Optimization of Electroporation Conditions for *Bacillus pumilus* 3–19 Strain

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Abstract
Electroporation is the process of using electrical impulses to create temporary pores in the plasma membrane which, in turn, enables the penetration of nucleic acids into the cytoplasm of a cell. This method is widely used for the rapid and efficient introduction of foreign DNA into a wide range of cells. Cell viability and electrotransfection efficiency depend on various experimental factors, including the impulse form, vector concentration, cell type (density), properties of the electroporation buffer, and the growth phase of the bacterial culture. In this work, we investigated the optimal conditions for the transformation of the *Bacillus pumilus* 3–19 strain using electroporation. Competent *B. pumilus* cells were obtained on the 4th hour of culture growth using PEB1 electroporation buffer and SOC1 (Super Optimal broth with Catabolic repressor) medium, while the electric field strength was 12 kV/cm. With these parameters, the transformation efficiency of bacillus cells was 56.3 transformants/μg DNA. Thus, the rational choice of pulsation conditions and buffering compositions is critical for the design of electroporation protocols to maximize the viability and efficiency of electrotransfection.

1 Introduction

Spore-forming gram-positive bacteria of the *Bacillus* genus belong to one of the most diverse and widely represented groups of microorganisms in nature. Besides their characteristics such as storage stability, manufacturability in the production process, and environmental safety, the natural ability of this group of bacteria to synthesize and secrete several important metabolites of industrial, agricultural, and medicinal significance makes bacilli an important object of industrial biotechnology. Due to the rapid development of post-genomic research, effective genetic tools are now necessary for targeted modification of the *Bacillus* genomes.

B. pumilus are gram-positive bacteria, known for their ability to produce industrially significant hydrolytic enzymes with the prospect of being used in producing gluten-degrading detergents, hydrolyzing bird feathers, and breaking down biofilms [1–5]. *B. pumilus* bacteria are also potential probiotic agents that positively affect the growth and development of farm animals and plants, as well as humans [6–9]. *B. pumilus* can produce biologically active substances with antibacterial [10], insecticidal (toxins acting on invertebrates) [11], and antifungal [12] activities, as well as inhibitors of metastasis [13] and substances for the biodegradation of industrial waste [14].

The high potential of the practical application of exoproteins and metabolites of *B. pumilus* actualizes the search for methods of genetic editing of the genome of these bacteria to increase the yield of useful products. On the path of genetic research, an important stage is the delivery of heterogeneous DNA into bacilli cells. Methods of transformation, electroporation, and conjugation are most often used for this purpose. These methods are used with high efficiency for laboratory strains; however, strains isolated from their natural habitat have natural defense mechanisms against the introduction of foreign DNA (DNA methylation, nucleases, antibiotic secretion). In this connection, transformation (and

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other methods of transfer of exogenous DNA) into *B. pumilus* cells could have some setbacks [15].

For gram-positive bacteria, chemical treatment of cells to increase competence is an ineffective method compared to other protocols, since the composition of the gram-positive cell wall prevents the absorption of foreign DNA, despite its possibility of use [16–18]. The electroporation method, originally developed for the transfection of eukaryotic cells, was subsequently adapted to transform a wide variety of bacteria, including gram-negative *Pseudomonas*, *Salmonellae*, *Vibrios*, *Serratiae*, and *Shigellae* and gram-positive *Clostridia*, *Bacilli*, *Lactobacilli*, and *Enterococci* [19]. The efficiency of transformation by electroporation is reportedly 10–20-fold higher than chemical competence or heat shock [20].

In the process of selecting the transformation conditions, we faced the problem of stable resistance of *B. pumilus* 3–19 to the acceptance of foreign DNA. The chemical transformation methods described for other strains of bacilli are inapplicable to *B. pumilus* 3–19. In using the electroporation method, few studies have been conducted on the transformation of *B. pumilus*. However, results showed a lower efficiency as compared to *B. subtilis* cells [15, 21]. Shen et al. developed an efficient transformation method for the *B. pumilus* DX01 strain by optimizing the electroporation conditions [22]. In this study, we designed transformation conditions for *B. pumilus* 3–19 by electroporation based on the method described in [22].

The development of an effective transformation system for *B. pumilus* will pave the way for the genome editing of this microorganism, creating recombinant strains with altered (enhanced and suppressed) functions of significant proteins.

2 Materials and Methods

We used the strains *B. pumilus* 3–19 (strR) and *E. coli* DH5α for the isolation of plasmids. Strains were obtained from the collection of the Agrobioengineering Research Laboratory (IFMaB, KFU). The plasmid vector pJOE9282.1 (9059 bps) used was acquired from Prof. Altenbuchner of the Institute of Industrial Genetics, Stuttgart University, Stuttgart, Germany.

LB agar and LB broth were used as nutrient media. The antibiotic kanamycin was added to the medium at a final concentration of 15 µg/ml. For electroporation, a SOC1 recovery medium (2% tryptone, 0.5% yeast extract, 0.05% NaCl, 2.5 mmol/l KCl and 10% sucrose, pH 7.0) and electroporation buffer PEB1 (0.5 mol/l sucrose, 0.1 mmol/l $KH_2{}^2PO_4{}^4/K_2HPO_4$, pH 7.4) were used [Shen et al., 2013].

Isolation of plasmid DNA from *E. coli* cells was performed using the GeneJET Plasmid Miniprep Kit (Thermo Scientific). To isolate plasmids, 1–5 ml of an overnight culture of *E. coli* DH5α was used according to protocol A, for a high copy number of plasmids. Centrifugation was carried out at 13,000 rpm.

Before plasmid DNA isolation from *B. pumilus* 3–19 strain, we harvested up to 2×10^9 bacterial cells in a 1.5- or 2-ml microcentrifuge tube by centrifugation for 10 min at 5000 g. Discarded the supernatant. Resuspended the pellet in 180 µl of Gram-positive bacteria lysis buffer (20 mM Tris–HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/ml immediately before use). Incubated for 30 min at 37 °C. After these manipulations, the GeneJET Plasmid Miniprep Kit (Thermo Scientific) was used for isolation of plasmid DNA from *B. pumilus* 3–19 cells.

To obtain competent cells, an overnight culture of *B. pumilus* 3–19 strain was introduced into LB medium (at $OD_{600} = 0.1$) and cultured at 37 °C with aeration (180 rpm) for 4 h (until $OD_{600} = 0.6$).

B. pumilus 3–19 competent cells were cooled on ice for 15 min, and then the pellet was collected by centrifugation at $4000 \times g$ for 10 min and washed twice with an equal volume of electroporation buffer (PEB1). Finally, the bacterial cells were suspended in 800 µl of electroporation buffer; 200 µl of competent cells and 30 µl of plasmid were carefully mixed in a 1.5-ml Eppendorf centrifuge tube. The mixture was added to a precooled 2-mm-gap electroporation cuvette (Bio-Rad, USA) and kept on ice for 40 min before electroporation.

Electroporation was performed using MicroPulser Electroporation equipment (Bio-Rad). Aliquots were electroporated at a field strength of 12 kV/cm.

The transformed *B. pumilus* cells were immediately mixed with 800 µl of SOC1 recovery medium and incubated for 4 h at 200 rpm and 37 °C for regenerative growth, and then plated on LB agar medium containing 15 µg/ml kanamycin [22].

DNA electrophoresis was performed in 2% agarose gel in tris–acetate buffer. A 1-Kb kit from SibEnzyme (M11, Russia) was used as markers.

3 Results and Discussion

Competence in many bacterial species occurs in a short period at a certain stage of growth. For example, *Streptococcus* cells are electrocompetent in the early exponential growth phase, *B. subtilis* cells — at later stages of the logarithmic growth phase, *Haemophilus* cells — at a stationary growth stage [23–25]. Shen et al. showed that the maximum transformation frequency was achieved at an OD_{600} of 1.0 (after incubation for 4 h) in the exponential phase of *B. pumilus* DX01 [22]. When the *B. pumius* 3–19 strain was cultured in 1000-ml flasks, we obtained competent cells after 4 h (until $OD_{600} = 0.6$) (Fig. 1). In some

Fig. 1 The growth dynamics of *B. pumilus* 3–19
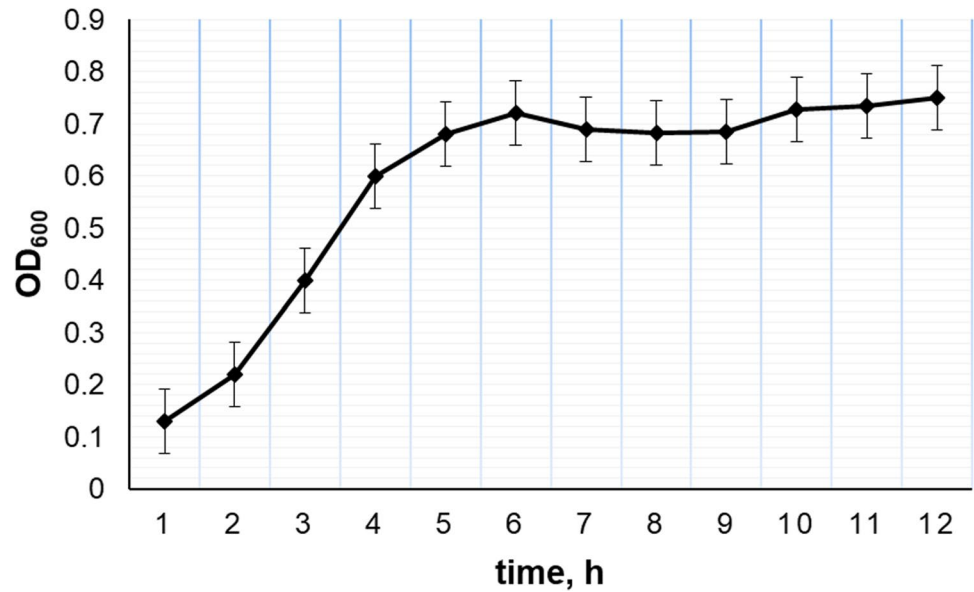
experiments, the *B. pumius* 3–19 strain culture reached a density of $OD_{600} = 1.0$ only after 8 h. At the same time, the efficiency of cell transformation under these growth conditions did not change.

A recovery medium with high osmolarity and optimized composition increases the frequency of electrotransformation of recipient cells, presumably because bacterial cells were osmotically or electrochemically sensitive after electroporation [26]. By using the PEB1 electroporation buffer and SOC1 recovery medium as recommended by Shen et al.,

we achieved a transformation efficiency of 56.3 transformants/µg DNA (Fig. 2). A mixture of competent cells and DNA resuspended in PEB1 was incubated on ice for 40 min before electroporation. The 10 min recommended by Shen et al. was insufficient for *B. pumilus* 3–19 cells. Subjected to Shen's protocol, the transformation of *B. pumius* 3–19 was unsuccessful.

High-voltage pulsed electric fields are widely used in the genetic transformation of bacteria [27]. Field strength affects the transformation rate, and for most prokaryotic cells, the corresponding electrical voltage range is from 6 to 14 kV [28, 29]. Shen et al. obtained the maximum transformation frequency at 12 kV/cm, which we adopted for the *B. pumilus* 3–19 strain. We also carried out the transformation of bacilli cells at an electric field voltage of 9 kV/cm. However, the transformation frequency was slightly lower. Confirmation of transformation was
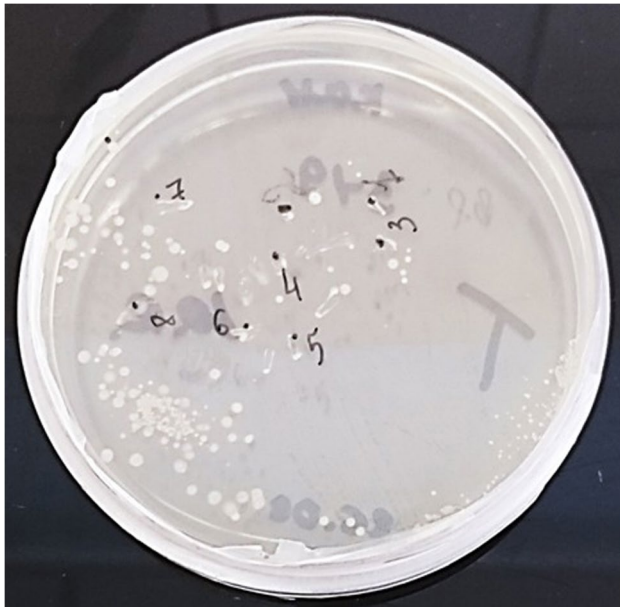

Fig. 2 Colonies of *B. pumius* 3–19 transformants, grown on a selective medium with kanamycin. The eight indicated clones were chosen for further validation (by pDNA isolation)
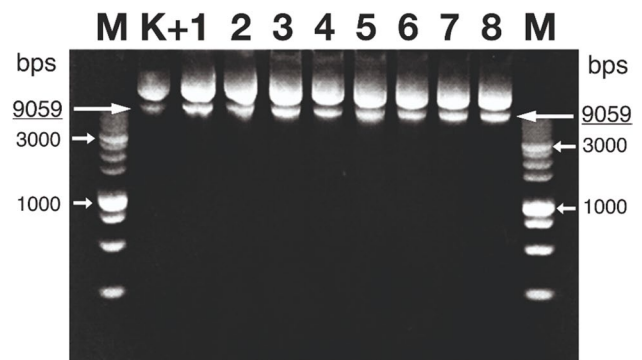

Fig. 3 Electrophoresis of plasmids isolated from colonies of transformants. M, marker; K+, positive control (plasmid pJOE9282.1, isolated from *E. coli* DH5α); 1–8, transformant colonies. Indicated by the corresponding numbers in Fig. 2

performed by the electrophoresis of pJOE9282.1 plasmids isolated from transformants (Fig. 3). All transformants obtained were observed to carry the vector pJOE9282.1. Thus, the transformation frequency for the *B. pumilus* 3–19 strain was increased by optimizing the electroporation conditions. Extension of the co-incubation time of competent cells and donor DNA before electroporation (for 40 min) was a critical parameter for the successful transformation of *B. pumilus* 3–19 cells.

Declarations

Conflict of Interest The authors declare no competing interests.

Research Involving Humans and Animals Statement The experiments were carried out without involving humans or animals.

Informed Consent All authors consent to the publication of the article.

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