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Saccharomyces cerevisiae BY4741 and W303-1A laboratory strains differ in salt tolerance

*Silvia PETREZSELYOVA, Jaromir ZAHRADKA, Hana SYCHROVA**

Department of Membrane Transport, Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i, Videnska 1083, 14220 Prague 4, Czech Republic

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ABSTRACT

Saccharomyces cerevisiae yeast cells serve as a model to elucidate the bases of salt tolerance and potassium homeostasis regulation in eukaryotic cells. In this study, we show that two widely used laboratory strains, BY4741 and W303-1A, differ not only in cell size and volume but also in their relative plasma-membrane potential (estimated with a potentiometric fluorescent dye diS-C_3(3) and as Hygromycin B sensitivity) and tolerance to alkali-metal cations. W303-1A cells and their mutant derivatives lacking either uptake (*trk1 trk2*) or efflux (*nha1*) systems for alkali-metal cations are more tolerant to toxic sodium and lithium cations but also more sensitive to higher external concentrations of potassium than BY4741 cells and their mutants. Moreover, our results suggest that though the two strains do not differ in the total potassium content, the regulation of intracellular potassium homeostasis is probably not the same in BY4741 and W303-1A cells.

Introduction

Ion and pH homeostases are fundamental to the physiology of all living cells, including yeast. As far as the homeostasis of alkali-metal cations is concerned, cells usually expend a substantial proportion of their energy to accumulate high amounts of potassium and maintain low cytosolic concentrations of toxic sodium or lithium cations. To ensure an optimal cytoplasmic K^+/Na^+ ratio, cells employ several systems transporting cations with differing affinities, capacities, substrate specificities and diverse mechanisms, both across the plasma membrane, and in eukaryotic cells also across the membranes of intracellular organelles. *Saccharomyces cerevisiae* cells have at least five K^+ transport systems at their disposal at their plasma membrane. For K^+ uptake, there are Trk1p and Trk2p as high-affinity specific transporters (Gaber *et al.* 1988; Ramos *et al.* 1994), and its efflux is mediated *via* the voltage-gated K^+-specific channel Tok1 (Ketchum *et al.* 1995), and two efflux systems that in addition to K^+, also transport Na^+ and Li^+: P-type ATPase (encoded by *ENA/PMR2* locus) (Haro *et al.* 1991) and the Nha1 antiporter (Prior *et al.* 1996).

A high intracellular K^+/Na^+ ratio is important for many cellular processes, such as the activation of enzymatic reactions, cell volume regulation, response to osmotic shock or buffering the intracellular pH (Sychrova 2004). The uptake and efflux of potassium cations is also indispensable for the preservation of a constant level of membrane potential ($\Delta\psi$). Membrane potential across the plasma membrane (negative inside) is built up by the activity of the H^+-ATPase Pma1 (Serrano *et al.* 1986), and consumed by the electrophoretic uptake of K^+ through Trk transporters and by many other secondary active systems (symporters and antiporters) including Nha1p. Plasma-membrane H^+-ATPase and potassium systems for influx and efflux act in synergy to fulfill their roles and damage to any of them is reflected in changes in intracellular pH and potassium content and in increased sensitivity to toxic alkali-metal cations or cationic drugs in general.

* *Corresponding author*. Tel.: +420241062667; fax: +420241062488.
E-mail address: sychrova@biomed.cas.cz

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If yeast cells are grown in the presence of high NaCl concentrations (salt stress) they must cope both with an elevated external osmotic pressure and an increasing amount of Na^+ entering the cells. Inside the cells, sodium replaces potassium, thus the cytosolic K^+/Na^+ ratio decreases with increasing external Na^+ and the cells must actively pump sodium out or sequestrate it in organelles (mainly the vacuole). To ensure this, *S. cerevisiae* cells mainly use Ena ATPases at the plasma membrane and the Nhx1, Vnx1 antiporters localized to the membranes of their late endosomes and vacuoles, respectively.

Numerous laboratory strains are used in yeast research, which in some cases results in discrepancies for comparable data. Within the framework of the exploration of salt tolerance and cation homeostasis in *S. cerevisiae*, we have observed specific differences among alkali-metal cation-transport deficient mutants derived from different parental strains. To elucidate the observed inconsistency, we employed two commonly used laboratory strains: BY4741 and W303-1A. While BY4741 is a derivative of S288C (Brachmann *et al.* 1998), used in the systematic sequencing of the *S. cerevisiae* genome (Goffeau *et al.* 1996), strains with a W303 background have served in many physiological and biochemical studies. The aim of the present study was to compare and describe the differences in salt-tolerance-associated phenotypes between the two strains and to find at least some of the factors determining the observed differences.

Experimental/materials and methods

Strains and growth conditions

The yeast *Saccharomyces cerevisiae* strains W303-1A (*MAT*a *leu2*-3/112 *ura3*-1 *trp*1-1 *his3*-11/15 *ade2*-1 *can*1-100) (Wallis *et al.* 1989) and BY4741 (*MAT*a *his*3Δ1 *leu*2Δ *met*15Δ *ura*3Δ; EUROSCARF, Germany) were used in this work, together with their derivatives lacking either the Trk systems for potassium uptake or the Nha1 antiporter for sodium and potassium efflux. W303-1A derivatives were W12 (*trk1*::*LEU2 trk2*::*HIS3* (Madrid *et al.* 1998), kindly provided by Dr. M.A. Bañuelos) and CW25 (*nha1*::*LEU2*; Kinclova-Zimmermannova *et al.* 2006). The BY4741 derivatives BYT12 (*trk1*::*loxP trk2*::*loxP*) and BYT4 (*nha1*::*loxP*) were constructed in this work by homologous recombination using the KanMX marker gene and Cre-*loxP* system (Guldener *et al.* 1996). The oligonucleotides used for deletion cassette amplifications and recombination diagnosis are listed in Table 1.

Cells were grown in YPD (1 % yeast extract, 2 % bacto peptone, 2 % glucose, 0.015 g L^{-1} adenine) or YNB (0.17 % yeast nitrogen base without amino acids, 0.5 % ammonium sulphate, 2 % glucose) with appropriate auxotrophic supplements added after autoclaving. For optimal growth of *trk1*Δ*trk2*Δ mutant strains (BYT12 and W12), KCl was added to the media (as indicated in the text, at least 50 mM). Solid media were supplemented with 2 % agar.

Salt-tolerance drop test

Growth phenotypes of strains in the presence of salts were tested on solid media. Yeast strains growing overnight in liquid medium without salts were washed and resuspended in sterile water to the same initial OD_{600} (approx. 1). Tenfold serial dilutions were prepared and 3 μL aliquots spotted on a series of plates containing increasing concentrations of salts (1.0–2.0 M KCl, 1.0–2.0 M NaCl, 0.1–0.5 M LiCl). Plates were incubated at 30 °C for 3–4 d. Representative results are shown.

Gradient plate assay

A gradient plate assay was performed as previously described (Maresova & Sychrova 2005). Briefly, a Hygromycin B gradient

Table 1 – List of oligonucleotides.

Gene deletion primers	
NHA1-kan-F	5′GTACATTATAAAAAAAAATCCTGAACTTAGCTAGATATTAttcgtacgctgcaggtcgac-3′
NHA1-kan-R	5′ATATACTAAAATAATATATCTTTGTGTATTAATAAATTACgcataggccactagtgga-3′
TRK1-kan-F	5′TCAAGGAAGTCATTCCTATCCATTTTACTTAAAGTTATTACCTTTTTTTGATAACTAACAttcgtacgctgcaggtcgac-3′
TRK1-kan-R	5′TTGAGTACGAAAACCTATTTCTAAAGAATGAGTATATATGgcataggccactagtggatctg-3′
TRK2-kan-F	5′GATGAGAAAAGAGGCTATTTTGTACTATTCACCGACGATAAGAGGCTGTAAGAACCACTCttcgtacgctgcaggtcgac-3′
TRK2-kan-R	5′ACGTTGGCTCTTATGTAGGTAAAGAGGGGTAAACTTGATTTTgcataggccactagtggatctg-3′
Diagnostic primers – deletion cassettes	
KANX-R1	5′CTCTGGCGCATCGGGC-3′
KANX-F1	5′CATTTGATGCTCGATGA-3′
Diagnostic primers – target gene	
NHA1-UP	5′CAACTCTGTGTGATATAG-3′
NHA1-DR	5′CAATGTGAACCCCAGTG-3′
NHA1-20	5′CCTGCGGCCACCGAGAGGAG-3′
TRK1-UP	5′GAGAGTAGATGTGGAGT-3′
TRK1-1	5′GATCAGAGGCAAGAATAG-3′
TRK1-DR	5′GGAGAACACCGGCGGG-3′
TRK2-UP	5′GATGCAAGCTGCCATG-3′
TRK2-1	5′GTTTGAAGTTGTTAGCGC-3′
TRK2-DR	5′CGTAGGGGACAAAATGC-3′

Lower case letters indicate nucleotides complementary to sequences of the template.