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Dictyostelium discoideum transformation by oscillating electric field electroporation

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Dictyostelium discoideum *has been used as a genetically tractable model organism to study many biological phenomena. High-efficiency transformation is a prerequisite for successful genetic screens such as mutant complementation, identification of suppressor genes, or insertional mutagenesis. Although exponential decay electroporation is the standard transformation technique for* D. discoideum*, its efficiency is relatively low and its reproducibility is weak. Here we optimized the oscillating electroporation technique for* D. discoideum *transformation and compared it to the exponential decay electroporation. A 20-fold increase in the efficiency was reproducibly achieved. This alternative electroporation technique should facilitate future genetic approaches in* D. discoideum.

INTRODUCTION

The social amoeba *Dictyostelium discoideum* lives in the soil as a unicellular organism feeding on bacteria. Upon starvation, *Dictyostelium* amoebae initiate a development program that leads to the formation of a multicellular organism and fruiting bodies containing spores (1). With a haploid genome of 34 Mb, *D. discoideum* represents a powerful genetic system to investigate many biological processes such as cell motility and development (2), phagocytosis and endocytosis (3), and host-pathogen interaction (4–7). Therefore, the availability of an efficient transformation technique is desirable to perform genetic screens, such as cDNA suppressor isolation or insertional mutagenesis, or to generate mutants by homologous recombination. Exponential decay electroporation has been used to introduce DNA molecules into *D. discoideum* cells for more than a decade (8). A significant increase in the transformation efficiency would help researchers and bring *D. discoideum* genetics to its full potential. A recent study demonstrated that *D. discoideum*

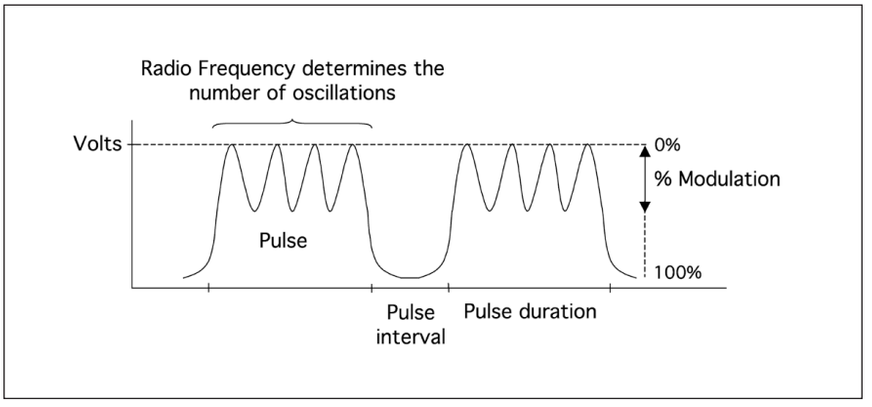

Figure 1. Scheme of the oscillating electric field electroporation. The electric field is applied as a succession of pulses. A rectangular pulse is obtained when modulation is set to 0%, and an oscillating pulse when modulation is set to 100%. Different parameters can be regulated: voltage, pulse duration, number of pulses, RF, pulse-interval duration, and percent of modulation.

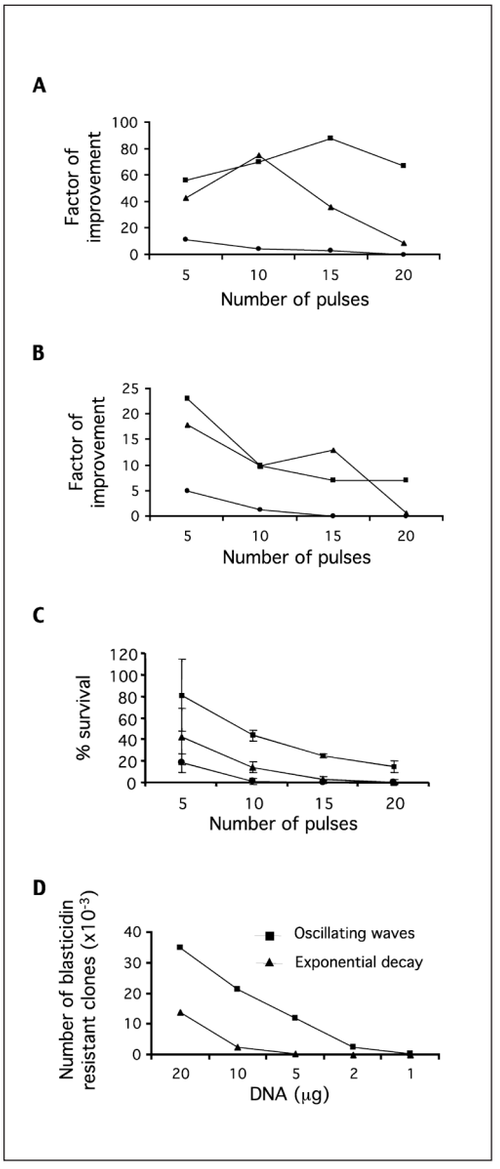

Figure 2. Optimization of the oscillating electric field electroporation. The following variables were kept constant: 400 V, 100% of modulation, RF of 50 Hz, and pulse interval of 1 s. (A and B) Transformation efficiencies compared to the exponential decay electroporation (two separate experiments). The factor of improvement was defined as the ratio of the number of blasticidin-resistant transformants of the oscillating electroporation technique and the number of transformants of the exponential decay electroporation procedures. The number of pulses was varied from 5 to 20 for each pulse duration (square, 2 ms; triangle, 3 ms; and circle, 4 ms). Ten micrograms of DNA were used. (C) Cell viability (% survival) after oscillating electroporation was determined by counting plaque formation on a *K. pneumoniae* lawn. The number of pulses was varied from 5 to 20 for each pulse duration (square, 2 ms; triangle, 3 ms; and circle, 4 ms). Ten micrograms of DNA were used. Means and standard deviations from three independent experiments are represented. (D) Titration of DNA amounts in oscillating and exponential decay electroporation procedures. The pulse duration and number of pulses were set constant to 2 ms and 5 pulses, respectively.

cell transformation could be improved by using a particle inflow gun (9). A new electroporation technique has been reported for mammalian cells (10–12). In this technique, the conventional direct current electric field is shifted by a radio frequency (RF) electric field. This results in a succession of oscillating pulses instead of a unique pulse of exponential decay (Figure 1). Many parameters can be modulated: voltage, pulse duration, number of pulses, RF, pulse-interval duration, and percent of modulation. This latter parameter allows the application of pulses from rectangular pulses like square wave (0% modulation) to sinusoidal pulses (100% modulation), with the maximum voltage at the selected voltage and the minimum voltage at 0 V (Figure 1).

Here we optimized the oscillating electric field electroporation technique for *D. discoideum* cell transformation and obtained a significant increase in the efficiency of transformation, compared to the exponential decay electroporation technique.

MATERIALS AND METHODS

Strains and Culture Conditions

The subclone DH1.10 (13) of the *D. discoideum* wild-type strain DH1 (14) was used in this study. Cells were grown at 21°C in HL5 medium containing 14.3 g/L peptone (Oxoid LTD, Basingstoke, Hampshire, UK), 7.15 g/L yeast extract (Brunschwig BD Difco, Basel, Switzerland), 18 g/L maltose (Fluka, Buchs, Switzerland), 3.6 mM Na_2HPO_4, and 3.6 mM KH_2PO_4, pH 6.7.

Klebsiella pneumoniae was used as a growth substrate when cells were plated on SM agar [10 g/L peptone, 1 g/L yeast extract, 16.2 mM KH_2PO_4, 5.7 mM K_2HPO_4, 4 mM $MgSO_4$, 1% glucose, and 20 g/L bactoagar (Brunschwig DB Difco)].

Electroporation Procedure

Cells were transformed with the integrative vector pUCBsrΔBam encoding the blasticidin selective marker (15). DNA was purified with the Endofree® Plasmid Maxi Kit (Qiagen, Basel, Switzerland) and resuspended in endotoxin-free water. After 10 min of incubation on ice, the cells were washed once with sterile ice-cold electroporation buffer (10 mM $NaPO_4$, pH 6.1, 50 mM sucrose) and resuspended to 5×10^7 cells/mL in the same buffer. Nonlinearized DNA was added at the indicated concentrations, and 0.4 mL (2×10^7 cells) of the suspension was transferred into the electroporation cuvette (Gene Pulser® Cuvette, 0.2-cm gap; Bio-Rad Laboratories, Hercules, CA, USA). Cells were electroporated at 0.8 kV, 3 µF using the Gene Pulser II system (Bio-Rad Laboratories) when a pulse of exponential decay was applied. These conditions have been previously optimized in our laboratory (unpublished results). Oscillating electric field electroporation was performed with the RF module (Bio-Rad Laboratories) connected to the Gene Pulser II system. The RF module delivers pulses of 400 V maximum. Immediately after the electroporation, the cells were processed as described below to evaluate the cell viability and the transformation efficiency.

Cell viability. One hundred of the 2×10^7 electroporated cells were distributed together with *K. pneumoniae* onto SM agar plates. The percentage of survival was estimated by counting the number of *D. discoideum* plaques growing on a *K. pneumoniae* lawn. In the case of 100% viability, 100 clones should grow, and none should grow in the case of 0% viability.

Transformation efficiency. One-hundredth of the electroporated cells (2×10^5 of 2×10^7) were distributed in a 9-cm Petri dish containing 10 mL HL5 medium. Blasticidin S hydrochloride (10 µg/mL; ICN Biomedicals, Aurora, OH, USA) was added 24 h later. After 5 days incubation with no further manipulation of the Petri dish, visible colonies of transformants attached to the plastic were counted. Each colony corresponds

to one independent clone. To obtain the absolute number of transformants per electroporation, the number of colonies has to be multiplied by 100.

RESULTS AND DISCUSSION

The oscillating electric field electroporation parameters (Figure 1) were optimized for *D. discoideum* stable transformation with the integrative nonlinearized vector pUCBsrΔBam. The RF and the pulse-interval duration were always set to 50 Hz and 1 s, respectively. The pulse duration and number of pulses were optimized in a first set of four independent experiments where the voltage and the modulation were kept constant at 400 V and 100%, respectively. These fixed parameters had been determined as close to optimum in preliminary experiments. In parallel, the classical exponential decay electroporation was performed to compare both electroporation methods with the very same batch of cells and DNA suspension. Although some variability was observed between the four independent experiments, the oscillating electric field electroporation always gave a higher transformation efficiency compared to the exponential decay electroporation procedure (Figure 2). Overall, the latter technique gave rise to 100–1000 clones per electroporation, whereas the oscillating electric field technique gave rise to 7000–28,000 clones. Figure 2, A and B, shows the oscillating electric field transformation efficiency relative to the exponential decay in two separate experiments. Two milliseconds of pulse duration appeared to be optimal, as the relative transformation efficiency ranged from 24- to 90-fold. Increasing the number of pulses from 5 to 20 did not give reproducible results. Indeed, a number of pulses higher than five sometimes gave very high efficiencies (Figure 2A) but also very poor efficiencies (Figure 2B). Altogether, in the four separate experiments, setting the pulse duration to 2 ms and the number of pulses to 5 resulted in a higher transformation efficiency that was reproducible. These conditions are now used in our laboratory, and at least 20-fold higher transformation rates are routinely obtained.

Table 1. Optimization of the Voltage and Modulation of the Oscillating Electric Field Electroporation

	No. Blasticidin-Resistant Clones ($\times 10^{-3}$)[a]			
% Modulation	100 V	200 V	300 V	400 V
100	0	0.9	21.5	28.6
70	0	0.4	24.0	14.4
30	0	8.6	4.7	0.3
0	0	16.0	2.3	0.2

[a]Electroporation was performed with 10 µg DNA and 5 pulses of 2 ms each.

Figure 2C shows that cell survival decreased rapidly as pulse duration and the number of pulses increased. The highest cell viability was obtained for pulse duration of 2 ms and 5 pulses. Pulse duration other than 2 ms or number of pulses greater than 5 appeared to be detrimental to cell viability. Average cell viability in the classical exponential decay electroporation was 75%. The cell viability correlated well with the reproducibility, as the more viable were the cells, the more reproducible were the efficiencies.

In a similar way, the voltage and the modulation were optimized by applying five pulses of 2 ms. The highest efficiencies were obtained with the voltage and the modulation at 400 V (maximum capacity of the RF module) and 100%, respectively (Table 1). These results confirm that the conditions set previously were optimal. Interestingly, the modulation and the voltage are tightly linked, as the best efficiencies are found on the diagonal (Table 1).

All the experiments described above were performed with 10 µg DNA per electroporation. Titration of the DNA amount was then performed (Figure 2D). Transformation efficiencies were improved by increasing the amount of DNA in both electroporation procedures. However, oscillating electroporations required much lower amount of DNA to reach exponential decay efficiencies. This should be particularly appreciated when the DNA amount to be transformed is rate limiting. Indeed, DNA amounts are often a limiting factor (e.g., in the case of cDNA libraries).

Altogether, our results demonstrate that oscillating electric field electroporation is a more efficient technique than the classical exponential decay electroporation to transform *D. discoideum* cells. By optimizing the electroporation parameters (voltage, 400 V; number of pulses, 5; pulse duration, 2 ms; RF, 50 Hz; pulse interval, 1 s; modulation, 100%), we were able to routinely obtain at least a 20-fold higher transformation efficiency.

This new electroporation procedure for *D. discoideum* cell transformation should allow researchers to perform larger and more efficient genetic screens. Additionally, the many parameters of the oscillating electroporation technique are a great advantage for transformation optimization of different wild-type or mutant strains exhibiting sensitivity or resistance to electroporation.

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