Electrotransformation of Bacteria by Plasmid DNA: Effect of Serial Electroporator Resistor

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Electroporation is a method widely used for the delivery of foreign DNA into plant, animal, and bacterial cell. We studied the influence of serial electroporator resistors (22 Ω, 330 Ω, and 510 Ω) on electroporation parameters (electrotransformation efficiency and frequency). Bacteria of *Salmonella typhimurium LB 5000* and DNA of plasmid pUC19 were used as a source of cells and DNA, respectively. The highest transformation frequency (number of transformants/number of survived cells) was achieved with the 22 Ω serial resistor; the highest transformation efficiency (number of transformants/μg DNA) was achieved with the 330 Ω serial resistor. For the relationship between the number of transformed cells (n_t) and the concentration of DNA (ρ) the equation $\ln n_t = a - b\{\rho\} + d\ln\{\rho\}$ (where a, b, d are constants) was used. It was shown that the dependence of n_t *vs.* ρ may be influenced by a serial electroporator resistor, too.

Electroporation is now widely used to introduce various kinds of molecules, including nucleic acids, into different eukaryotic and prokaryotic cells [1—5]. It enables to transform many bacterial species including some that are resistant to chemical transformation procedures. This procedure also eliminates several time-consuming steps that are required for transformation by other procedures [6, 7]. Electrotransformation is of particular importance for genetics, recombinant DNA technology, and biotechnology progress.

Electrotransformation involves the application of a high-voltage electric field pulse of short duration to a suspension of cells and DNA. The process results in membrane permeabilization and the subsequent uptake of exogenous DNA. The molecular aspects of electrotransformation that enable DNA to enter bacteria are still poorly understood [1—3, 8—11]. Therefore, the optimization of the process of bacterial electrotransformation remains empirical and the experimental conditions vary from one microorganism to another. Thus great attention has been paid to the determination of electrical variables important to electroporation (especially field strength and time constant) and to the question how to manipulate them to achieve high electrotransformation efficiency and frequency [1, 2, 5]. The aim of this paper was to study the influence of some physical parameters (resistors) on electrotransformation efficiency and frequency.

THEORETICAL

Two different types of electrical pulses have been used to electroporate cells: exponential decay [12] or square wave [13]. For practical reasons, capacitor discharge circuits are most commonly employed to reproducibly deliver pulses of an intensity and duration suitable for electroporation. The pulse produced by the discharge of a capacitor has an exponential decay waveform and is described by

$$E(t) = E(0)\exp(-t/\tau) \qquad (1)$$

where $E(t)$ is the electric field strength (V cm^{-1}) at any time t(s), $E(0)$ is the initial field strength, and τ is the resistance—capacitance (RC) time constant (s). The electrical field strength E is determined as $E = U/d$, where U is the voltage applied across parallel electrodes separated by a distance d. Of key importance for bacterial electroporation are $E(0)$ and τ. The results published in literature demonstrate an increase of the transformation efficiency as the electric field strength increases (the time constant was held

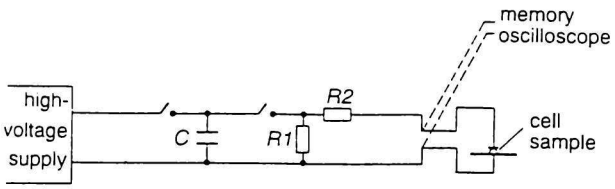

Fig. 1. The electrical circuit of a capacitor discharged device. C – capacitor, R_1 – parallel resistor, R_2 – serial resistor.

constant), or as the time constant increases (electric field strength was held constant). As a matter of fact, the electric field strength and the time constant have a compensatory effect. It means that higher voltages require shorter time constants and longer pulses reduce the voltage requirement [1]. Field strength can be adjusted by varying the voltage to which the capacitor is charged (at a constant interelectrode distance). The time constant depends on the total resistance $R(\Omega)$ and capacitance $C(\mathrm{F})$ of the system as follows

$$\tau = RC \tag{2}$$

Manipulation of the time constant therefore involves adjusting the resistance of the system, as well as the size of the capacitor. The design of the electroporation apparatus used is shown in Fig. 1. The total resistance R is described by the equation

$$1/R = 1/R_1 + 1/(R_2 + R_{\mathrm{sample}}) \tag{3}$$

where R_1 is the parallel resistor, R_2 the serial resistor, and R_{sample} resistance of the sample (it depends on the composition of the sample and on the geometry of the cuvette).

To limit the current the resistor R_2 was placed in series with the sample cuvette. When the sample resistance is very large ($R_{\mathrm{sample}} \gg R_2$), the total resistance and thus the time constant is determined by the parallel resistor R_1. Thus the additional resistance R_2 has a negligible effect on the time constant or voltage applied to the sample.

EXPERIMENTAL

Bacterial Strain and Plasmid

The bacterial strain *Salmonella typhimurium LB 5000* with mutations in a host restriction system was used for electrotransformation. The electrocompetent cells were prepared according to the authors of paper [14]. The plasmid DNA pUC19 (2686 bp) carrying the gene for ampicillin resistance was prepared from *E. coli JM109* with alkaline lysis according to [15], purified through a Biotrap membrane (Schleicher Schull), and resuspended in 10^{-3} M-NaCl before electroporation.

Electroporation

The exponential decay pulser and the pulse controller were home-made according to modified published diagrams [14, 16]. The capacitance was discharged through parallel (R_1 = 1500 Ω) and different serial resistors (R_2 = 22 Ω, 330 Ω, and 510 Ω). The voltage and current in the sample drop were measured with a memory oscilloscope. Twenty mm^3 of chilled cells of a constant quantity (4×10^8) dissolved in 10 % glycerol were mixed with 1 mm^3 of 10^{-3} M-NaCl containing various amounts of plasmid DNA (ranging from 0.1 ng to 1500 ng), and the mixture was transferred onto a chilled electrode. The voltage used was 13.2—13.8 kV cm^{-1} with a time of approximately 4 ms (4 μF and 6 μF capacitor below 100 ng and over 100 ng of DNA amounts, respectively). Following electroporation, the cells were mixed with 1 cm^3 of SOC medium [14] and shaken for 90 min at 37 °C. The ampicillin-resistant transformants were recovered in 2 % (8×10^6) of surviving cells after plating on L-agar supplemented with ampicillin (50 μg cm^{-3}) in 3—4 independent experiments.

Calculation

For the quantitative expression of the relationship between the number of electrotransformed cells (n_t) and the concentration of DNA (ρ) the following equation was derived [11].

$$\ln n_t = a - b\{\rho\} + d\ln\{\rho\} \tag{4}$$

where a, b, d are constants. For low DNA concentrations we can write eqn (4) in the simplest form as follows

$$\ln n_t = a + d\ln\{\rho\} \tag{5}$$

The regression coefficients in eqn (4) were tested on hypothesis b, $d = 0$ and $d = 1$, respectively, according to the procedure presented by the authors of [17].

RESULTS AND DISCUSSION

The aim of the electroporation process optimization is to find the experimental conditions leading to the highest efficiency of transformation (number of transformants/μg DNA). Due to the absence of detailed knowledge of the physical processes during electroporation the optimization of electroporation conditions is empirical. Different optimization ways may be used.

As was mentioned above, $E(0)$ and τ parameters are of key importance for bacterial electrotransformation. A change of these variables can be achieved by manipulation with capacitance C or resistance R_1. However, manipulation with variables is quite limited by the biological system properties. Higher values of $E(0)$ and τ decrease the number of surviving bacterial

Table 1. Electrotransformation of *Salmonella typhimurium LB 5000* by Plasmid pUC19 DNA, Number of Survived Cells and Transformation Efficiency

Serial resistor R_2/Ω	22	330	510
$\bar{x}/10^7$	0.61^a	2.97^b	3.16^b
$s_x/10^6$	0.21	0.61	0.93
n	63	45	50
$\bar{x}_P/\%$	2.4^a	8.4^b	7.1^b
t.e. $(10^{-6}/\mu g)$	1.1^a	1.9^b	1.7^b

$\bar{x}$ – the average number of survived cells, $\bar{x}_P/\%$ – the average number of survived cells in % (the number of survived cells/the number of cells in control group without electrical pulse), s_x – standard deviation, n – number of variables in the set, t.e. – transformation efficiency (number of transformants/mass of DNA). *a*, *b*) Statistically significant difference for $P < 0.05$ (the means indicated with the same letters are identical).

cells. The increase of the pulse duration by increasing the capacitor size is limited by the heat generated. Practically at first $E(0)$ is selected at which the highest efficiency was achieved. Then the time constant can be optimized by testing various combinations of capacitors and the parallel resistors R_1.

Contrary to the above-mentioned process we proved another way of optimization of the experimental conditions. In Theoretical it was supposed that the serial resistor R_2 (if $R_{sample} \gg R_2$) had no influence on $E(0)$ and τ values. For optimal $E(0)$ and τ parameters (determined according to the above-mentioned process) it was found that the change of serial R_2 resistor influenced the course of electrotransformation. From literature it is known [1, 11] that the electroporation is highly dependent on DNA concentration. For this reason the influence of a change of R_2 resistor on both survived cell and the number of electrotransformants was studied for DNA concentration range of $\rho = 0.005$—$71.4\ \mu g\ cm^{-3}$. The average values of the number of survived cells and the transformation efficiency (number of electrotransformants/μg DNA) are given in Table 1. The number of survived cells was not affected by DNA concentration. The transformation efficiency was the highest for 330 Ω resistor. This phenomenon can be explained by the fact that the change of serial resistor value affects the increase of pulse length and field strength. Small changes in field strength below or above the optimum level can result in a decrease of the transformation efficiency. It is in agreement with the published results [1, 18]. For all tested R_2 resistor sets the transformation efficiency was constant in the DNA concentration range $\rho = 0.005$—$33.3\ \mu g\ cm^{-3}$ and then it was going down.

The quantitative relationship between the number of transformed cells (n_t) and DNA concentration (ρ) expressed by eqn (*4*) was checked by regression analysis. The results are given in Table 2. For mutual correlations the limit of allowable precision was taken to be a value of the correlation coefficient $r > 0.9$ [19]. In a previous paper [11] we derived the value of regression coefficient in eqn (*5*) $d = 1$. Eqn (*5*) was derived for low concentration of DNA. It was valid for linear section of the curve relationship n_t *vs.* ρ if DNA concentration ρ was below the saturation concentration ρ_{sat} (above the saturation DNA concentration the number of electrotransformants remained constant). The assumption $d = 1$ was fulfilled if $R_2 = 330\ \Omega$. For this reason, according to previously published assumption [11], in sets $R_2 = 22\ \Omega$ and $510\ \Omega$, controlling process is not only the binding of DNA molecules on the cell surface. The transport of DNA through the cell membrane must be taken into consideration. The maximal DNA concentration (ρ_{max}) and the maximal number of electrotransformants (n_{tmax}) were calculated by derivation of eqn (*4*). For $\rho > \rho_{max}$ the number of electrotransformants decreased. The maximal number of electrotransformants was achieved using an $R_2 = 330\ \Omega$ resistor.

Table 2. The Effect of Plasmid pUC19 DNA Concentration on the Transformation of *Salmonella typhimurium LB 5000*. Regression Analysis for $\ln n_t = a - b\{\rho\} + d\ln\{\rho\}$ Correlation

R/Ω	$\ln n_t =$	n	I/r	s	F_b	F_d	$t_{d=1}$	ρ_{max}	n_{tmax}	ρ	t.f.$_{max}$
								$\mu g\ cm^{-3}$		$\mu g\ cm^{-3}$	
22	$10.436 - 0.036\{\rho\} + 1.034\ \ln\{\rho\}$	63	0.991	0.276	673.01^A	2453.0^A	–	28.9	3.6×10^5	0.005–71	6.7×10^{-2}
	$10.167 + 0.900\ \ln\{\rho\}$	52	0.977	0.464	–	227.46^A	3.605^A	–	–	0.003–33	–
330	$10.732 - 0.037\{\rho\} + 1.131\ \ln\{\rho\}$	45	0.975	0.513	217.91^A	577.21^A	–	30.7	1.1×10^6	0.005–71	2.4×10^{-2}
	$10.502 + 1.034\ \ln\{\rho\}$	34	0.991	0.331	–	1775.2^A	1.395	–	–	0.005–33	–
510	$10.472 - 0.020\{\rho\} + 0.875\ \ln\{\rho\}$	50	0.956	0.563	230.28^A	263.66^A	–	43.8	4.4×10^5	0.005–71	1.3×10^{-2}
	$10.332 + 0.800\ \ln\{\rho\}$	38	0.968	0.483	–	542.53^A	5.903^A	–	–	0.005–33	–

n – number of values in the set, I – index of correlation, r – correlation coefficient, s – standard deviation, F_b, F_d – Fischer—Snedecor's criterion of coefficient b or d, respectively, of regression equation $\ln n_t = a - b\{\rho\} + d\ln\{\rho\}$, $t_{d=1}$ – Student's characteristic for the coefficient d (the coefficient was tested on hypothesis $d = 1$), n_t – number of electrotransformants, n_{tmax} – maximal number of electrotransformants, ρ – concentration of DNA ($\mu g\ cm^{-3}$), ρ_{max} – maximal concentration of DNA ($\mu g\ cm^{-3}$), f.t.$_{max}$– frequency of transformation (maximal number of transformants/number of survived cells), A – statistically highly significant difference for $P < 0.01$.

The frequency of transformation (number of transformants/number of survived cells) was also influenced by the values of the serial resistor. It decreased with the value of serial resistor R_2. The maximum of transformation frequency was found out for a resistor $R_2 = 22\ \Omega$ (*i.e.* for the set with a lower number of survived cells and electrotransformants than for $R_2 = 330\ \Omega$ and $R_2 = 510\ \Omega$ resistors). This corresponds to the course of the dependence of the maximal DNA concentration (ρ_{max}) on the value of serial resistor R_2. The ρ_{max} (at which the maximal number of electrotransformants n_{tmax} was achieved) increased with the value of serial resistor R_2 (Table 2). The course of these relationships was analogical to the relationships n_t *vs.* ρ (which is comprehensible as the number of survived cells was constant for each set).

From these results it is clear that an accurate optimization of the experimental conditions is necessary. Further experiments are required to increase the usefulness of this system. Better understanding of the mechanism of electroporation will enable a wider application of this technique for genetic improvement of many industrially important microorganisms in the future.

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