Note

Genetic Transformation of *Bacillus brevis* with Plasmid DNA by Electroporation

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Bacillus brevis HPD31¹⁾ has been shown to be an excellent host bacterium for the extracellular production of foreign proteins such as thermophilic α -amylase from *Bacillus stearothermophilus*,²⁾ swine pepsinogen³⁾ and human epidermal growth factor,⁴⁾ because of its high protein-productivity and that it has no detectable extracellular protease activity.¹⁾

The Tris-PEG transformation method,⁵⁾ which was developed for introducing plasmid DNA into *B. brevis* 47, has several disadvantages when applied to *B. brevis* HPD31. This method showed a low efficiency as to the transformation of strain HPD31 with plasmid pBAM101⁶⁾ and no transformants were obtained with plasmids with a replication origin of pHW1⁷⁾ and pWT481.⁸⁾

Electroporation, which was originally developed for the genetic transformation of eukaryotic cells, was recently used to introduce DNAs into bacteria of several species.^{9~12)} We explored the possibility of efficient transformation of various strains of *B. brevis* with plasmid pBAM101 by electroporation. The transformation efficiency was determined by determining the number of neomycin-resistant and amylase positive colonies.

The standard transformation procedure for the electroporation of *B. brevis* HPD31 was as follows. An overnight culture in T2 medium¹⁾ was diluted (1:100) with fresh broth and then grown at 37°C for 4 hr to the early stationary phase of growth (optical density at 660 nm, 2.5). Cells were harvested by centrifugation (7,000 $\times$ g, 5 min, 4°C), washed with an equal volume of cold electroporation buffer (SHC; 15% glycerol, 272 mM sucrose, 16 mM HEPES (pH 7.0), 1 mM CaCl₂) and then suspended in a 1/20 volume of cold SHC. Aliquots of the suspension were frozen immediately at -70°C. This cell suspension could be stored without loss of transformation efficiency for at least 3 months. A Gene PulserTM apparatus (Bio-Rad) was used to induce transformation. 0.8 ml or 0.4 ml of a cold

competent cell suspension prepared as above was mixed with 10~50 μ l of a plasmid DNA solution in a 0.4 cm electroporation cuvette (interelectrode distance, 0.4 cm) or a 0.2 cm cuvette (interelectrode distance, 0.2 cm). Electroporation was performed as follows: with the 0.4 cm cuvette, the Gene Pulser apparatus was set at 2.5 kV and 25 μ F, and the pulse controller was not used. With the 0.2 cm cuvette, it was set at 1.5 kV and 25 μ F, and the pulse controller was set at 1,000 Ω . After keeping on ice for a few minutes, the cell suspensions were mixed with 1 ml (0.4 cm cuvette) or 0.5 ml (0.2 cm cuvette) of MTB (T2 medium supplemented with 20 mM MgCl₂ and 0.01% bovine serum albumin) and then held at 37°C for 1 hr. Cells were spread on selection agar plates and incubated at 37°C.

At a constant capacitance of 25 μ F, with the 0.4 cm cuvette, the transformation efficiency increased with the electric field strength, the highest efficiency being obtained at a field strength of 6.25 kV/cm (set at 2.5 kV), the upper limit of the apparatus. At a constant electric field strength of 6.25 kV/cm, the highest efficiency was obtained at a capacitance of 25 μ F, the upper limit of the apparatus. Under these conditions, the transformation efficiency was 1.3×10^4 transformants per μ g of pBAM101. A higher electric field strength could be applied when the 0.2 cm electroporation cuvette was used. Under these conditions, the highest efficiency was obtained at 7.5 kV/cm (set at 1.5 kV), 25 μ F and a resistance of 1,000 Ω (time constant, 5 msec). The transformation efficiency was 2.6×10^4 transformants per μ g of pBAM101.

Cells treated with lysozyme, proteinase, sodium dodecyl sulfate and alkaline buffer (50 mM Tris-HCl, pH 8.5) prior to electroporation were inferior to intact cells (data not shown). The transformation efficiency was greatly in-

Fig. 1. Effect of the Growth Phase of *B. brevis* HPD31 on Transformation by Electroporation.

B. brevis HPD31 was grown in T2 broth at 37°C with shaking and cells were harvested periodically. Electroporation was performed by the standard procedure. $\circ$, transformation efficiency (transformants per μ g pBAM101 DNA); $\bullet$, growth (OD₆₆₀); $\blacktriangle$, survival rate (%) (ratio of viable cells before and after electroporation).

Table I. TRANSFORMATION EFFICIENCIES OF VARIOUS *B. brevis* STRAINS WITH PLASMID pBAM101 ON ELECTROPORATION

Strain	Transformation efficiency ^a	Survival rate (%) ^a
HPD31	1.3×10^4	35
HPD52	1.0×10^1	32
144	1.3×10^3	8
HP1301	2.8×10^3	21
444	4.0×10^1	4
HP1502	2.0×10^1	5
HP030	0	3
HP1304	0	12
HP1302	0	25
HP032	0	2
47	0	107
481	0	40
899	0	13
HP601	0	2
HP602	0	1
HP603	0	2
HP033	0	4
HP037	0	8
ATCC9999	0	1
ATCC8185	0	2
ATCC8246	0	3

^a See the legend to Fig. 1.

fluenced by the growth phase at which the cells were harvested, as shown in Fig. 1. The efficiency was optimal with cells at the early stationary phase and almost no transformants appeared with cells at the early to mid exponential phase.

The transformation efficiencies of *B. brevis* HPD31 with other plasmids, pTS5 (6.6 kb in size, carrying the human salivary amylase gene¹³) and the replication origin of pHW1, pHY481⁸) (3.7 kb, carrying the replication origin of pWT481 isolated from *B. brevis* 481) and pHY300PLK¹⁴) (4.9 kb, carrying the replication origin of pAM α 1) were 4.2×10^3 , 1.4×10^3 and 3.2×10^3 transformants per μ g DNA, respectively. Moreover, transformants were obtained with DNA in a ligation mixture by electroporation (data not shown).

Eighteen strains of protein-hyperproducing *B. brevis*, and 3 type and reference *B. brevis* strains were further examined as to the transformation efficiency on electroporation under the optimal conditions described above. As shown in Table I, 6 strains were transformed, although the efficiency varied with the strain. All the transformants were found to carry the same plasmid as pBAM101. It is notable that no transformants were obtained from strain

47 by electroporation. Protein-producing *B. brevis* strains are classified morphologically into two groups. The cell wall of group I strains, such as HPD31 and HPD52, consists of a peptidoglycan layer and one protein layer, the so-called S-layer, whereas that of group II strains, such as 47 and HP033, consists of a peptidoglycan layer and two S-layers.¹⁵ Cells having two S-layers may be resistant to electroporation. Therefore, the data obtained so far suggest a direct relationship between the cell wall structure and transformability through electroporation.

Though electroporation is not applicable to all *B. brevis* strains, it is a very useful method for transforming some strains of *B. brevis*.

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