A Simple and Rapid Method for DNA Transformation of Intact Cells of *Bacillus cereus* by Electroporation

Morimasa OHSE, Takehiro YAMASHITA,
Yasuhiro KADOWAKI and Hideo KUSAOKE

SYNOPSIS

An electroporation preocedure for the plasmid-mediated genetic transformation of intact cells *Bacillus cereus* was performed. The method was optimized for *B. cereus* IFO3466, and the transformation of IFO3466 with plasmid pUB110, using the mixture of 0.3 M sucrose, 1 mM $CaCl_2$, and 1 mM sodium citrate (pH 7.0) as a buffer for pulsation, resulted in a pulse duration of 500 μsec with an initial electric field strength of 12 kV/cm. Plasmid DNA isolated from transformants was indistinguishable from authentic preparation of plasmid pUB110 on gel electrophoretic analysis. The electroporation was simply sampled at 20 μl-small volume of cell suspension ($\sim$ 1 x 10^{10} cells/ml, *ca.* 4 pg DNA/ μl).

INTRODUCTION

Electroporation has been used to introduce DNA into both eucaryotic and procaryotic cells.[1,2] Transformation by electroporation is a recent method that has been successfully employed with a number of bacterial species. The technique is less tedious and time-consuming.

Formerly we succeeded in gene transfer into intact cells as well as protoplasts of *Bacillus subtilis*.[3] Even transformation of intact cells resulted in a maximum efficiency of 2.8 x 10^4 transfromants per μg plasmid pUB110 DNA.[4] Shivarova *et al.*[5] reported that *B. cereus* protoplasts in the presence of polyethylene glycol can be transformed by electroporation. Takagi *et al.*[6] also reported that the transforamtion efficiency of *B. brevis* intact cells was 1.3 x 10^4 transformants per μg

* Department of applied physics and chemistry

plasmid pBAM101. *Bacillus* strains offer many potential advantages in the productioin of cloned products, which are secrected to the growth medium, and have been widely used for production of industrial enzyme in large-scale fermentation proceses.

In this paper, we report the successful application of electroporation to the transformation of strains of *B. cereus*.

EXPERIMENTAL

Strain and plasmid. *Bacillus cereus* IFO 3466 was used as a transformation host strain. Plasmid pUB110 (4.5 kbp, Km^R) was kindly provided by Y. Yoneda (Nippon Gene Co. LTD.). Plasmid DNA was isolated from *B. subtilis* Marburg 168 as well as transformants according to the alkaline lysis method described by Maniatis *et al.*[7] and purified for use in electroporation by centrifugation to equilibrium in cesium chloride/ethidium bromide density gradients.

DNA transformation by electroporation. Usually the mixture of 0.5 M sucrose, 0.1 mM $MgCl_2$, and 0.1 mM maleic acid (pH 7.0) was used for washing buffer of intact cells, and the mixture of 0.3 M sucrose, 1 mM $CaCl_2$, and 1 mM sodium citrate (pH 7.0) was employed as an electroporation-suspension buffer. SMMP medium (3.5 % Pen, 17.1 % sucrose, 0.23 % maleic acid, 0.41 % $MgCl_2$) was used for dilution and expression medium. Bacterium was stationary culture overnight on pen medium at 37 ℃, and a portion of the bacterium was inoculated onto 100 ml of Pen medium and the cells were growth with shaking at 37 ℃ to OD_{660}=0.4 ~0.5 (~1 x 10^8 cells/ml) The cell suspension was cooled at 0 ℃, and the 196 μl-portion was transferred to a 0.5 ml Eppendorf tube. Four μl of plasmid DNA solution (0.2 μg/ml) were added, followed by careful mixing with a pipette. Twenty μl of the cell suspension were placed in a chamber. The cells were subjected to electroporation. After the electric treatment, the cells were left for 5 min at room temperature. The cells were pipetted out of the chamber to an Eppendorf tube. The suspension in tube was suitably diluted in SMMP medium and then placed on Pen agar medium. Prior to

plating on media containing antibiotics, the diluted cells were inoculated at 30 ℃ for 1.5 hrs to allow the phenotypic expression of antibiotic resistance markers. Plates were inoculated at 37 ℃ for 1 to 2 days, the numbers of regenerated colonies were counted. Determination of transformation efficiency and cell survival was made as described in our previous paper.[3)]

Electroporation apparatus. A high voltage generator (SHIMADZU SSH-1 TYPE) that can supply electric pulse of squared waves was constructed as a electroportion apparatus. Chamber (electric distance, 1 mm; volume, 20 μl) were used to obtain pulse duration of 10 to 50 μsec and various field strengths up to 28 kV/cm at 0 ~ 50 ℃.

RESULTS AND DISCUSSION

Effect of electric field strength on transformation. We first investigated the effect of electric field strength on transformation. The results are shown in Fig. 1. The transformation efficiency increased with increasing electric field strength in the voltage of 3 to 12 kV/cm, but slightly decreased with increasing electric field strength over 12 kV/cm. Maximum transformation efficiency was given as 1.2×10^2 transformants per μg plasmid DNA with an electric field strength of 12 kV/cm under the pulse duration of 500 μsec at 0 ℃.

Luchansky *et al*.[8)] reported that the electric transformation of intact *Bacillus cereus* cells with plasmid pGK12 resulted in an efficiency of 6.5×10^1 transformants per μg DNA. Belliveau and Trevors[9)] also reported that transformation frequency of intact *B. cereus* 569 with plasmid pC194 resulted in a maximum of 2×10^{-5} transformants per viable cell. Our transformation value was higher than that from these studies. The survival with duration of 500 μsec decreased from 80 to 20 %with an increase of electric field strength from 4 kV/cm to 6 kV/cm (data not shown). This shows that electric breakdown of cell membranes may occur at the electric field strength over 5 kV/cm. The increase of the number of pulses used for transformation by

electroporation was not effected to transformation efficiency (data not shown).

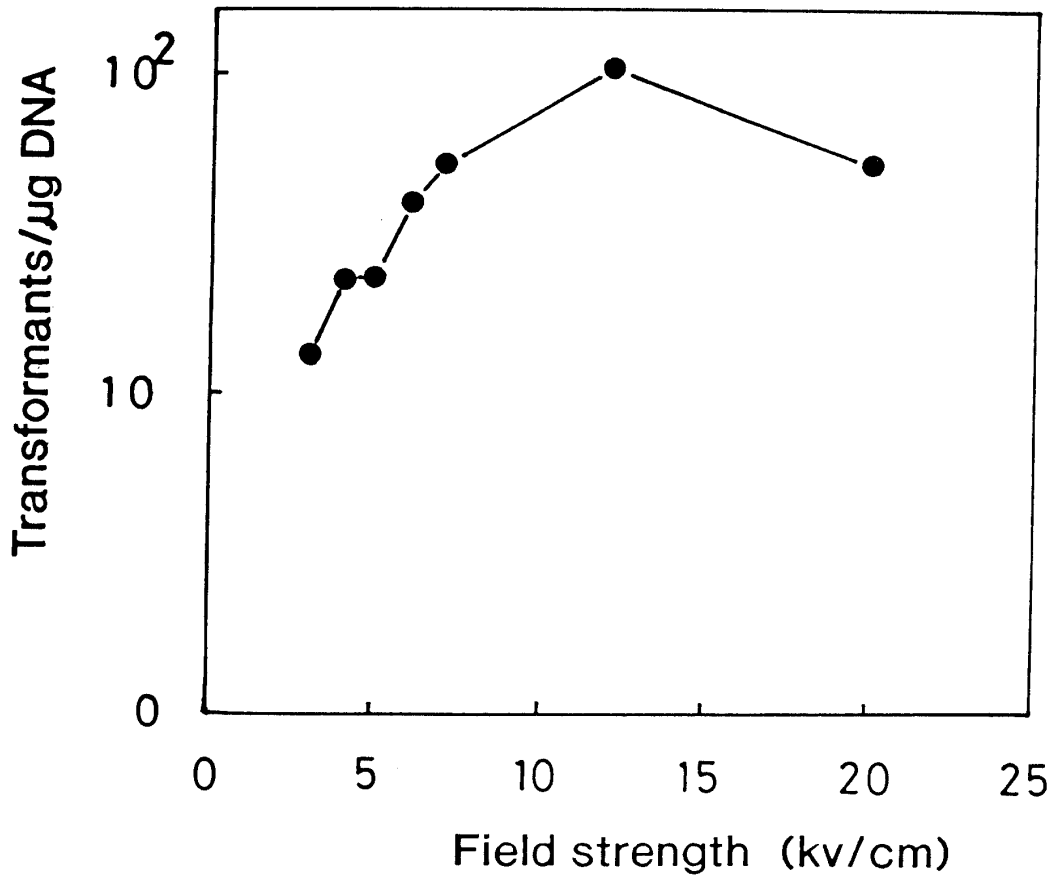


Fig. 1 Efficiency of the electric field strength on transformation efficiency with *Bacillus sutilis*.
Cell suspension (196 μl, 5 x 10^9/ml) of *B. cereus* in buffer containing 0.3 M sucrose, 1 mM $CaCl_2$, and 1 mM sodium citrate (pH 7.0) were mixed with 4 μl of pUB110 (0.2 μg/ml) and subjected to electroporation of the indicated electric field strength at a pulse duration of 500 μsec, at pulsation temperature at 0 ℃, and at pulsation of one time.

Effect of pulse duration on transformation. The effects of the pulse duration are shown in Fig. 2. Transformation efficiency increased with increasing pulse duration untill 300 μsec, but longer pulses than 300 μsec slightly decreased the efficiency at a given field strength of 7 kV/cm. The survival decreased from 63 % to 22 % with an increase of pulse duration from 100 to 500 μsec. The increase of the pulse duration may present the irreversible damage to cells, but the longer

duration seems to cause the ion flux for membrane breakdown on pulsation.

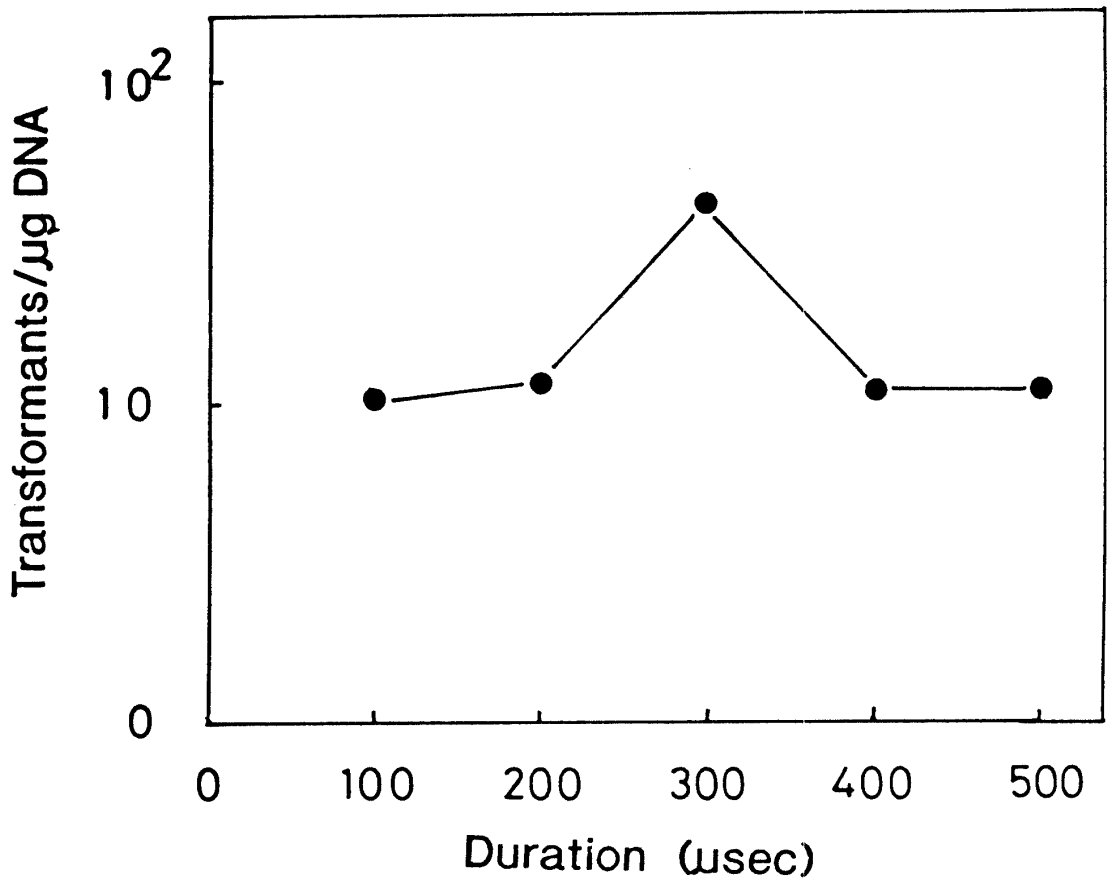
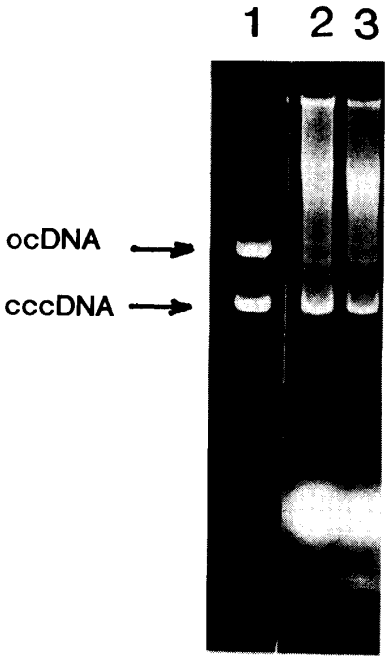


Fig. 2 Effects of the pulse duration on transformation efficiency with *B. cereus.*
Cells with plasmid DNA were suspended in buffer and exposed to a single pulse of various duration at electric field at 7 kV/cm. Other conditions were the same in Fig. 1.

Isolation of plasmid DNA from transformants. Plasmid DNA was isolated from transformants and analyzed by agarose gel electrophoresis (see Fig. 3). The electrophoresis mobility of plasmid DNA isolated from transformants was identical to that of authentic preparation isolated from *B. subtilis*. Twenty transfromants with plasmid pUB110 DNA examined, and all yielded a plasmid profile identical to that in native *B. subtilis* strain. Transfer of pUB110 into several *Bacillus* cells by electroporation was evaluated, as shown in our previous paper.[4] Intact cells of *B. subtilis* ISW1214 strain were

transformed at maximum efficiency of 2.8 x 10^4 transformants per μg DNA, using a buffer of 0.3 M sucrose, 1 mM sodium citrate, and 1 mM $CaCl_2$ (pH 7.0). Transformation of *B. cereus* results in efficiencies as low as 1.2 x 10^2 transformants per μg DNA. It speculats that the introduction of DNA through the formation of poration by electroporation may be difficult, because the rod cells of *B. cereus* form the chains.



Fi. 3 Agarose gel electrophoresis of plasmid pUB110 isolated from *B. subtilis.*

Plasmid DNA was isolated from *B. subtilis* transformed with pUB110 and then electrophoresed in 0.7 % agarose gel. Lane1, authentic plamid pUB110 isolated from *B. subtilis*; lanes 2 and 3, pUB110 isolated from transformants. Abbreviations of ocDNA and cccDNA present the molecular structure of open circular and covalent closed circular, respectively.

The results we have presented show that *B. cereus* intact cells can be transformed by electroporation. Electroporation is simple and easy to perform, and requires minimal sample preparation. As advances in electroporation technology are made, the technique may also replace well established methods of bacterial transformation.

Rreferences

1) H. Inaba, M. Kasai, and K. Sato, *Protein, Nucleic Acid and Enzyme,* **32**, 10(1987).
2) K. Shigekawa and W. J. Dower, *BioTechniques*, **6**, 742(1988).
3) H. Kusaoke, Y. Hayashi, Y. Kadowaki, H. Kimoto, and H. Kusaoke, *Agric. Biol. Chem.*, **53**, 2441(1989).
4) M. Ohse, K. Takahashi, Y. Kadowaki, and H. Kusaoke, *Biosci. Biotech. Biochem.*, **59**, 1433(1995).
5) N. Shivarova, W. Forster, H. E. Jacob, and R. Grigorova, *Z. Allg. Microbio.*, **23**, 595(1983).
6) H. Takagi, S. Kajiyama, K. Kadowaki, N. Tsukagoshi, and S. udaka, *Agric. Biol. Chem.*, **53**, 3099(1989).
7) T. Maniatis, E. F. Fritsch, J. Sambrook, "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, NY., 1982.
8) J. B. Luchansky, P. M. M. Muriana, and T. R. Klaenhammer, *Molecular Biology*, **2**, 637(1988).
9) B. H. Belliveau and J. T. Trevors, *Appl. Environ. Microbiol.*, **55**, 1649 (1989).

(Received July 17, 1995)