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JÓZEF STANISŁAW SZOPA
KRYSTYNA KOWAL
GRZEGORZ ZWOLIŃSKI
ŁUKASZ SIKORSKI*)
FRANCISZEK MOSIŃSKI*)
BEATA GAŃCZYK

EFFECT OF VOLTAGE SURGE PULSES ON FUSION OF *SACCHAROMYCES CEREVISIAE* PROTOPLASTS

Institute of Fermentation Technology and Microbiology, Technical University, Łódź

*) Department of High Voltage, Technical University, Łódź

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The effect of electric force pulses on the survival rate of protoplasts of auxotrophic haploid *Saccharomyces cerevisiae* yeasts was investigated. Increases of electric force were found to decrease the survival rate of protoplasts which was down to 50-60% at 22.5 kV/cm. 5 kV/cm pulses of electric force increase the frequency of haploid protoplasts fusion by about 50%.

Studies of the effect of electric field intensity on cell suspensions, in particular the effect of short electric pulses on permeability of biological membranes of yeasts and bacteria protoplasts, were pioneered by Sale and Hamilton [9] in 1967. These authors demonstrated disruptions of the membrane potential of protoplasts leading to drastic changes in their permeability due to electroporosity, i.e. the formation of micropores in the membrane [2-6, 8, 9, 12]. Although the mechanism of these changes is still unclear, the phenomenon is used to fuse protoplasts and to obtain new organisms from different species and cell kinds marked by features advantageous in biotechnology.

Electric pulses expanding membrane micropores in the protoplasts facilitate their fusion which, when traditional methods are applied, takes place with the low frequency of 10^{-5} to 10^{-4}.

Electric pulses which do not cause electroporosity may be used to stimulate the activity of enzymatic proteins of membranes responsible for active transport of substrates to the cell interior. The results of Berg et al. [1] and Weber et al. [11] who studied the effect of electric pulses on the fusion of yeast protoplasts in the presence of PEG according to the method proposed by Maraz [7] indicate that voltage surges significantly stimulate this process.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

The experiments were performed with haploid auxotrophic *Saccharomyces cerevisiae* strains obtained from the Institute of Agricultural Sciences in Zamość:

S. cer. SP_{20}, leu 1, ade 1, a

S. cer. AH_{22}, leu_{2-3}, leu_{2-112}, his_{4-519}, a

MEDIA

The cultures were maintained in YPG medium and in minimal medium containing (per dm^3): 2 g KH_2PO_4, 1 g $MgSO_4$, 1 g $(NH_4)_2SO_4$, 20 g glucose, 200 μg thiamine, 2 μg biotin, and 25 g agar. The media were stabilized with 0.6 M KCl.

PROTOPLASTS

Protoplast formation were obtained from cells from the logarithmic growth phase (16th-18th hour of cultivation in the YPG medium). The yeasts were washed twice with distilled water and then treated with a buffer solution of Tris-SO_4 (pH 9.4). The cell suspension in Tris-SO_4 buffer with 2-mercapto-ethanol was then preincubated at 30°C for 15 min, and then again centrifuged and washed with Na-citrate buffer with sorbitol (pH 5.8). After centrifugation, the yeasts were treated with a 1% solution of *Helix pomatia* enzyme in 0.6 M KCl in order to adjust cell density to about $5 \times 10^7/cm^3$. Digestion was carried out at 30°C for 45 min, following which the protoplasts were centrifuged off, washed twice with a 0.6 M KCl solution, and suspended in 0.6 M KCl.

FUSION OF PROTOPLASTS

Suspensions of protoplasts of AH_{22} and SP_{20} yeasts in 0.6 M KCl (cell density ca. $5 \times 10^7/cm^3$) were mixed in 1 : 1 proportion. After centrifugation the yeast were treated with a 40% solution of polyethylene glycol (molecular weight 4000) in 0.6 M KCl. Ca. 1-cm^3 portions of the obtained suspension were subjected to electric pulses in a high-voltage pulse generator manufactured especially for this purpose at the Łódź Technical University.

The protoplasts mixture was placed in a test vessel inside the high voltage chamber (Fig.). The fusion of protoplasts was stimulated by voltage pulses which, given the 1-cm distance between electrodes in the test vessel, corresponded to the electric field intensity expressed in kV/cm (2-22.5 kV/cm).

After being subjected to the electric pulses, the protoplasts suspensions were incubated at 28°C for 30 min and then transferred to minimal medium containing leucine.

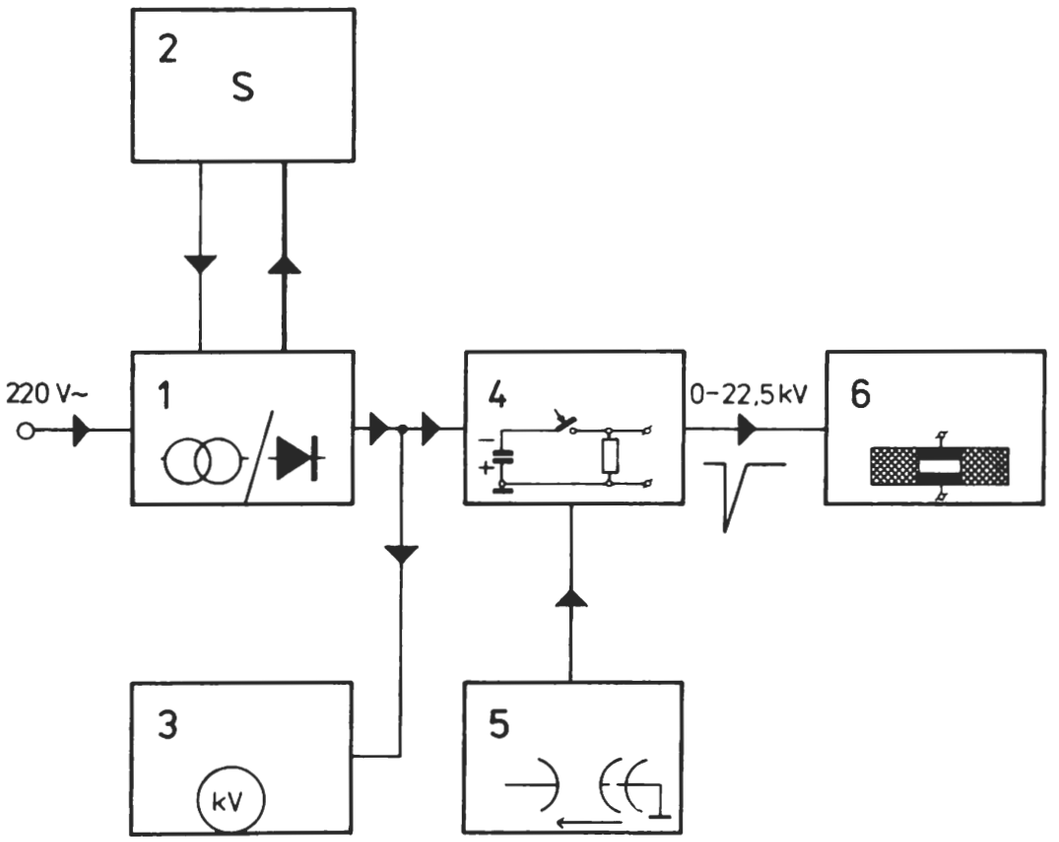

Fig. Block diagram of high voltage pulse generator; 1 — power supply, 2 — control circuit, 3 — peak voltmeter, 4 — pulse generator, 5 — triggered spark gap, 6 — high voltage chamber with test vessel

Parallel tests were carried out to compare the obtained results with regeneration, survival rate, and frequency of fusion of protoplasts in the presence of polyethylene glycol alone.

HIGH VOLTAGE PULSE GENERATOR

The electrofusion apparatus was manufactured by the Department of High Voltage of Łódź Technical University. It is a single-stage generator of current pulses supplied by 220 V mains. It operates by a pulse discharge of electrical energy stored in condensers through a test vessel containing yeast protoplasts suspensions (Fig.).

The generator is a portable device featuring controls enabling adjustment of voltage and a peak voltmeter. In addition to continuous adjustment of peak voltage it is also possible to switch the polarity of voltage and abruptly change the energy of the generator. Since the electrodes in the test vessel in the high voltage chamber are 1 cm apart, the readings on the voltmeter of the real value of the voltage pulse correspond to the value of electric field intensity expressed in kV/cm. The basic parameters of the generator as as follows: peak charging voltage — 22.5 kV, principal capacity — 2 × 33 nF, maximum energy — 17 Ws.

RESULTS AND DISCUSSION

The effect of voltage pulses on the fusion of protoplasts of haploid yeast auxotrophs was investigated using the generator of electric pulses in the electric field intensity range of 0-22.5 kV × cm^{-1}.

Haploid auxotrophs of *S. cerevisiae* SP_{20} and AH_{22} were subjected to protoplastization and exposed to electric pulses of 5, 10, 15 and 22.5 kV × cm^{-1} field intensity (Table 1). It was found that in the applied field intensity range, the survival rate ot protoplasts drops to about 50% in the case of the strongest pulse (22.5 kV × cm^{-1}). The phenomenon was observed in both the investigated strains. At 5 kV × cm^{-1} the survival rate of protoplasts decreases only slightly (4-10%).

Table 1. Survival rate of *S. cerevisiae* protoplasts after treatment with high voltage pulses

Electric field intensity (kV/cm)*	Survival rate of protoplasts (%)	
	strain AH_{22}	strain SP_{20}
5	90	96
10	83	74
15	73	62
22.5	59	52

* Given that the electrodes in the test vessel are 1 cm apart, the reading of the voltmeter measuring the real value of the high voltage pulse corresponds to the electric field intensity expressed in kV/cm

These results differ considerably from those obtained by Weber et al. [11] in their study of yeast cells. They registered a survival rate 7-27%. The sensitivity of yeast cells to electric pulses may differ widely depending on the species and strain.

The experimental fusion of protoplasts of two auxotrophic yeast haploids using electric pulses was meant to determine the field intensity stimulating protoplasts combination to the greatest extent. The fusion products were screened in minimal medium with leucine. This medium guaranteed the growth of just the diploid forms resulting from the fusion of haploids of strains *S. cerevisiae* AH_{22} and *S. cerevisiae* SP_{20}.

The obtained results indicate that the intensity of electric field stimulating the frequency of fusion is in the range 3-7 kV × cm^{-1} (Table 2). The greatest number of colonies was obtained in the minimal medium with leucine at 5 kV × cm^{-1}; in this case stimulation amounted to 52%.

The achieved stimulation of fusion, although significant, particularly as regards the obtaining of triploid and tentraploid forms of *S. cerevisiae*, is unimpressive when compared with, for example, the stimulation obtained with *Saccharomycopsis lipolytica* (10-20×) 11. Nevertheless, the stimulation of protoplasts fusion by means of electric pulses may greatly facilitate the obtaining of strains with genomes suitably modified to satisfy biotechnological processes.

Table 2. Effect of high voltage electric pulses on frequency of fusion of *S. cerevisiae* AH_{22} and SP_{20} protoplasts

Electric field intensity (kV/cm)*	No. of colonies formed in minimal medium by 0.1 cm^3 of mixture of protoplasts after fusion**
0 (control)	224
2	236
3	306
4	326
5	340
6	254
7	262
8	200
10	180
15	172
22.5	150

* see note to Table 1
** mean values from three experiments

Our experiments also demonstrated that the generator of electric pulses we used is well suited to stimulate protoplasts fusion.

CONCLUSIONS

1. Electric pulses from a high voltage generator of pulses affect the survival rate of protoplasts to a various degree. At the highest voltage applied, the rate drops to 50-56%.

2. The optimal value of peak intensity of electric field, 5 kV/cm, increases the frequency of fusion of haploid protoplasts of *Saccharomyces cerevisiae* by about 50%.

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J. St. Szopa, K. Kowal, G. Zwoliński, Ł. Sikorski[*)], *F. Mosiński*[*)], *B. Gańczyk*

WPŁYW IMPULSÓW NATĘŻENIA POLA ELEKTRYCZNEGO NA FUZJĘ PROTOPLASTÓW *SACCHAROMYCES CEREVISIAE*[**)]

Instytut Technologii Fermentacji i Mikrobiologii, Politechnika, Łódź
[*)] Katedra Wysokich Napięć, Politechnika, Łódź

Streszczenie

Badano wpływ elektrycznych impulsów napięciowych na protoplasty auksotroficznych drożdży *Saccharomyces cerevisiae*. Protoplasty drożdży otrzymywano w wyniku trawienia ścian komórkowych wyciągiem enzymatycznym z *Helix pomatia*. Gęstość zawiesiny protoplastów zastosowanej do badania wpływu elektrycznych impulsów natężenia pola wynosiła $5 \times 10^7/cm^3$. Protoplasty stabilizowano za pomocą 0,6 M roztworu KCl. Stwierdzono, że ze wzrostem wielkości impulsu elektrycznego maleje liczba zregenerowanych protoplastów osiągając ok. 50% przeżywalności przy wartości 22,5 kV/cm natężenia pola.

Wykazano, że impulsy natężenia pola w przedziale 2-7 kV/cm wpływają stymulująco na fuzję protoplastów drożdży *Sac. cerevisiae*. Optymalne natężenie pola wynoszące 5 kV/cm wywoływało zwiększenie zachodzących fuzji protoplastów o ok. 50%.

[*)] Badania wykonano w ramach problemu CPBP 04.11.