

INVESTIGATION OF *S. CEREVISIAE* PLASMA MEMBRANE AND CELL WALL INTERACTION AFTER PEF TREATMENT

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Saccharomyces cerevisiae are viewed as a prototype of eukaryotic cells, ideally suited for use in studies of many phenomena of eukaryotic life. Yeasts are surrounded by a cell wall, which provides them with protection. Pulsed electric field (PEF) treatment is known to cause plasma membrane permeabilization, an effect known as electroporation. However, the dynamic of cell wall and plasma membrane interaction during and after PEF treatment is a still unanswered question. Our previous work used tetraphenylphosphonium bromide [TPPBr] to measure the kinetics of yeast cell wall recovery after PEF treatment [1]. This study aims to expand the fundamental knowledge about plasma membrane and cell wall dynamic and recovery after PEF treatment.

The use of a potentiometric ion-selective electrode is a convenient method for the quantitative evaluation of the permeability of the yeast cell wall and membrane. In this study we have constructed an ion selective electrode by adapting Zimkus *et. al.* methodology [2]. Then, we

have employed a wild type (WT) and a mutant strain derived from WT, MNN11, to measure cell wall recovery via TPP⁺ ion uptake by electroporated yeast cells'. PEF parameters: single square pulse, duration of 150μs and field strength of 2.9, 4.5 or 5.9kV/cm.

We have found that non-electroporated WT cells can absorb a maximum of about 2μM and MNN11 about 1,5μM (Fig. 1. a,b). PEF treatment did not impact the maximum absorption, but it significantly reduced the time of TPP⁺ absorption. The higher electric field strength we applied, the faster TPP⁺ absorption happened. Furthermore, we measured cell wall recovery after PEF. What we found is that cell wall behaves in a similar fashion as plasma membrane does, but the time it takes to recover is slower (Fig. 1. c,d).

Further work includes measuring plasma membrane potential changes by employing a DisC₃(3) fluorescence probe to further investigate PEF effect on the yeast cell exterior.

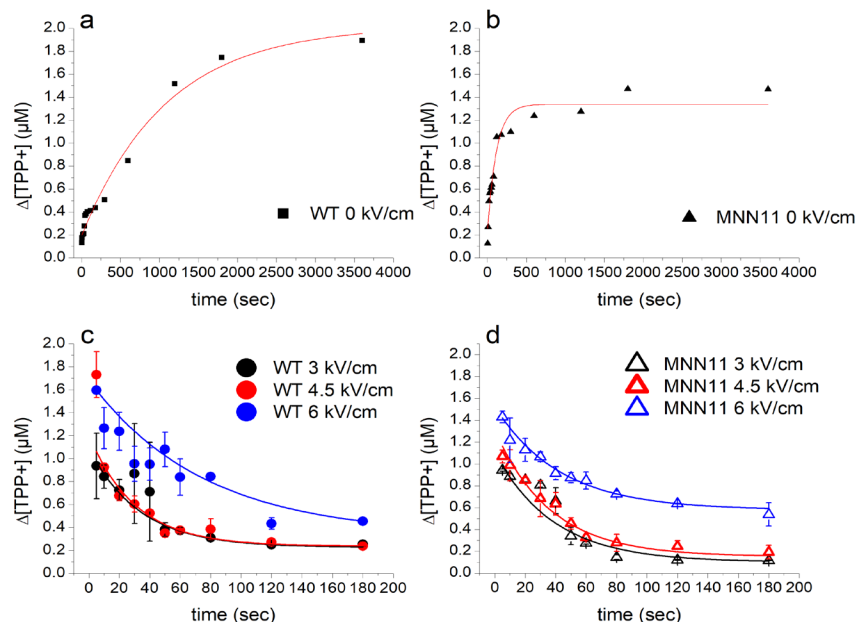


Fig. 1. TPP⁺ absorption of a. WT and b. MNN11 yeasts; cell wall recovery after PEF treatment of c. WT and d. MNN11 yeasts.

References

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2. A. Stirke, A. Zimkus, A. Ramanaviciene, S. Balevicius, N. Zurauskiene, G. Saulis, L. Chaustova, V. Stankevicius, and A. Ramanavicius. Electric Field-Induced Effects on Yeast Cell Wall Permeabilization. *Bioelectromagnetics*, 35:136-144, (2014).