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Effect of lithium and sodium cations on the permeability of yeast *Saccharomyces cerevisiae* cells to tetraphenylphosphonium ions

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The capacity of lithium cations to induce changes in yeast cell permeability under transformation conditions was investigated. Permeability properties of yeast cells were estimated as the accumulation of tetraphenylphosphonium ions (TPP^+) by a TPP^+-selective electrode. The results demonstrated that the treatment of yeast cells with either 0.1 M Li^+ or 0.1M Na^+ cations did not inhibit the growth of yeast cells under physiological conditions. Treatment with 0.1 M Li acetate increased the permeability of yeast cells to TPP cations 1.5 times compared with cells incubated in buffer without cations. The amount of TPP accumulated after treatment with Na cations was lower and reached only 15% compared with cells incubated in buffer without cations.

Key words: permeability, alkaline cations, tetraphenylphosphonium (TPP), *Saccharomyces cerevisiae*

INTRODUCTION

Monovalent cations are very important in yeast metabolism. However, high internal concentrations of Na^+ or its analogue Li^+ are generally toxic to cells [1]. Lithium is highly toxic to yeast when grown in galactose medium, mainly because phosphoglucomutase, a key enzyme of galactose metabolism, is inhibited [2]. High salt concentrations (0.3–0.5 M NaCl) inhibit most enzymes because of the perturbation of the hydrophobic-electrostatic balance between the forces maintaining protein structure. Many metabolic reactions and membrane functions to seems be strongly affected by these salt concentrations [3, 4]. In addition to these relatively nonspecific salt effects, some enzymes may be a specially sensitive to Na^+ or Cl^- at much lower concentrations. Incubation of wild-type yeast cells with NaCl and LiCl, but not with KCl, inhibited intracellular nucleotidase witch responsible for dephosphorilation of PAP (3-phosphoadenosine 5-phosphate) [5]. The Na^+/H^+ antiporter responsible for sodium and lithium tolerance of the fission yeast *Schizosaccharomyces pombe* is encode by the *sod2* gene and is believed to be the sole_sodium extrusion system in this yeast [6]. On the other hand, in the yeast *Saccharomyces cerevisiae*, the most efficient sodium-eliminating system is Na^+-ATPase (encoded by the *ENA1-4/PMR2A-E genes)* [7].

Transport systems mediated the efflux and influx of cations are widely studied [2-8], however, the physiological function of alkali-metal-cations on the permeability of yeast cells has not been investigated. It is well known that intact bacterial *E. coli* [9] cells take up plasmid DNA only after treatment with divalent metal cations. More effective in inducing competence for yeast cells were alkali metal cations Li^+, Na^+, Rb^+, however, Li^+ was eight times the most effective than the other cations tested, and the transformation efficiency with LiCl or Li acetate exceeded that obtained by the conventional protoplast method [10]. Ca^{2+}, which induces competence in *E. coli* cells, was inert with yeast cells.

This report presents data on the permeability properties of *Saccharomyces cerevisiae* SEY6210 strain to lipophilic tetraphenylphosphonium cations after treatment with Li^+ and Na^+ cations.

MATERIALS AND METHODS

Yeast strains and cultivation. The *Saccharomyces cerevisiae* strain SEY6210 (*MATα, leu2-3, ura3-52, his3-Δ200, lys2-801, trp-Δ901, suc2-Δ9*) was used in this study: 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 an absorbance at 660 nm of 0.3–0.4 (exponential phase). Then the yeast cells were concentrated 10 times in 50 mM Tris-HCl buffer pH 7.85. An equal volume of 0.2 M of each cation was added. After 1 h of incubation at 30 °C, the with shaking cells were centrifuged and used in the next experiments.

Tetraphenylphosphonium accumulation measurements. The yeast cells were washed with 50 mM Tris-HCl buffer pH 7.85 two times, concentrated 10 times in the same buffer, TPPBr was added to the final concentration of $3 \cdot 10^{-7}$ M. After 30 min of incubation at 30 °C, the 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 50 mM Tris-HCl buffer, pH 7.85 (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.

Growth assessment. The lithium and sodium tolerance of intact yeast cells were determined in liquid YEPD media and in 50 mM TrisHCL-buffer, pH 7.85, supplemented with the indicated amounts of Li and Na cations. To assess their growth, the yeast cells were incubated at 30 °C with vigorous aeration for 3 hours. Growth was assessed every 30 min by measuring the increase in absorbance of the cell suspension at 600 nm. The results presented are typical of at least three experiments.

Statistical analysis. The effect of the study cations on TPP accumulation was analyzed by comparing the amount of TPP accumulated after incubation with cations and in their absence. The data points in Fig. 3 and text are expressed as the mean of three experiments ± SE. Statistical analysis of the data was done by Student *t*-test. Statistical significance was assumed at $p < 0.05$.

RESULTS AND DISCUSSION

The aim of the study was to determine the influence of treatment of alkali cations on the permeability of the yeast *Saccharomyces cerevisiae* cells under transformation conditions.

Permeability properties of the yeast cells were estimated as the accumulation of the tetraphenylphosphonium ions (TPP^+) by a TPP^+-selective electrode. We have previously observed that a maximum of accumulated TPP^+ was determined after 30 min of incubation; continuous incubation did not cause a significant increase in the amount of accumulated TPP^+[11].

The results observed by us demonstrate that incubation of yeast cells with 0.1 M Li^+ and Na^+ cations for 180 min did not inhibite the growth of yeast cells under physiological conditions then the cells were suspended in YEPD medium (Fig. 1). At the same time, then yeast cells were suspended in 50 mM Tris-HCl buffer, the growth was inhibited but the viability of cells was not affected (Fig. 2).

The changes induced in yeast cell permeability to TPP cations by Li^+ and Na^+ are presented in Fig. 3. The yeast cells incubated without cations were used as control; they accumulated 27 nmol of TPP for 1 mg of yeast protein.

The incubation of the yeast cells with Li acetate and LiCl 1.5 and 1.3 times increased the accumulation of TPP cations compared with cells incubated in a buffer with-

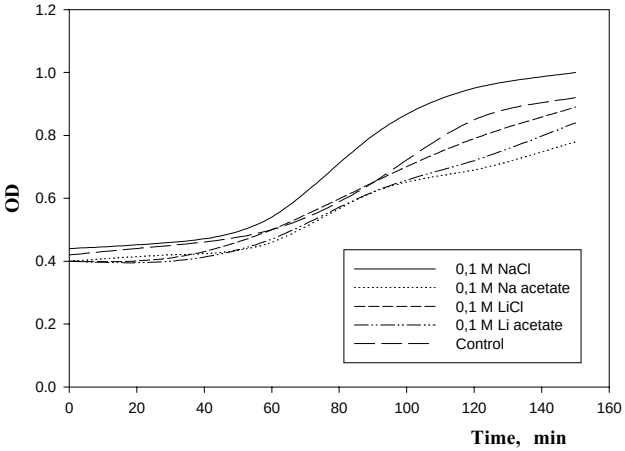

Fig. 1. Growth of yeast *Saccharomyces cerevisiae* SEY6210 strain in liquid YEPD medium in the presence of cations. Cells were prepared as described in Materials and Methods

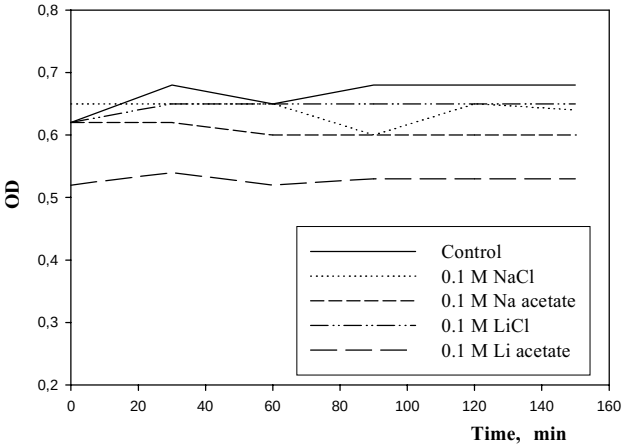

Fig. 2. Growth of yeast *Saccharomyces cerevisiae* SEY6210 strain in 50 mM Tris-HCl buffer, pH 7.85 in the presence of cations. Cells were prepared as described in Materials and Methods

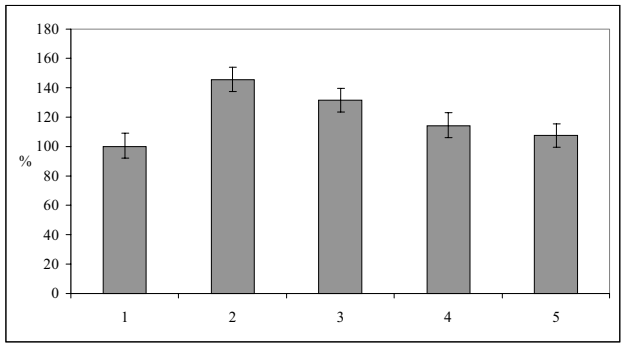

Fig. 3. Accumulation of TPP^+ by *Saccharomyces cerevisiae* SEY6210 strain after incubation with cations.
1 – control, 2 – 0.1 M Li acetate, 3 – 0.1 M LiCl, 4 – 0.1 M NaCl, 5 – 0.1 M Na acetate. The TPP^+ accumulation assay was performed as described in Materials and Methods. Cells were prepared as described in Materials and Methods. Values are the average ± standard errors of three independent experiments (100% = 27 ±1nmolTPP^+/mg yeast protein)

out cations. The amount of TPP accumulated after treatment with Na cations was lower and reached only 15% compared with cells incubated in a buffer without cations.

Our results showed that treatment with lithium cations increased the permeability of intact yeast cells, and this effect was presumable due to a facilitate entrance of DNA through the holes or pores generated by treatment with metal cations. Another agent faciliting the penetration of DNA in the process of yeast transformation, polyethylene glycol (PEG), caused changes in membrane charges by interactions among negatively charged PEG, lithium cations, and the yeast cell surface[10]. We do not exclude the possibility that other effects of lithium cations are also involved in the structural changes of the cell wall or membrane. Efforts to identify additional functionally important effects of alkaline cations on the yeast cell envelope in progress.

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LIČIO IR NATRIO KATIJONAIS PAVEIKTŲ MIELIŲ *SACCHAROMYCES CEREVISIAE* LAIDUMAS TETRAFENILFOSFONIO JONAMS

Santrauka

Pristatomame darbe buvo tirtas mielių *Saccharomyces cerevisiae* SEY6210 kamieno, paveikto ličio ir natrio katijonais, sugebėjimas akumuliuoti lipofilinius tetrafenilfosfonio (TPP^+) jonus. Akumuliacija buvo registruojama TPP jonams selektyviu elektrodu. Nustatyta, kad augimo terpėje esantys 0,1 M ličio ar 0,1 M natrio katijonai neinhibavo ląstelių augimo fiziologinėmis sąlygomis. Paveikus mielių ląsteles 0,1 M Li acetatu, 1,5 karto padidėjo jų sugebėjimas akumuliuoti TPP^+, lyginant su katijonais nepaveiktomis ląstelėmis. Ląstelės po inkubacijos su Na katijonais pasižymėjo truputį didesniu akumuliacijos lygiu (15%).