium; (ii) how the electrocompetent cells are prepared; (iii) the time constant (the duration of the electrical pulse) and the field strength (defined as the voltage applied to the electrodes divided by the distance between the electrodes [gap size] of the cuvette); (iv) the amount of DNA added and the concentration of cells used for each electrotransformation; and (v) the recovery medium used after electroporation and the growth medium used to select for the transformants. Optimization of these parameters should result in the highest efficiency of transformation.

Electroporation of both \textit{S. aureus} RN4220 [1,9] and \textit{S. epidermidis} Tu3298 [1] cells has been previously reported, but transformation efficiencies of only $7.5 \times 10^5$ per $\mu$g of plasmid pDH5060 DNA and $3 \times 10^5$ per $\mu$g of plasmid pC194 DNA, respectively, were obtained. The two procedures used to obtain these results differed in the wash protocols used to prepare electrocompetent cells and the plasmid DNA used to transform the cells. The purpose of this study was to maximize the transformation efficiency of \textit{S. aureus} cells by utilizing modifications of previously developed procedures and to determine the effect of 0.1-cm cuvettes on the efficiency of transformation.

3. MATERIALS AND METHODS

3.1. Strains and growth conditions

\textit{S. aureus} RN4220 [10], \textit{S. aureus} ATCC 29213 and \textit{S. epidermidis} 12228 were used as bacterial hosts. Plasmids are listed in Table 1. \textit{S. aureus} RN4220 cells were the source of plasmid DNA. The plasmid DNA was isolated by the method of Novick et al. [14], purified by CsCl-ethidium bro-

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Origin of replication</th>
<th>Selective marker (^b)</th>
<th>Size/copy number</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>pJ258</td>
<td>pJ258</td>
<td>Erm</td>
<td>29.0 kbp/3</td>
<td>14</td>
</tr>
<tr>
<td>pMH109</td>
<td>pUB110</td>
<td>Km</td>
<td>7.4 kbp/10</td>
<td>7</td>
</tr>
<tr>
<td>pSK265</td>
<td>pC194</td>
<td>Cm</td>
<td>2.9 kbp/90</td>
<td>15</td>
</tr>
<tr>
<td>pSK270</td>
<td>pE194</td>
<td>Erm</td>
<td>2.9 kbp/35</td>
<td>S. Kahn, pers. comm.</td>
</tr>
</tbody>
</table>

\(^a\) = all plasmids were isolated from \textit{S. aureus} RN4220 cells. \(^b\) = the antibiotics and concentrations used are: Km, kanamycin; Cm, chloramphenicol; Erm, erythromycin. The antibiotics and concentrations used are: (i) chloramphenicol (6 $\mu$g/ml); (ii) kanamycin (20 $\mu$g/ml); (iii) erythromycin (100 $\mu$g/ml).

mide density gradient centrifugation and subsequently resuspended in TE buffer.

Cells were grown in one of three different media. B2 broth is a modification of the medium of Augustin and Gotz [1] and consists of 1.0% casein hydrolysate (Sigma Co.), 2.5% yeast extract (Difco), 0.1% $K_2$HPO\(_4\), 0.5% glucose, 2.5% NaCl (pH 7.5). The other media were CY broth [8] and SOC broth [5]. To determine the total number of viable transformants, electrotransformed cells were plated on NYE agar [2], which is composed of 1.0% casein hydrolysate (Sigma Co.), 0.5% yeast extract (Difco), 0.5% NaCl, containing one of the following antibiotics: (i) chloramphenicol (6 $\mu$g/ml); (ii) kanamycin (20 $\mu$g/ml); (iii) erythromycin (100 $\mu$g/ml).

3.2. Cell growth and preparation of cells for electroporation

An overnight culture grown in B2 broth with constant aeration at 37°C was diluted 1/25 into 25 ml of fresh B2 broth in a 300 ml Klett flask. The cells were grown with constant aeration at 37°C until they reached mid-log phase of growth (260 Klett units [Ku] using a #66 red filter) and were then harvested by centrifugation.

Upon harvesting the cells from the B2 broth, the cells were washed three times in an equal volume of deionized water, followed by two washes with 1/5 and 1/10 volumes of 10% glycero1 solution. Following resuspension of the cells in the second 10% glycero1 wash solution, the cell suspension was incubated for 15 min, centrifuged and the cell pellet resuspended in 800 $\mu$l of a
10% glycerol solution. The final cell concentrations obtained were between 1 and $3 \times 10^{10}$ cells per ml. All wash solutions and incubations were at 20°C. All centrifugations were at 8k, 10 min, 20°C. The electrocompetent cells were used within 1 h after preparation. Alternatively, 70-μl aliquots of electrocompetent cells were frozen in microfuge tubes at −80°C immediately after preparation.

3.3. Electroporation procedure

For electroporation, 70 μl of electrocompetent cells were mixed with the appropriate amount of DNA (usually 1 μg) and 60 μl of the cell suspension-DNA mixture transferred to a 0.1-cm gap electroporation cuvette. The cells and DNA were electroporated at 20°C, 100 ohms resistance, 25 μF capacitance (optimum time constant = 2.5 ms), and 2.3 kV in a Gene Pulser™ apparatus with pulse controller (Bio-Rad Laboratories, Richmond, CA). The cells were then immediately resuspended in 390 μl of B2 broth, transferred to a test tube (glass or plastic) and incubated for 1 h at 37°C. The cells were then plated on NYE agar plates containing the appropriate selective agent and incubated at 37°C for 24–48 h. When electrocompetent cells stored at −80°C were used, the cells were thawed at 20°C and used immediately.

Unless stated to the contrary, all electroporation experiments utilized the optimum electroporation procedure described above with 1 μg of pSK265 DNA, a 0.1-cm cuvette and S. aureus RN4220 cells. Plasmid pSK265 is a copy number mutant of plasmid pC194 [13] and also contains the pUC19 multicloning site [15].

4. RESULTS

4.1. Effect of the field strength, time constant and cuvette gap size on transformation efficiency

The effects of increasing field strength at several time constants on the efficiency of transformation were tested. Four time constants (10, 5, 2.5, 1 ms) were selected and at each time constant, five field strengths (10, 15, 20, 22.5, 25 kV/cm) were tested. The optimum time constant was 2.5 ms and the optimum field strength was between 22.5 and 25 kV/cm (Fig. 1). Further experiments determined that the optimum field strength was 23.0 kV/cm (data not shown). These results agree with those obtained by Augstin and Gotz [1] and Kraemer and Landolo [9] as the optimum conditions for electrotransformation of electrocompetent cells of S. epidermidis Tu3298 and S. aureus RN4220, respectively.

Utilizing a field strength of 23 kV/cm and time constant of 2.5 ms, we determined that the transformation efficiency of electrocompetent cells electroporated in 0.2-cm cuvettes was 12-fold less than that produced by electrocompetent cells electroporated in 0.1-cm cuvette.

4.2. Effect of cell concentration on the transformation efficiency

The effect of different cell concentrations (from $1 \times 10^{9}$ to $6 \times 10^{10}$ cells per ml) on the transformation efficiency was determined. The optimum concentration of electrocompetent cells
was between 1–3 × 10^10 cell per ml. Cell concentrations greater than 3 × 10^10 cell per ml resulted in reduced efficiency of transformation (data not shown). When 3 × 10^10 electrocompetent cells per ml were electroporated, only 2% of the cells survived electroporation. Yet, about 1/3 of the cells which survived electroporation were transformed with plasmid DNA (data not shown).

### 4.3. Effect of DNA concentration on the transformation efficiency

The number of transformants increased linearly as the amount of DNA added to cuvette increased 100-fold (from 0.001 to 0.1 μg pSK265 DNA). The number of transformants increased by only five-fold as the amount of DNA added to the cuvette increased an additional ten-fold (from 0.1 to 1.0 μg pSK265 DNA) and finally remained constant thereafter (from 1 to 3 μg pSK265 DNA; Fig. 2). Similar results were obtained by Augustin and Gotz [1] and Kraemer and Landolo [9], except that in these studies, the total number of transformants increased linearly with the amount of DNA added up to a maximum of about 1 μg of plasmid DNA. Additional experiments established that the electrocompetent cells prepared in the present study were not naturally competent.

### 4.4. Effect of growth medium on transformation efficiency

The transformation efficiency of cells grown in B2 broth was 300-fold greater than that obtained with cells grown in either CY or SOC medium. Specifically, the transformation efficiencies for B2, CY, and SOC medium grown cells were 4.0 × 10^8, 5.6 × 10^5 and 6.3 × 10^5 transformants per μg of pSK265 DNA, respectively. B2 broth contains elevated levels of both yeast extract and NaCl. Additional experiments determined that yeast extract is the growth medium component primarily responsible for the improved efficiency of transformation (data not shown).

### 4.5. Effect of temperature on transformation efficiency

Augustin and Gotz [1] determined for *S. epidermidis* Tu3298 cells that 10% glycerol wash solutions and 0.2-cm cuvettes kept at 20°C produced greater transformation efficiency than the same solutions and cuvettes pre-cooled to 4°C. Kraemer and Landolo [9] used sucrose wash solutions and 0.2-cm cuvettes, which were all pre-cooled to 4°C, to obtain electrot transformation of *S. aureus* RN4220 cells. Experiments were done to ascertain which temperature (4°C or 20°C) was optimum for *S. aureus* RN4220 cells. The transformation efficiency of 4°C treated cells was only 15% of that obtained using 20°C treated cells (data not shown).

Freshly prepared electrocompetent cells were stable for at least 1 h at 20°C. However, the transformation efficiency decreased linearly over time (data not shown). After 1 h incubation at 20°C, the transformation efficiency of electrocompetent cells was ten-fold less than that ob-

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**Fig. 2.** The effect of amount of DNA added to the cuvette on the total number of transformants obtained. 60 μl of electrocompetent cells (cell concentration was 1 × 10^10 cells per ml) were electroporated (23 kV/cm, 25 μF, 100 ohms, 20°C) with varying amounts of pSK265 DNA. Cells were plated on NYE agar with 6 μg/ml of chloramphenicol at 37°C.
tained with freshly prepared, electrocompetent cells (data not shown).

4.6. Effect of recovery treatment on transformation efficiency

Incubation of cells in recovery medium for 1 or 2 h increased the transformation efficiency by only 1.5- and two-fold, respectively, over that observed for cells plated immediately after resuspension in recovery medium (data not shown). Identical results were obtained using either NYE or B2 broth as the recovery medium (data not shown). It was not necessary to resuspend electroporated cells in SMMP [3] to achieve a high efficiency transformation as previously reported [1,9].

4.7. The effect of freezing electrocompetent cells on the transformation efficiency

Electrocompetent cells, which had been frozen at −80°C, thawed and then immediately electroporated, produced transformation frequencies equal to that obtained with freshly prepared electrocompetent cells (Table 2). The cells were stable for at least two months at −80°C (data not shown). Previous studies obtained similar results when electroporation experiments were done with electrocompetent cells of *S. aureus* RN4220 [1] and *S. epidermidis* Tu3298 [9] stored at −70°C.

4.8. Transformation efficiencies of plasmids other than pSK265

For each plasmid tested, both freshly prepared and electrocompetent cells previously stored at −80°C produced similar transformation efficiencies, which were significantly reduced compared to that observed when pSK265 DNA was used (Table 2). With respect to plasmid pIL258, the efficiency obtained in the present study was 3500-fold higher than that reported previously for transformation of this plasmid into *S. aureus* RN4220 [9].

4.9. Electroporation of *S. aureus* RN4220, *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228

The optimized electroporation procedure produced the highest transformation efficiencies with *S. aureus* RN4220 cells. Electrocompetent cells of *S. aureus* RN4220, *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 12228 produced $4.0 \times 10^8$, $6.1 \times 10^5$ and $5 \times 10^3$ transformants per µg of pSK265 DNA, respectively.

5. DISCUSSION

The improved procedure described here is superior to previously described methods in several ways. First, high efficiency transformation of Gram-positive bacteria was obtained for the first time. Second, there is no need to utilize the complex and expensive recovery medium, SMMP. Third, there is no need to pre-incubate the electrocompetent cells with DNA before electroporation.

Plasmids pMH109 (pUB110), pSK265 (pC194) and pSK270 (pE194) belong to incompatibility class I and pIL258 belongs to incompatibility class II [13]. Each plasmid used in this study possesses a unique origin of replication originally isolated from *S. aureus*. The fact that each of these plasmids efficiently transformed *S. aureus* indicates that this procedure is not limited to one plasmid with a unique origin of replication, selective marker or incompatibility group.

Kraemer and Landolo [9] determined that plasmid size and not the selective marker utilized was the discriminatory factor which influenced the transformation efficiency obtained with each plasmid. In the present study, plasmids pSK265 and pSK270 are essentially the same size and

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effect of freezing electrocompetent cells on the transformation efficiency</th>
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</thead>
<tbody>
<tr>
<td>Plasmids</td>
<td>Transformation efficiency</td>
</tr>
<tr>
<td></td>
<td>Freshly prepared cells</td>
</tr>
<tr>
<td>pIL258</td>
<td>$6.0 \times 10^5$</td>
</tr>
<tr>
<td>pMH109</td>
<td>$6.4 \times 10^5$</td>
</tr>
<tr>
<td>pSK265</td>
<td>$4.0 \times 10^6$</td>
</tr>
<tr>
<td>pSK270</td>
<td>$1.3 \times 10^6$</td>
</tr>
</tbody>
</table>

a = plasmid DNA was isolated from *S. aureus* RN4220 cells.

b = for each batch of electrocompetent cells electroporated, 1 µg of plasmid DNA was used.
purified by the same procedure, but the copy number of pSK265 is much greater than that of pSK270 (Table 1). In addition, pSK265 confers resistance to chloramphenicol, while pSK270 confers resistance to erythromycin. The transformation efficiency of cells electroporated with pSK270 DNA was much less than that obtained when pSK265 DNA was used (Table 2). It is possible that the copy number of the plasmid electrotansformed into cells and the selective marker used may be significant factors in determining the efficiency of the transformation.

In the present study, when the optimum concentration of electrocompetent cells was used (1-3 × 10^10 cells per ml) only 2% of the cells survived electroporation (data not shown). Kraemer and Landolo [9] and Augustin and Gotz [1] performed similar studies with S. epidermidis Tu3298 and S. aureus RN4220, respectively, and determined that the optimum concentration of electrocompetent cells of either species was determined to be between 3-5 × 10^10 cells per ml. However, Augustin and Gotz [1] had determined that after electroporation under optimum conditions, 50% of the S. epidermidis electrocompetent cells survived, while Kraemer and Landolo [9] determined that after electroporation, 2–8% of the S. aureus RN4220 electrocompetent cells survived. Meletzus and Eichenlaub [12] also obtained a similar low survival rate when cells of the Gram-positive bacterium, Clavibacter michiganense, were electroporated under optimum conditions.

Lastly, the results of the present study demonstrate that it is desirable to optimize electroporation procedures for each strain of bacteria used and that the growth medium utilized is important in optimizing electroporation conditions.

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REFERENCES