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Note

Efficient transformation of *Staphylococcus aureus* using multi-pulse electroporation

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ABSTRACT

A new multi-pulse electroporation system was evaluated to transform *Staphylococcus aureus*. Compared to the conventional electroporation system, it yielded high transformation efficiency to obtain more than 3.9×10^5 *S. aureus* RN4220 transformed cells/1 μg plasmid DNA using a single electroporation by manipulating the poring pulse and transfer pulse.

Acceleration of bacterial transformation using a plasmid with calcium chloride was first demonstrated by Cohen et al., 1972. Since then, bacterial transformation has opened the way to explore and study gene products of any creature expressed these genes in *Escherichia coli*. This bacterial transformation became a fundamental technique in molecular biology. In the early '80s, physical transfer of plasmid DNA into eukaryotes (Neumann et al., 1982) and prokaryotes (Dower et al., 1988; Taketo, 1988; Chassy and Flickinger, 1987; Fiedler and Wirth, 1988; Miller et al., 1988) using electric discharge called electroporation was introduced. Electrical discharge destabilizes membranes of target cells in a small cell and transiently creates pores or aqueous pathways through which DNA molecules enter the cells. This method enables transformation of a variety of microorganisms unlike the transformation with calcium chloride-induced competent Gram-negatives that were limited to *E. coli* and *Salmonella* spp. (Mandel and Higa, 1970). Natural competency is also used for bacterial genetic manipulation in natural environment and for genome editing technology (Lorenz and Wackernagel, 1994; Jiang et al., 2013). Natural competency can be observed and used in Gram-negatives such as *Helicobacter pylori* and *Neisseria meningitidis*, Gram-positive bacilli and Streptococci (Gorrell et al., 2005; Alexander et al., 2004; Kumpfmuller et al., 2013; Morrison et al., 2015). However, other pathogenic bacteria are resistant to natural transformation. Electroporation with established protocols works well with authentic laboratory strains of *E. coli* and high efficiency of transformation is obtained (Dower et al., 1988; Tung and Chow, 1995). This method is an alternative to transform bacteria that are resistant to calcium chloride-induced competency and natural competency. However, electroporation induced transformation efficiency significantly varies among strains even in *E. coli* and limited transformation efficiency in other bacteria is one of the challenging obstacles to overcome.

Staphylococcus aureus is a common human pathogen causing a variety of infectious diseases from mild to life threatening. Primary and the most common site of infection is the skin where it causes skin and soft tissue infections. Entering the blood stream, it infects virtually every tissue causing a variety of diseases including bone/joint infection and endocarditis. *S. aureus* is also known to produce a great variety of protein toxins including superantigens such as Toxic Shock Syndrome Toxin-1 (TSST-1)/enterotoxins that often cause toxic shock syndrome (Pinchuk et al., 2010).

Most of pathophysiological studies of genetically engineered *S. aureus* were conducted using a limited number of laboratory or prototype strains such as 8325-4, COL and others (Novick, 1990). One of the

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reasons why we use such strains is the difficulty of transformation with most clinical strains (Monk et al., 2015). In clinical strains, the laboratory strain RN4220 deficient in restriction system has been used as an intermediate strain for the transfer of plasmid DNA into the target clinical strain (De Azavedo et al., 1985) but we often encounter difficulty in transforming clinical strains even with RN4220. For the transformation of *S. aureus*, electroporation is indispensable and single-pulse electroporators such as Gene-Pulser (Bio—Rad Laboratories, Inc.) and ECM (BTX, Harvard Apparatus, Inc.) have been used (Kraemer and Iandolo, 1990). Recently multi-pulse electroporation system ELEPO21 was introduced and widely used to transfect eukaryotic cells and organ tissue. The novel multi-pulse with natural voltage decay results in high transformation efficiency and high viability. This electroporation starts with multiple high-voltage pulses of short duration to form temporary pores in cell membrane (poring pulses) and subsequent multiple low-voltage pulses for delivering nucleic acids into target cells with minimum damage (transfer pulses) (Miyahara et al., 2013). The electrode polarities for poring pulses and transfer pulses can be reversed

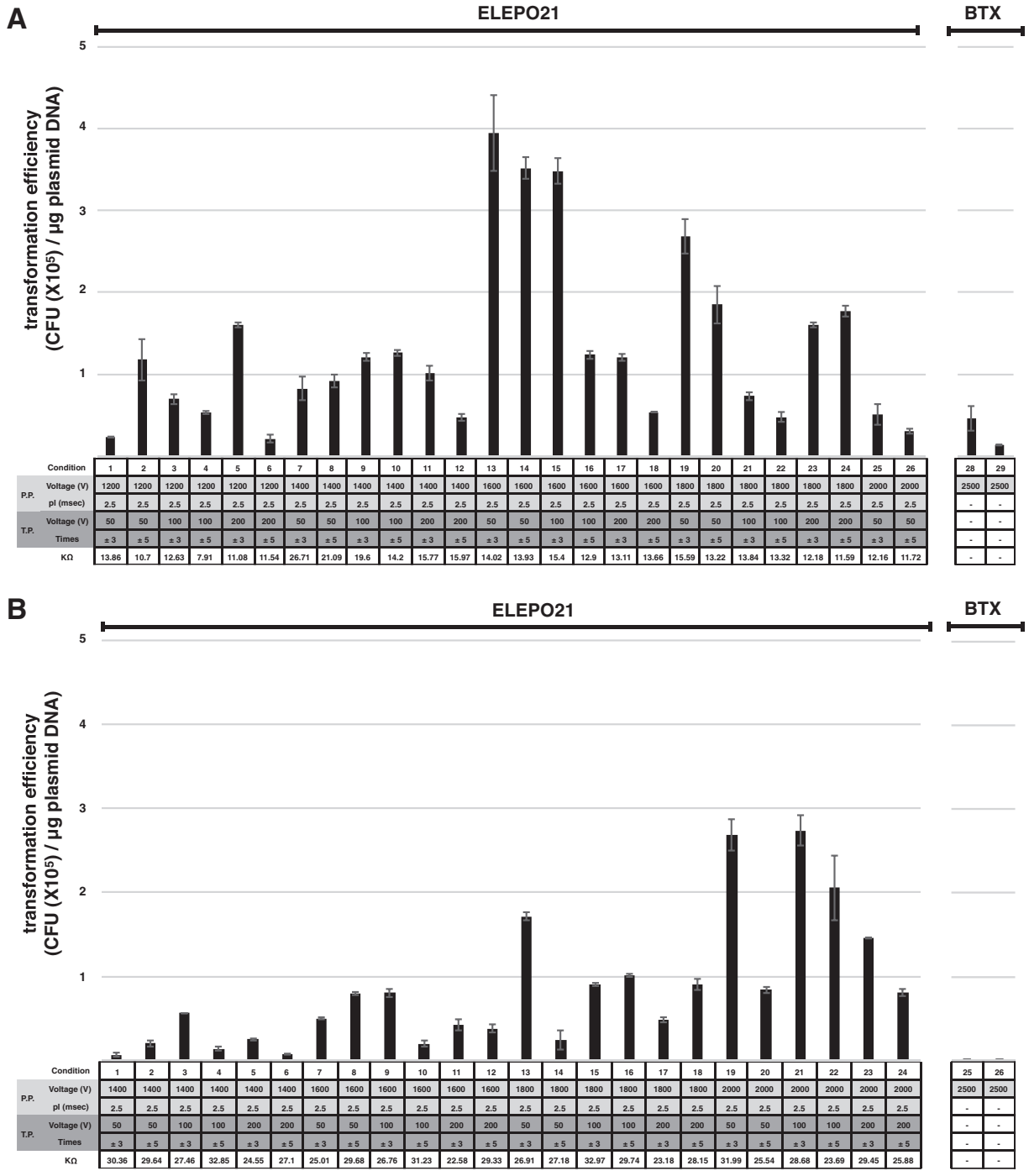

Fig. 1. Optimization of pulse conditions for electroporation. P.P., poring pulse; T.P., transfer pulse. The number of transformants was assessed by manipulating the voltage of the poring pulse (P.P.), the transfer pulse (T.P.) and frequency of the discharge transfer pulse. Measured conductance is shown (KΩ). The sample mixture was pre-incubated at room temperature (A) or on ice (B) before electroporation. Transformation efficiency was calculated as CFU ($\times 10^5$)/μg plasmid DNA electroporated using a cuvette with a 1 mm gap. Data are means ± SD of values from three independent experiments with assays in duplicate.