Application of Electroporation for Transformation in A Thermophilic Bacterium, *Bacillus stearothermophilus*

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It was found that plasmid DNA can be introduced into a thermophilic intact bacterium, *Bacillus stearothermophilus* by electroporation. The transformation efficiency depended upon the host strain of *B. stearothermophilus*, the electric field strength, the capacitance of the electric discharge, and the electroporation-buffer. Transformation of strain IAM11001 with plasmid pUB110 resulted in a maximum number of transformants of 2.3 x 10^2 per μg DNA by a single decayed wave pulse with an electric strength of 10 kV/cm and a capacitance of 1 μF, using electroporation-buffer containing 1 mM magnesium chloride and 1 mM maleic acid (pH 7.0). Transformants of *B. stearothermophilus* obtained by electroporation contained structurally intact plasmid molecules.

INTRODUCTION

Electroporation is a relatively new method for bacterial transformation that is rapidly gaining acceptance. The method has opened the way to genetic analysis and manipulation of the majority of bacteria have been successfully transformed.[1] Although efficiencies vary from strain to strain, electroporation has become the best technique available for the introduction of DNA. In general, the gram-negative bacteria can be efficiently transformed by electrical methods. But the majority of the gram-positive bacteria had not been transformed, or had been transformable only by tedious protoplast techniques, prior to the introduction of electrotransformation. The efficiencies of transformation observed with gram-positives are generally lower than those observed for gram-negatives. Because, gram-positives generally have thicker cell walls than gram-negatives.

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In our previous studies, an efficiency of 9.3×10^4 transformants per μg plasmid pUB110 was obtained in electroporation of a gram-positive bacterium, *Bacillus subtilis* ISW1214. The efficiency of 2.3×10^3 transformants per μg of DNA was also obtained routinely, even by using a plasmid pBDR331T with a large molecular size of 12.6 kbp. Electrotransformation methods have been applied to at least seven species of *Bacillus*.[1] However, electrotransformation of thermophilic bacteria has not yet proven possible. Imanaka *et al.*[2] reported that the effective transformation of *B. stearothermophilus* with plasmid was done by the protoplast procedure. However this procedure is difficult to handle and the transformation efficiency is often different by investigators. If the transformation for a thermophile with plasmids could be done easily by electroporation, the cloning of specific genes of thermostable enzymes would be made possible and the mode of gene expression at higher temperature could be examined.

In this work, we employed electroporation to transform three strains of *B. stearothermophilus* using a simple commercially available system, and we describe a rapid and general method for the transformation of a thermophilic bacterium, *B. stearothermophilus* that yields colonies of transformants with electric pulses.

EXPERIMENTAL

Bacillus stearothermophilus strain CU21 was obtained from T. Imanaka (University of Osaka, Osaka), strain IAM11001 was from Institute of Molecular and Cellular Bioscience, The University of Tokyo, and strain ATCC12980 was from Japan Collection of Microorganisms. The each strain was subcultured in LG medium[3] at 55 ℃. LG agar medium containing antibiotic was used as the selection medium for transformants. Plasmid pUB110 obtained from Y. Yoneda (Nippon Gene Co. Ltd.) was prepared by the method as described in our previous paper[4] and used as a insertion DNA.

Electroporation was done by the method of Kusaoke *et al.*[4] An Electric Gene Transfer Equipment GTE-10 (Shimadzu Corp.) was mainly used as a high voltage generator that could supply an electric pulse of a decayed wave

with capacitance in steps of 1,3,10, and 35 μF. Somatic Hybridizer SSH-1 Type (Shimadzu) was also used a high voltage generator that can supply an electric pulse of a squared wave with 10-500 μsec of pulse duration. Disposable sterilized 1 mm-cuvettes with a capacity of 50 μl were used as a sample chamber. The measurement of transformation efficiency and cell survival was done as described in our previous paper. [3]

RESULTS AND DISCUSSION

We investigated the growth curve of three strains, IAM11001, ATCC12980, and CU21 of *B. stearothermophilus* in order to obtain the host strain for efficient transformation (Fig. 1). OD_{660} showed the values of 1.91 in

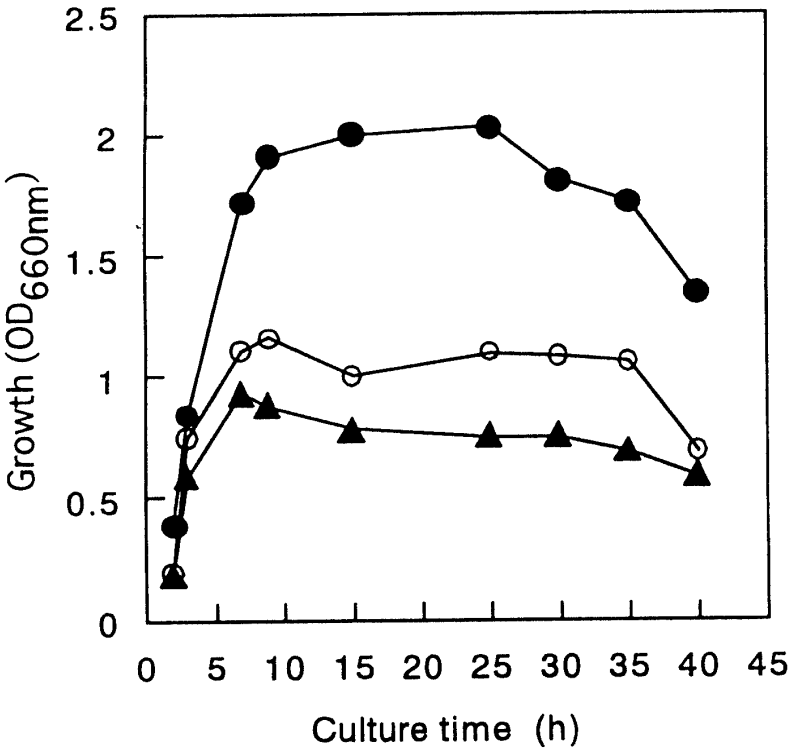

Fig. 1. Growth Curves of Three Strains of *B. stearothermophilus*.
Symbols: ○, ATCC12980; ▲, CU21; ●, IAM11001.

IAM11001, 1.16 in ATCC12980, and 0.88 in CU21 at growth time of 9 h. Electroporation was done by using a single squared pulse of 500 μsec with an electric field strength of 20 kV/cm. Table I shows the transformation efficiency and cell survival of three strains of *B. stearothermophilus*. Values are means from triplicate trials. Transformation efficiency of 1.2×10^2 transformants per μg DNA was obtained in IAM11001, though CU21 and

ATCC12980 could not be quite transformed. It is clear that the efficient transformation can be obtained by using the strain which is higher in survival and growth potency. Consequently IAM11001 was used in the following experiments.

Table I. Effect of Three Strains of *Bacillus stearothermophilus* on Transformation Efficiency and Cell Survival.

Strain	Efficiency (Transformants/ μg DNA	Survival (%)
ATCC 12980	0	18.7
CU 21	0	18.9
IAM 11001	120	45.6

To determine the optimum electric pulse for gene transfer, we suspended cells (*ca*. 1 x 10^{10} cells/ml) in LG medium and then exposed them to single decayed pulses of field strength between 5 kV/cm and 15 kV/cm at a fixed capacitance of 10 μF. As shown in Fig. 2, transformation efficiency increased with increasing field strength untill 12 kV/cm. However, transformation efficiency with electric strength of 15 kV/cm decreased because of the irreversible death of cells caused by higher electric pulsation. The amplitude of the electric pulse therefore had a effect on the efficiency of electroporation. Fig. 3 shows the transformation efficiency as a function of capacitance of discharge capacitor when the initial intensity of the single electric field pulse was fixed at 10 kV/cm. The transformation efficiency gave a maximum of value of 2.3 x 10^2 at the low capacitance of 1 μF. Hashimoto *et al*.[5] also reported that transformation of yeast resulted in a maximum yield in the capacitance of 1 μF. The capacitance of the

Cells for electroporation were prepared to the concentration of approximately 1.0 x 10^{10} cells/ml. Plasmid DNA was added to the cells suspended in electroporation-buffer as described in the text. Cell suspension was exposed to single squared pulses of the field strength of 20 kV/cm and pulse duration of 500 μsec.

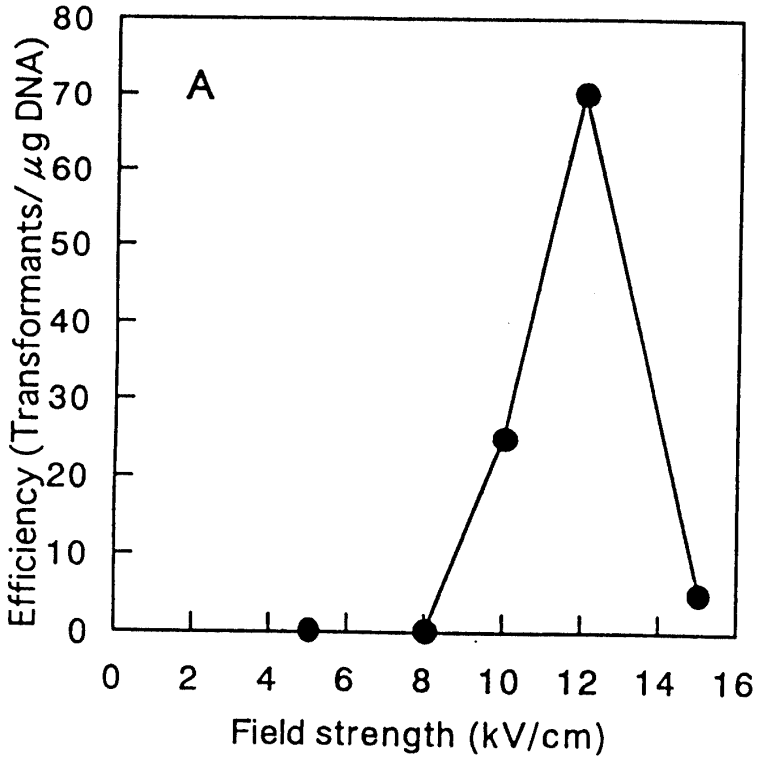

Fig. 2. Dependence of Transformation Efficiency on Electric Field Strength.
Using single decayed wave pulses, high voltage densities at a fixed capacitance of 10 μF were applied to the cell suspension (2.7×10^9/ml) mixed with the plasmid DNA.

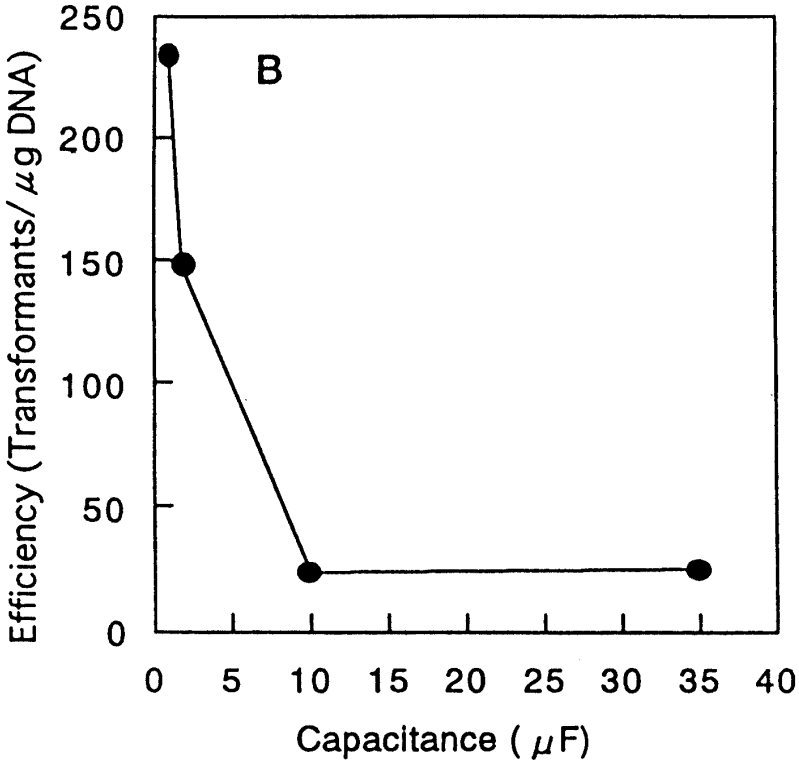

Fig. 3. Dependence of Transformation Efficiency on Capacitance.
The initial field strength was 10 kV/cm, and the capacitance of 1, 3, 10, and 35 μF set by an external capacitor assembly was applied to the cell suspension (1.0×10^{10} cells/ml) mixed with the plasmid DNA.

electric pulse therefore had a dramatic effect on the efficiency of electroporation.

It is also important to note that electroporation-buffer was effective to transform *B. stearothermophilus* IAM11001. In our previous paper[3], we obtained the effective transformation of *B. subtilis* ISW1214 with plasmid

DNA when buffer A containing 0.3 M sucrose, 1 mM magnesium chloride, and 1 mM maleic acid (pH 7.0) as a electroporation-buffer was used. In this paper, we investigated the effect of some buffer systems on the transformation efficiency, using buffer system A as a basal buffer (data not shown). When buffer system B containing 1 mM magnesium chloride and 1 mM maleic acid (pH 7.0) was used, the transformation efficiency showed the high value of 2.3×10^2 per μg DNA. Xie *et al*.[6] reported that electroporation is efficiently to be transformable to cells by the $MgCl_2$-facilitated cell surface binding of DNA. Magnesium ions may be efficient to transform *B. stearothermophilus* without sucrose as a isotonic agent in buffer. Though we used various buffer systems based on the combination of sucrose, magnesium chloride, calsium chloride, and maleic acid, electroporation with these systems was not effectively transformable to *B. stearothermophilus*.

pUB110-introduced transformants selected for kanamycin resistance were plasmid-screened by agarose gel electrophoresis. Transformants were obtained by pulsation with decayed wave pulses (Fig. 4-A) and squared wave pulses (Fig. 4-B). Electroporation of both a decayed wave and a squared wave pulses gave all isolates that contain supercoiled DNAs of plasmids with the same relative mobility as authentic plasmids in the agarose gel, as shown in Fig. 4. All transformants (individual 5-10 strains of plasmid-containing transformants) analyzed had electrophoretically the same plasmid pattern characteristics as the authentic plasmids. The restriction endonuclease cutting patterns observed were also identical in all transformants (data not shown). The agarose gel electrophoretic results indicated that the transferred plasmid DNAs were completely replicated in the cell and did not undergo significant modifications such as rearrangements or detections in *B. stearothermophilus*.[4]

We first investigated that *B. stearothermophilus* can be transformable by electroporation, though the transformation efficiency is lower than that of *B. subtilis*. We have also described that electroporation is a rapid and easy means of transformation that will be readily applicable to *B. stearothermophilus*.

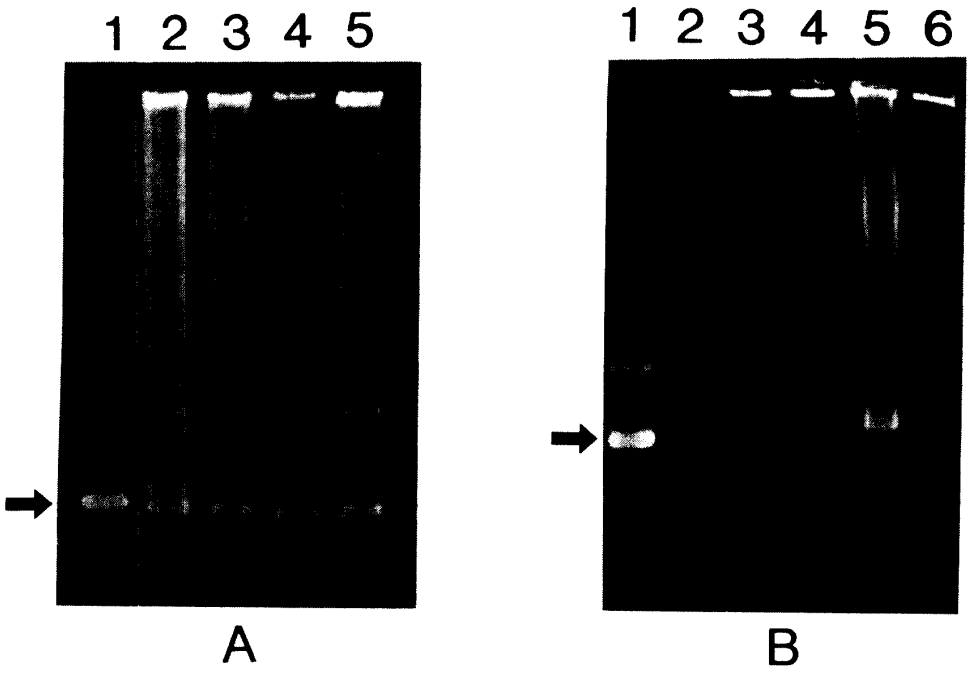

Fig. 4. Agarose Gel Electrophoresis of Plasmid DNAs Isolated from Transformants of *B. stearothermophilus* IAM11001.
(A) Electroporation was done with decayed wave pulses under the conditions of electric field strength of 12 kV/cm at capacitance of 1 μF. Lane1, authentic plasmid pUB110; lanes 2 - 5; DNAs isolated from pUB110-transferred transformants. (B) Electroporation was done with squared wave pulses under the conditions of electric field strength of 20 kV/cm at duration of 500 μsec. The arrows show supercoiled DNA molecules.

The electric field pulse induces the anchoring of DNA to the cell membrane and cell wall, and the plasmid then crosses them.[7] It is important to clarify the electroporation mechanisms of gene transfer into bacteria with cell-walled systems. We will study the mechanisms of electrotransformation for the bacilli with DNA in detail later.

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