

Comparative physiology of salt tolerance in *Candida tropicalis* and *Saccharomyces cerevisiae*

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The salt tolerance of the respiratory yeast *Candida tropicalis* and the fermentative yeast *Saccharomyces cerevisiae* have been compared in glucose media. *C. tropicalis* showed a better adaptation to Na⁺ and Li⁺ and maintained higher intracellular K⁺:Na⁺ and K⁺:Li⁺ ratios than *S. cerevisiae*. However, *C. tropicalis* showed a poorer adaptation to osmotic stress (produced by KCl and sorbitol) and exhibited reduced glycerol production as compared to *S. cerevisiae*. In media with the non-repressing sugar galactose as carbon source, *S. cerevisiae* exhibited reduced glycerol production and increased sensitivity to osmotic stress. Under these conditions, *S. cerevisiae*, but not *C. tropicalis*, utilized trehalose as a more important osmolyte than glycerol. These results suggest that the relative tolerance of yeast to the osmotic and cation toxicities of NaCl, and the underlying relative capabilities for osmolyte synthesis and cation transport, are modulated by the general catabolite control exerted by glucose.

Keywords: salt stress, yeast, sodium, glycerol, trehalose

INTRODUCTION

The progressive salinization of irrigated land compromises the future of agriculture in the most productive areas of our planet (Ashraf, 1994). In this respect, a full understanding of the mechanisms of salt tolerance utilized by model organisms such as yeast could provide tools for the genetic engineering of salt-tolerant crops (Serrano & Gaxiola, 1994). Micro-organisms, like many yeasts (Tokuoka, 1993), that can tolerate high saline environments, develop systems to counteract the deleterious effects of the two components of salt stress: intracellular ion toxicity and osmotic stress (turgor and water loss). In yeast, defence responses to salt stress are based on osmotic adjustment by osmolyte synthesis and cation transport systems for sodium exclusion. Polyols, and especially glycerol, are the major osmolytes produced by yeast (Blomberg & Adler, 1992, 1993; Brown, 1990). Both a Na⁺-ATPase and a H⁺/Na⁺ antiporter have been described as mechanisms of sodium extrusion (Serrano, 1996). Depending on the relative activities of

these systems, either the osmotic or the ionic toxicities of NaCl may dominate growth inhibition by salt stress.

The identification of the physiological factors which modulate the defence of yeast against salt stress is an important goal of salt tolerance studies. One important factor in yeast physiology is the type of sugar metabolism used, fermentative or respiratory (Gancedo & Serrano, 1989). Glucose exerts a general modulation of metabolism and stress responses in yeast (Thevelein, 1994; Serrano, 1996). In addition, to meet with the energy requirements for metabolic and transport reactions (including stress-activated futile cycles; see Jennings, 1993), glucose metabolism has regulatory effects which are collectively described as either 'general catabolite control' (Gancedo & Serrano, 1989) or 'glucose-induced signal transduction' (Thevelein, 1994). This phenomenon occurs in the so-called 'fermentative yeast', such as the genus *Saccharomyces*, where high rates of glucose catabolism down-regulate respiration (Gancedo & Serrano, 1989) and defence responses against stresses such as heat shock, starvation (Thevelein, 1994; Serrano, 1996) and high salt concentrations (G. Ríos & R. Serrano, unpublished data). In the so-called 'respiratory yeast', such as the genus *Candida*, glucose catabolism is

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slow, the general catabolite control is not operative and most of the sugar is respired (Gancedo & Serrano, 1989).

In this study we have compared the relative tolerance to osmotic stress and to alkali cation toxicity of the fermentative yeast *Saccharomyces cerevisiae* and the respiratory yeast *Candida tropicalis*. The results indicate that the operation of general catabolite control in *S. cerevisiae*, but not in *C. tropicalis*, correlates with a high capability for glycerol synthesis and osmotic tolerance but a low capability for sodium and lithium extrusion and cation tolerance. In addition, we have determined that, in the absence of glucose repression, trehalose behaves as an important osmoreponsive solute in *S. cerevisiae*.

METHODS

Yeast strains and growth. *Candida tropicalis* strain NCYC 2512 was isolated by R. Ali from a saline Pakistani soil on the basis of its tolerance to high salt concentrations (15% NaCl). *Saccharomyces cerevisiae* strain DBY746 (*MAT_{his3-Δ1} leu2-3,112 ura3-52 trp1-289*) was obtained from the Yeast Genetic Stock Center, Berkeley, California. YPD medium containing 1% yeast extract (Difco), 2% Bacto Peptone (Difco) and 2% glucose was used for routine culture. Growth experiments were on SD minimal medium containing 2% glucose, 0.7% Yeast Nitrogen Base (without amino acids; Difco) and 50 mM MES adjusted to pH 6.0 with Tris. Histidine (30 $\mu\text{g ml}^{-1}$), leucine (100 $\mu\text{g ml}^{-1}$), uracil (30 $\mu\text{g ml}^{-1}$) and tryptophan (100 $\mu\text{g ml}^{-1}$) were also included in the case of *S. cerevisiae*. Saturated cultures of *C. tropicalis* and *S. cerevisiae* (grown for 3 d and with OD_{660} values of 5 and 2, respectively) were diluted 50- and 30-fold, respectively, at time zero in normal medium or in medium containing NaCl (1 M), KCl (1 M), LiCl (0.2 M) or sorbitol (1.5 M) as indicated. Growth was followed in 3 ml cultures by OD_{660} using a Spectronic 20D (Milton Roy) spectrophotometer. The growth temperature was 28 °C and the tubes were shaken by rotation on a tube roller (New Brunswick Scientific).

Intracellular cation determination. For Li^+ accumulation kinetics, 0.1 M LiCl (final concentration) was added to a mid-exponential culture (OD_{660} 0.4–0.6; 500 ml in 2 l shaken flask) and 20 ml aliquots were collected at different times. Twenty millilitres of ice-cooled 180 mM sorbitol and 20 mM MgCl_2 were added and immediately centrifuged at 7000 r.p.m. in a Beckman JA-20 rotor at 4 °C. After resuspending in 10 ml of the same ice-cooled sorbitol solution, cells were collected on glass microfibre filters (Whatman GF/C) and washed twice on the filter with the same solution. Extraction was performed in 1 ml 20 mM MgCl_2 at 95 °C for 12 min.

For Na^+ accumulation kinetics, 1 M NaCl (final concentration) was added as above and 20 ml aliquots were collected at different times. Twenty millilitres of ice-cooled 1.5 M sorbitol and 20 mM MgCl_2 were added and cells were harvested by centrifugation at 7000 r.p.m. in a Beckman JA-20 rotor at 4 °C. Pelleted cells were washed four times by centrifugation and resuspension in the same sorbitol solution. Extraction was performed as described above for lithium.

The ion content in the clarified extracts was determined with an Atomic Absorption Spectrometer (Varian) at flame emission mode. Internal water of *C. tropicalis* was determined by measuring the difference between total water of a cell pellet

and extracellular water (Conway & Armstrong, 1961). The latter was determined with $\text{D-[U-}^{14}\text{C]sorbitol}$, a non-permeable and non-metabolizable compound for this yeast species (R. Ali, unpublished data). Determination of intracellular K^+ content was performed in the same extracts utilized for Li^+ and Na^+ determination. One OD_{660} unit of *C. tropicalis* suspension corresponded to an intracellular water content of 2.4 $\mu\text{l (ml suspension)}^{-1}$. For *S. cerevisiae* one OD_{660} unit was equivalent to 1.6 $\mu\text{l intracellular water (ml suspension)}^{-1}$ (Gaxiola *et al.*, 1992).

Glycerol determination. Samples (1 ml) were withdrawn from mid-exponential cultures supplemented with 1 M NaCl. Cells were collected by filtration through a glass microfibre filter (Whatman GF/C) and washed twice on the filter with a solution containing 1.5 M sorbitol and 20 mM MgCl_2 . Cells were resuspended in 1 ml 0.5 M Tris/HCl, pH 7.6, and heated at 95 °C for 12 min. After centrifugation, the supernatant was used to measure the internal glycerol content. For determination of extracellular glycerol, samples (1 ml) were withdrawn from the culture, cells were removed by centrifugation and the clarified supernatant was used for the assay. Glycerol content was determined by means of an enzymic assay kit (Boehringer Mannheim, cat. no. 148270).

Trehalose determination. Extracts were prepared as described for glycerol determination except that water was used as the extracting medium. Separation of soluble carbohydrates was by high-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAE-PAD, Waters), using a CarboPac PA1 (4 × 250 mm) column (Dionex) maintained at 22 °C (Rocklin & Pohl, 1983).

RESULTS AND DISCUSSION

Growth of *C. tropicalis* and *S. cerevisiae* in presence of different solutes

Growth curves of *C. tropicalis* and *S. cerevisiae* in the absence and presence of 1 M NaCl are shown in Fig. 1. *C. tropicalis* was able to grow much faster than *S. cerevisiae* due to its respiratory metabolism (Gancedo &

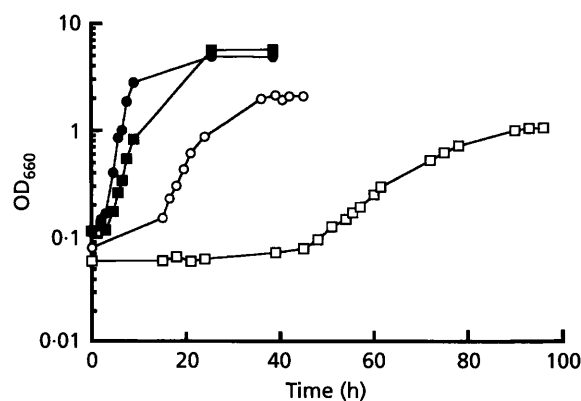


Fig. 1. Growth of *C. tropicalis* (●, ■) and *S. cerevisiae* (○, □) in the absence (●, ○) and presence (■, □) of 1 M NaCl. Saturated cultures of *C. tropicalis* and *S. cerevisiae* (OD_{660} values of 5 and 2, respectively) were diluted 50- and 30-fold, respectively, at time zero. Similar results were obtained in two independent experiments.

Table 1. Relative effect of different solutes on growth of *C. tropicalis* and *S. cerevisiae*

Growth of *C. tropicalis* and *S. cerevisiae* was recorded after 14 and 26 h, respectively. Results are expressed as a percentage of the OD₆₆₀ value reached by control cultures in normal medium without additions. The absolute values were 3.5 for *C. tropicalis* and 1.5 for *S. cerevisiae*, corresponding to late-exponential phase (see Fig. 1). Values are the means of two determinations differing by less than 10%.

| Solute | Relative growth (%) | |
|----------------|----------------------|----------------------|
| | <i>C. tropicalis</i> | <i>S. cerevisiae</i> |
| None | 100 | 100 |
| 0.2 M LiCl | 10 | 2 |
| 1 M NaCl | 20 | 5 |
| 1 M KCl | 30 | 50 |
| 1.5 M Sorbitol | 3 | 30 |

Serrano, 1989). In the absence of NaCl the growth rate of *C. tropicalis* was 0.43 h⁻¹, whereas that of *S. cerevisiae* was 0.26 h⁻¹. In the presence of 1 M NaCl, *C. tropicalis* showed a 30% reduction in growth rate while in the case of *S. cerevisiae* the reduction of growth rate was much greater, a 70% decrease. The lag phase was also dramatically increased (to 40 h) in the case of *S. cerevisiae* in the presence of 1 M NaCl. These results suggest that *C. tropicalis* grows better than *S. cerevisiae* at high NaCl concentrations, not only because of its more vigorous respiratory metabolism but also because of its higher salt tolerance.

We have investigated the sensitivities of both yeast species to different chloride salts (NaCl, KCl, LiCl) and to sorbitol. Lithium is much more toxic than sodium while KCl is not toxic and only poses osmotic stress similar to sorbitol (Serrano, 1996). All these solutes produced an increase in both the generation time and the lag phase of yeast cultures (see Fig. 1 for NaCl). We determined relative inhibitions by measuring growth at a fixed time-point, when control cultures had reached late exponential phase (Table 1). KCl was the least inhibitory solute for both *S. cerevisiae* and *C. tropicalis*. Taking KCl as a reference, sorbitol was strongly inhibitory for *C. tropicalis* but much better tolerated by *S. cerevisiae*. NaCl and LiCl were much better tolerated by *C. tropicalis* than by *S. cerevisiae*.

These results indicate that these yeast species differ in their relative sensitivities to osmotic stress (sorbitol) and cation toxicity (sodium and lithium). The low relative toxicity of KCl indicates that chloride poses no toxicity problem for either yeast species. The fact that KCl is much less inhibitory for *C. tropicalis* than sorbitol could be explained if K⁺ uptake, but not sorbitol uptake, could be utilized for osmotic adjustment in this species (see below). The difference between KCl and sorbitol toxicities is much less apparent in *S. cerevisiae*.

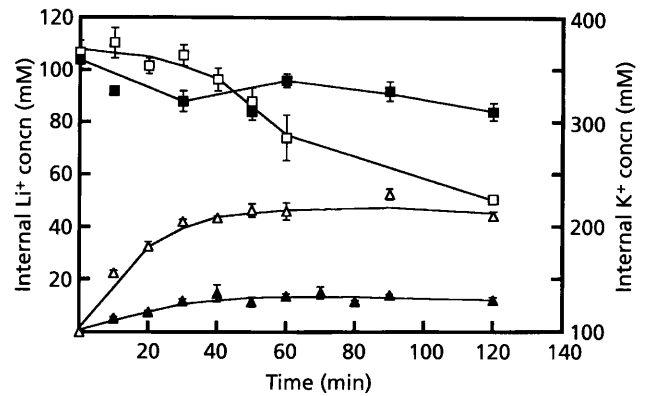


Fig. 2. Kinetics of Li⁺ uptake (△, ▲) and K⁺ efflux (□, ■) in *C. tropicalis* (▲, ■) and *S. cerevisiae* (△, □). Cell cultures growing exponentially (OD₆₆₀ 0.4–0.6) were supplemented at time zero with 100 mM LiCl and samples were taken at the indicated times for intracellular cation determination as described in Methods. Values are the means of three independent determinations and bars represent the SD.

Cation uptake and homeostasis

The greater sensitivity of *S. cerevisiae* to Na⁺ and Li⁺ as compared to *C. tropicalis* suggested that these toxic cations could be accumulated to higher levels in the former yeast species. As indicated in Fig. 2 (Li⁺ accumulation) and Fig. 3 (Na⁺ accumulation) this is actually the case. *C. tropicalis* accumulated much less Li⁺ than *S. cerevisiae*, the differences being apparent from the earliest time point at only 10 min (Fig. 2). Therefore the cation uptake system must have different relative activities for Li⁺ and K⁺ (present in the medium) in the two yeast species. Li⁺ accumulation caused no significant changes in intracellular K⁺ levels during the first hour. At longer times the K⁺ content of *S. cerevisiae* cells, but not of *C. tropicalis* cells, started to decrease. This could be a secondary effect of metabolic poisoning by high levels of intracellular Li⁺.

Initial Na⁺ influx during the first 30 min was very similar in both yeast species (Fig. 3). However, at longer times *C. tropicalis* was able to maintain a lower intracellular steady-state Na⁺ level than *S. cerevisiae*. According to the kinetic model developed for net accumulation of Li⁺ and Na⁺ in yeast (Rodríguez-Navarro & Asensio, 1977; Rodríguez-Navarro & Ortega, 1982), this suggests that the major differences in sodium transport systems between the two yeast species are concerned with active efflux of the cation. Both *C. tropicalis* and *S. cerevisiae* suffered a massive (almost 50%) and rapid (completed in less than 10 min) loss of internal K⁺ after addition of 1 M NaCl (Fig. 3). This K⁺ efflux was not observed in the case of 0.1 M LiCl (Fig. 2) and it may be due to osmotic shock. Gustin *et al.* (1988) reported the presence of putative mechanosensitive channels in *S. cerevisiae* opened by the shrinkage produced by osmotic stress. These channels have a higher selectivity for K⁺ than for other ions such as Na⁺.

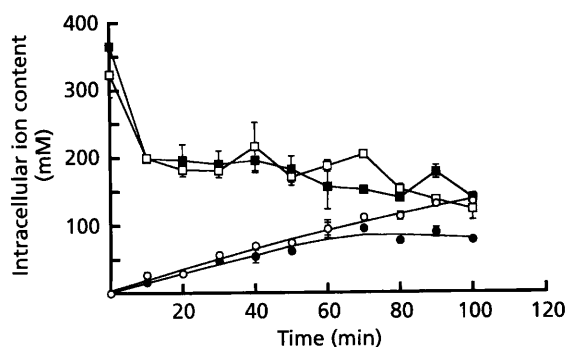


Fig. 3. Kinetics of Na^+ uptake (\circ , \bullet) and K^+ efflux (\square , \blacksquare) in *C. tropicalis* (\bullet , \blacksquare) and *S. cerevisiae* (\circ , \square). Cell cultures growing exponentially (OD_{660} 0.4–0.6) were supplemented at time zero with 1 M NaCl and samples were taken at the indicated times for intracellular cation determination as described in Methods. Values are the means of three independent determinations and bars represent the SD.

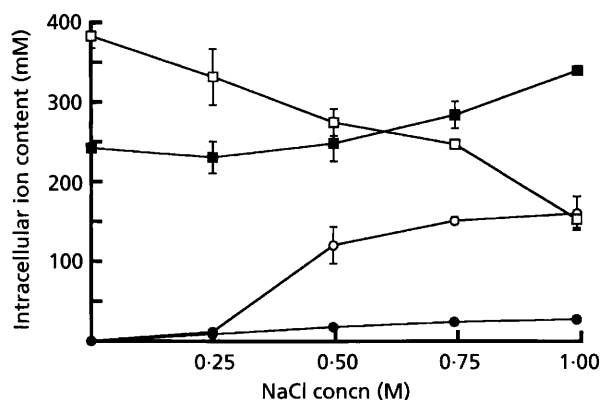


Fig. 4. Intracellular steady-state levels of Na^+ (\circ , \bullet) and K^+ (\square , \blacksquare) in *C. tropicalis* (\bullet , \blacksquare) and *S. cerevisiae* (\circ , \square). Samples of cell cultures growing exponentially (OD_{660} 0.4–0.6) with the indicated NaCl concentrations were taken for intracellular cation determination as described in Methods. Values are the means of three independent determinations and bars represent the SD.

It is known that intracellular $\text{Na}^+:\text{K}^+$ ratios above 0.5 are toxic to yeast cells (Camacho *et al.*, 1981; Gaxiola *et al.*, 1992). For *S. cerevisiae* this ratio increased up to a value of 1.1 at the end of the experiment shown in Fig. 3. On the other hand, *C. tropicalis* maintained a $\text{Na}^+:\text{K}^+$ ratio lower than 0.5 during the whole uptake experiment.

The results of these kinetic experiments were corroborated by studies of the steady-state levels of Na^+ and K^+ during growth. For external NaCl concentrations of ≥ 0.5 M, *S. cerevisiae* accumulated higher amounts of Na^+ than *C. tropicalis* (Fig. 4). K^+ contents decreased at high NaCl concentrations in *S. cerevisiae* but increased in *C. tropicalis*. Therefore, after the initial K^+ loss triggered by NaCl addition in both yeast species (Fig. 3),

there must be an adaptative response in *C. tropicalis* to recover the intracellular K^+ level and even to increase it at high NaCl concentrations. In *S. cerevisiae* $\text{Na}^+:\text{K}^+$ ratios were increased up to a value of about 1 in presence of 1 M NaCl. However, in *C. tropicalis* the decrease in Na^+ uptake and the increase in K^+ retention resulted in low $\text{Na}^+:\text{K}^+$ ratios even at high NaCl concentrations (0.1 at 1 M NaCl). Moreover, *C. tropicalis* was able to grow at a NaCl concentration of 1.5 M, whereas *S. cerevisiae* could not. Under this condition, the intracellular $\text{Na}^+:\text{K}^+$ ratio was only 0.2 (M. J. García, unpublished data).

In *S. cerevisiae* the intracellular Na^+ level obtained in kinetic experiments with 1 M NaCl (0.15 M; Fig. 3) was very similar to the steady-state level achieved during growth at the same NaCl concentration (Fig. 4). In *C. tropicalis*, however, the steady-state level after 1–1.5 h of incubation with 1 M NaCl (0.09 M; Fig. 3) was much higher than the steady-state level during growth at this NaCl concentration (0.03 M; Fig. 4). This suggests the operation in *C. tropicalis* of an adaptative mechanism to increase sodium efflux or restrict sodium uptake during continuous growth in media with NaCl.

Taken together, these results are evidence of a higher adaptation of *C. tropicalis* to ionic stress as compared to *S. cerevisiae*. It is known that Na^+ and Li^+ ions enter *S. cerevisiae* cells through the K^+ transporter (Gaber, 1992; Serrano, 1996). Cation uptake is more efficient at producing salt tolerance in *C. tropicalis* as compared to *S. cerevisiae* because (a) the initial rate of Li^+ uptake is decreased (Fig. 2) and (b) K^+ accumulates to higher levels in response to NaCl stress (Fig. 4) despite similar efflux (Fig. 3). In *S. cerevisiae* cation uptake is mediated by the *TRK1,2* system (Gaber, 1992; Serrano, 1996). This system discriminates against Na^+ and Li^+ in favour of K^+ and this discrimination increases during salt stress (Haro *et al.*, 1993). If the same system were operative in *C. tropicalis*, it should have an even greater discriminatory power and, in addition, should respond to salt stress by increased K^+ accumulation. A different K^+ transporter encoded by the *HAK1* gene has recently been identified in the yeast *Schwanniomyces occidentalis* (Bañuelos *et al.*, 1995). This system is more efficient than the *S. cerevisiae* *TRK1,2* system and therefore the presence of *HAK1*-related transporters in *C. tropicalis* should be investigated.

As discussed above, sodium efflux in *C. tropicalis* is more efficient than in *S. cerevisiae*. Sodium efflux in *S. cerevisiae* is mediated by the *ENA1* ATPase (Haro *et al.*, 1991), which is induced upon salt stress (García de Blas *et al.*, 1993). *C. tropicalis* could have a similar ATPase but with higher activity and better up-regulation by salt stress. In *S. cerevisiae*, expression of the *ENA1* gene is controlled by multiple signal transduction pathways (Marquez & Serrano, 1996) which could be more active in *C. tropicalis*. Alternatively, a H^+/Na^+ antiporter has been described in *Schizosaccharomyces pombe* (Jia *et al.*, 1992) and *Zygosaccharomyces rouxii* (Watanabe *et al.*, 1995) and a high activity of this kind of transporter

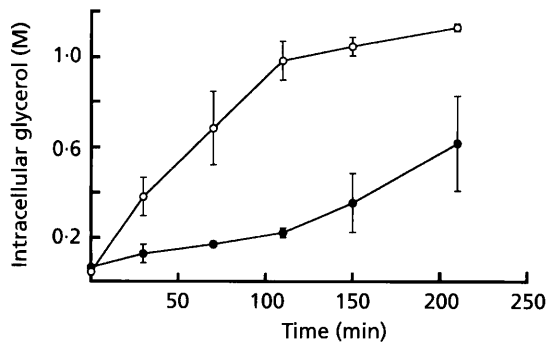


Fig. 5. Kinetics of intracellular glycerol accumulation in *C. tropicalis* (●) and *S. cerevisiae* (○) upon salt stress. Cell cultures growing exponentially in glucose medium (OD_{660} 0.4–0.6) were supplemented at time zero with 1 M NaCl and samples taken at the indicated times for intracellular glycerol determination as described in Methods. Values are the means of three independent determinations and bars represent the SD.

Table 2. Intracellular concentrations of glycerol and trehalose in *S. cerevisiae* and *C. tropicalis*

Cells were grown to exponential phase (OD_{660} 0.4–0.6) in media with either glucose or galactose as carbon source and with 1 M NaCl when indicated. Samples were taken for glycerol and trehalose determination as described in Methods. Values are the means of two determinations differing by less than 10%. ND, not determined.

| Growth medium | Intracellular concentration (M) | | | |
|------------------|---------------------------------|-----------|----------------------|-----------|
| | <i>S. cerevisiae</i> | | <i>C. tropicalis</i> | |
| | Glycerol | Trehalose | Glycerol | Trehalose |
| Glucose | 0.04 | < 0.02 | 0.06 | < 0.02 |
| Glucose + NaCl | 1.2 | 0.05 | 1.3 | < 0.02 |
| Galactose | < 0.02 | 0.11 | ND | < 0.02 |
| Galactose + NaCl | 0.48 | 0.75 | ND | < 0.02 |

could also explain the capability of *C. tropicalis* for sodium efflux.

Glycerol production under salt stress

In the case of *S. cerevisiae* growing on glucose, Na^+ toxicity is the major problem associated with salinity and this can be explained by the reduced capability for sodium exclusion and potassium accumulation of *S. cerevisiae* compared with *C. tropicalis*. In the latter yeast species, however, the osmotic component of salt stress poses the greater problem during growth on NaCl. This could be explained by a reduced capability of *C. tropicalis* for osmotic adjustment. As shown in Fig. 5, *S. cerevisiae* started to accumulate glycerol immediately after salt shock and reached steady-state levels in 2 h. In *C. tropicalis* glycerol production was much slower and did not reach a steady-state level during the time course

of the experiment. However, during steady-state growth with 1 M NaCl *C. tropicalis* was able to accumulate glycerol up to a concentration of 1.3 M, which is similar to that reached by *S. cerevisiae* (Table 2). This suggests that osmotic adjustment in glucose medium is achieved in both yeast species by glycerol accumulation, although in *C. tropicalis* glycerol synthesis is much slower than in *S. cerevisiae*. External glycerol concentration (less than 2 mM) did not change during the whole experiment in either of the strains tested (data not shown).

Studies carried out on over 20 different yeasts and filamentous fungi show that glycerol seems to be the main osmolyte accumulated upon salt stress (Blomberg & Adler, 1992, 1993). Glycerol production involves NADH-dependent reduction of dihydroxyacetone phosphate to glycerol 3-phosphate by cytoplasmic glycerol-3-phosphate dehydrogenase (GPD) and subsequent dephosphorylation of glycerol 3-phosphate to glycerol. Growth in the presence of NaCl increased GPD activity about sevenfold (André *et al.*, 1991; Blomberg & Adler, 1989; Albertyn *et al.*, 1994a) and a great part of this regulation is at the level of transcription (Albertyn *et al.*, 1994b). It seems that high rates of glycolysis, as occur during catabolic repression by glucose in *S. cerevisiae*, are optimal for glycerol production. In *C. tropicalis* the rate of glycolysis is lower, no catabolic repression occurs and respiration occurs at high rates. Slow glycolysis will reduce the supply of dihydroxyacetone phosphate and the high respiration rate will consume both dihydroxyacetone phosphate and NADH, thus competing with their utilization for glycerol synthesis (Gancedo & Serrano, 1989). Although GPD is repressed by glucose in *S. cerevisiae*, salt induction overcomes the repression effect and the enzyme has high activity in media with glucose and NaCl (Albertyn *et al.*, 1994a).

We have recently observed that *S. cerevisiae* growing in galactose media has high osmotic sensitivity because sorbitol and KCl are as toxic as NaCl at equivalent osmotic concentrations. In addition, glycerol synthesis is greatly reduced under these conditions (G. Ríos & R. Serrano, unpublished data). Galactose is metabolized more slowly than glucose and is not as repressive as glucose. Therefore, the energy metabolism of *S. cerevisiae* on galactose medium is mostly respiratory (Gancedo & Serrano, 1989).

One unexpected finding indicated in Table 2 is that trehalose is a better osmolyte than glycerol for *S. cerevisiae* growing on galactose medium with NaCl. Trehalose is not accumulated in *C. tropicalis* under any conditions. Trehalose is not considered as an osmoregulatory osmolyte in *S. cerevisiae*, mostly because it is not accumulated during osmotic stress on glucose medium (Brown *et al.*, 1986). Trehalose accumulation has only been observed upon heat shock (Hottiger *et al.*, 1987) and starvation during sporulation or stationary phase (Gancedo & Serrano, 1989). Our results indicate that, when the repressing effect of glucose is removed, as in galactose medium, trehalose is an osmoregulatory solute contributing more to the osmotic adjustment than glycerol (Table 2). Trehalose phosphate synthase

(Gancedo & Serrano, 1989) and trehalose phosphate phosphatase (Gounalaki & Thireos, 1994) are repressed by glucose while trehalase is activated by glucose (Gancedo & Serrano, 1989). Therefore, glucose should strongly oppose trehalose synthesis, even in the presence of stresses such as NaCl which induce trehalose synthesis. Only in non-repressing carbon sources such as galactose can the osmoregulatory role of trehalose be observed. We have observed that a *S. cerevisiae tps1* mutant, devoid of trehalose-6-phosphate synthase and unable to grow on glucose medium (Thevelein, 1994), is more sensitive to osmotic stress produced by sorbitol or KCl on galactose medium than wild-type (G. Rios & R. Serrano, unpublished observations). This corroborates the suggested role of trehalose as an osmoresponsive compatible solute on galactose medium.

As indicated in Table 2, trehalose is not accumulated in *C. tropicalis* despite the lack of catabolic repression of this yeast (Gancedo & Serrano, 1989). It has been suggested that trehalose biosynthesis in *S. cerevisiae* plays a crucial role in modulating glycolysis (Thevelein, 1994). Apparently, the high rate of glucose phosphorylation of *S. cerevisiae* requires a pathway for consuming sugar phosphates which is provided by trehalose biosynthesis. This hypothesis is in accordance with the lack of trehalose biosynthesis in a yeast with slow glucose phosphorylation and glycolysis such as *C. tropicalis*.

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