The permeability of *Saccharomyces cerevisiae* yeast strains with different cell wall architecture to lipophilic cation tetraphenylphosphonium and their transformation capability

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The permeability properties of the yeast strains that have a defective cell wall SEY6210(*kre1*), (*kre2*), (*kre6*) or an impaired mitochondrial system SEY6210(*rho°*) or both of these mutations SEY6210(*kre6*/*rho°*) and XCY42-30D(*mnn1*) were studied. The accumulation of the tetraphenylphosphonium cation (TPP^+) by yeast cells was measured with a TPP-selective electrode. Our results have shown that the amount of lipophilic cations accumulated by yeast *Saccharomyces cerevisiae* cells was different and depended on the properties of the cell wall structure. The permeability of the strains was compared with the capability of yeast cells to be transformed.

Key words: permeability, lipid-soluble ions (LSI), lipophilic cations (LC), tetraphenylphosphonium (TPP), *kre* mutants, *Saccharomyces cerevisiae*

INTRODUCTION

Lipophilic cations (LC) such as fluorescent dyes or tetraphenylphosphonium are frequently used as probes for the membrane potential ($\Delta\Psi$) of prokaryotic and small animal cells, organelles, and vesicles [1]. The use of lipophilic cations for determining membrane voltage in intact plant and fungal cells was causing some controversy that in walled species the equilibrium and steady-state distribution of lipophilic cations are complicated and only indirectly image $\Delta\Psi$ [2–4].

TPP^+ and cationic fluorescent dyes do not distribute to equilibrium with plasma membrane voltage in intervals of minutes in intact *S. cerevisiae* cells. To reach the steady state at some strains need 0.5 to 2 hours [3, 5]. On the other hand, it appears that TPP^+, if applied at sufficiently low concentrations (<1 μM), can be used as a quantitative probe for the membrane potential in some *S. cerevisiae* strains [2]. Yeast cell wall characteristics and integrity seem to play a significant role in LC accumulation processes.

Recently it has been determined that the transformation efficiency of yeast *S. cerevisiae* cells depends not only on the conditions of the experiments but also on the properties of the strains, such as cell wall structure [6–9]. The major components of fungal cell walls are polysaccharides and glycoproteins. In *S. cerevisiae*, the cell wall contains β(1–3)-D-glucan, β(1–6)-D-glucan, chitin, and mannoproteins. All the four major components are linked together. *KRE* and *KRE*-related genes involved in cell wall 1,6-glucan biosynthesis have been isolated through mutations that confer resistance to the K1 killer toxin, which binds to cell wall β 1,6-glucan [10–13]. On the external surface of the wall there are mannoproteins, which are extensively O- and N- glycolysated. They are densely packed and influence wall permeability. The layered structure of the cell wall, being a general phenomenon in yeasts, modifies the surface properties such as hydrophobicity, electrical charge, sexual agglutinability, and porosity [11, 13, 14].

This report presents the data on permeability of different *Saccharomyces cerevisiae* strains to lipophilic cations tetraphenylphosphonium and the transformation efficiency of the yeast strains studied.

MATERIALS AND METHODS

Yeast strains and cultivation. The following *Saccharomyces cerevisiae* strains were used in this study: SEY6210 (*MATα, leu2-3, ura3-52, his3-Δ200, lys2-*

801, trp-Δ901, suc2-Δ9) as a parental strain, SEY6210 ΔKre1 (*MATα, leu2-3, ura3-52, his3-Δ200, lys2-801, trp-Δ901, suc2-Δ9, ΔKre1::HIS*), SEY6210 ΔKre2 (*MATα, leu2-3, ura3-52, his3-Δ200, lys2-801, trp-Δ901, suc2-Δ9, ΔKre2::TRP*), SEY6210 ΔKre6 (*MATα, leu2-3, ura3-52, his3-Δ200, lys2-801, trp-Δ901, suc2-Δ9, ΔKre6::HIS),* XCY42-30D (*MATα, ade2-101, adex, ura3, trp1, lys2, leu2-3,112, Δmnn1::LEU2).* The strains were kindly gifted by A. Meškauskas (Vilnius). The yeast strains SEY6210(*rho^o*) and SEY6210(*kre6/rho^o*) were produced by mutagenesis, which was performed with ethidium bromide (EthBr) according to Machler and Perlman [15]. Yeast cells were grown in complete YEPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30 °C on a reciprocal shaker at 150 rpm to the logarithmic growth phase (OD_{590} 0.2–0.5).

Tetraphenylphosphonium accumulation measurements. The yeast cells were washed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.8,) two times, concentrated 200 times in the same buffer, TPPBr was added to the final concentration 3.10^{-7} M. After 1 h of incubation at 30 °C yeast cells were precipitated and the supernatant was used for measuring the residual TPP^+ concentration. 200 µl of the supernatant was added to 400 µl of TE buffer (with 3 10^{-7} M TPPBr), with a TPP^+ selective combination electrode immersed. The electrode potential drift was estimated with a Hanna pH213 ionometer, and the yeast absorbed quantity of TPP^+ was calculated.

The protein concentration was determined by the Lowry method [16].

RESULTS AND DISCUSSION

Interaction of lipid-soluble ions (LSI) with bacterial and eukaryotic cells has become one of the widely used methods to investigate the principles of cell permeability and physiological functions [2, 3].

The lipophilic cation TPP^+ under a high concentration exerts a discharging effect on the membrane potential [17]. In our experiments the concentration of TPP^+ was 3.10^{-7} M and did not influence the membrane potential. Yeast strains with a defective cell wall SEY6210(*kre1*), (*kre2*), (*kre6*) or an impaired mitochondrial system SEY6210(*rho^o*) or with both of these mutations SEY6210(*kre6/rho^o)* and XCY42-30D(*mnn1*) were used. SEY6210 was used as a parental strain. The cells were incubated without glucose or any other carbon souses. Therefore, our data on the accumulation of TPP^+ do not represent the real level of membrane potential in the strains studied.

Results presented in Table show that the mutations that deal with yeast cell 1,6-glucan biosynthesis enhanced the permeability of the lipid-soluble TPP^+ ion. The yeast strains SEY6210*(kre1)* and SEY6210*(kre6)* absorbed TPP^+ 1.7 and 1.5 times more effectively than the parental strain. This correlates with a reduced (to 60%) level of alkali-insoluble cell wall β(1-6)-glucan a in strain with *kre1* mutation [12]. Deletion of *KRE6* gene, supposed to be specifically involved in the elongation of β(1-6)-glucan chains [11, 12], also caused a five-fold increase in chitin level and a 16% decrease in β-glucan, too.

Table. **Accumulation of TPP+ by different *Saccharomyces cerevisiae* strains**

Strains	TPP^+ accumulated	Per cent to SEY6210
SEY6210	7.132 ± 0.366	100
SEY6210*(kre1)*	12.357 ± 1.342	173
SEY6210*(kre2)*	12.600 ± 0.576	177
SEY6210*(kre6)*	10.460 ± 1.168	147
SEY6210*(rho^0)*	6.690 ± 0.5010	93
SEY6210*(kre6/rho^0)*	10.597 ± 0.418	146
XCY42-30D(*mnn1*)	8.053 ± 0.621	113

Results presented as millimols of TPP per milligram of yeast protein 10^{-9}. Values are the average ± standard errors of five independent experiments

β(1-6)-glucan is the central molecule that keeps together the other components of the cell wall, including β(1-3)-glucan, mannoprotein, and part of the chitin. Thus, it is not surprising that defects in β(1-6)-glucan formation, found in several mutants, can interfere with cell wall assembly and have severe effects both on cell growth and accumulation of TPP cations.

A moderate increase in TPP^+ accumulation was observed in XCY42-30D(*mnn1*) strain with a mutation involved in N- and O-glycosylation of mannoproteins. The strain SEY6210*(kre2)* with a defective synthesis of a-1,2-mannosyltransferase, which involved in protein *O*-glycosylation [18] caused TPP^+ accumulation for 170%. Our data are in good agreement with data of De Nobel et al. [19] showing that the external protein layer, the N-linked sidechains of mannoproteins in particular, determines the permeability of the yeast cell wall.

Accumulation of TPP^+ cations was slightly decreased by respiratory-deficient yeast cells of SEY6210*(rho^0)* strain as compared to the parental strain. On the other hand, a significant increase in TPP^+ accumulation (147%) was determined in strain SEY6210*(kre6/rho^0)*, which had two mutations. An increase of accumulation in this strain possessing double mutation is associated with mutation in glucan synthesis (*kre6)* rather than with mutation in mitochondrial metabolism.

Measurement of membrane potential using the equilibration of potential-responding lipophilic cations and/or fluorescent probes between the cell and the external medium is often hampered by the barrier properties of the cell wall [3]. Some authors even expressed severe doubts as to the usefulness of small lipid soluble cations (including fluorescent dyes) for measuring membrane potential in walled cells, while others strongly advocate their use [4, 5].

Our results point to the critical role of b(1-6)-glucan and mannoprotein in the barrier properties of the cell wall. Differences in the lipophilic cation or fluorescent probe uptake may reflect not only the membrane potential but also a number of morphological dissimilarities, cell wall structure and thickness included.

We have tried to compare two independent processes related to the properties of the cell envelope: the accumulation of TPP^+ cations, and the penetration of plasmid DNA during transformation of the yeast *S. cerevisiae*. The question arises: is there a correlation between yeast cell wall permeability and plasmid DNA transfer? Previous results [9] showed that the transformation efficiency was strongly strain-dependent and varied from 3783 transformants for strain SEY6210 to 770 for strain SEY6210(*kre6*). Based on the results above-presented, we can assume that it is not possible to compare these two processes in this way, and confirm our suggestion that the mechanism of the penetration of plasmid DNA is more multiple and differs from lipophilic cation accumulation.

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MIELIŲ *SACCHAROMYCES CEREVISIAE* KAMIENŲ TURINČIŲ SKIRTINGĄ SIENELĖS STRUKTŪRĄ, LAIDUMO LIPOFILINIAMS KATIJONAMS TYRIMAI BEI PALYGINIMAS SU TRANSFORMACIJOS EFEKTYVUMU

Santrauka

Tirti mielių kamienai, turintys genetiškai pažeistą lastelių sienelės struktūrą SEY6210(*kre1*), (*kre2*), (*kre6*), XCY42-30D(*mnn1*) arba neefektyvią mitochondrinę sistemą SEY6210(*rho^o*), taip pat abi genetines pažaidas kartu SEY6210(*kre6/rho^o*).

Lipofilinio katijono tetrafenilfosfonio (TPP) akumuliacija ląstelių viduje buvo registruojama TPP jonams selektyviu elektrodu. Mūsų rezultatai parodė, kad mielės *S. cerevisiae* akumuliavo skirtingą lipofilinio katijono kiekį, kuris priklausė nuo ląstelės sienelės struktūros savybių. Buvo palygintos tirtų mielių kamienų laidumo sąvybės ir jų gebėjimas transformuoti plazmidinę DNR.