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Metabolic flux response to salt-induced stress in the halotolerant yeast *Debaryomyces hansenii*

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The toxic effect of NaCl and KCl on growth of the marine yeast *Debaryomyces hansenii* on glucose or glycerol was studied. Above a threshold value, both salts reduced the specific growth rate, specific glucose and glycerol respiration rates and specific glucose fermentation rate, as well as biomass yields. The exponential inhibition constant, *k*, and minimum toxic concentration, c_{min}, were similar for all physiological parameters assayed. The effect of either salt on the specific activity of several glycolytic enzymes showed a similar inhibition pattern, although at much lower salt concentrations compared with the physiological parameters. In agreement with published results on glycerol phosphate dehydrogenase stimulation by salt, we present evidence that a general glycolytic flux deviation could occur naturally during salt stress, due to the intrinsic sensitivity of the glycolytic enzymes to intracellular ion concentrations.

Keywords: *Debaryomyces hansenii*, halotolerance, metabolic flux, growth parameters, enzyme sensitivity

INTRODUCTION

Debaryomyces hansenii, like other osmotolerant yeasts exposed to osmotic stress, produces and accumulates glycerol as the major compatible solute (Gustafsson & Norkans, 1976; Adler & Gustafsson, 1980). Glycerol is assumed to be synthesized via the reduction of dihydroxyacetone phosphate to glycerol 3-phosphate by the cytoplasmic NAD-dependent glycerol-3-phosphate dehydrogenase, followed by dephosphorylation of glycerol 3-phosphate to glycerol (Adler *et al.*, 1985). Nevertheless, it is not yet completely understood how the yeast senses osmotic stress and modulates glycerol production.

Since *D. hansenii* is one of the most salt-resistant species of yeast, its study could contribute to a better understanding of the phenomenon of osmoregulation. The present work aims to elucidate how the main metabolic fluxes react to salt stress, shifting glucose metabolism towards glycerol production. For this purpose, we studied the influence of salt-induced stress on growth, glucose respiration, and fermentation rates and glycerol respiration rates, as well as on intracellular sodium and potassium concentrations, and *in vitro* specific activity of some glycolytic enzymes, in particular glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), responsible for the prosecution of glycolysis at the metabolic crossroads which lead to glycerol production.

This work is dedicated to the memory of Professor Nicolau van Uden.

Abbreviations: μ_{max}, maximum specific growth rate; μ_s, specific substrate consumption rate for growth; *Y*, yield coefficient.

METHODS

Micro-organism and media. *D. hansenii* type strain IGC 2968 (CBS 767) was maintained on YEPD solid medium. Cells were grown in mineral liquid medium (van Uden, 1967) with 2% (w/v) glucose or glycerol, as the sole carbon and energy source, supplemented with NaCl or KCl at the desired concentrations.

Culture conditions. Growth was performed in a 1:1 ratio of liquid:air, at 25 °C and 170 r.p.m. in an orbital shaker. Growth was monitored by OD_{640} (Bausch & Lomb Spectronic 21) and by dry weight determinations. Samples of 10 ml were filtered through a GF/C 2·5 Whatman membranes, followed by extensive washing and drying overnight at 80 °C. All determinations were done in duplicate or triplicate for calculation of maximum specific growth rates (μ_{max}) during exponential growth. Yield coefficients (*Y*) were based on dry weight determinations and substrate consumption in stationary phase. Glucose and glycerol concentrations in culture media were estimated enzymically using, respectively, Boehringer Mannheim biochemical test combinations nos 124036 and 148270. Enzymic performance in the presence of salt was tested by producing calibration curves for either reaction in the presence of 2 M NaCl or KCl. Specific substrate consumption rates for growth (μ_s) were calculated as the ratio μ_{max}/Y.

Measurement of respiratory and fermentative fluxes. Cells growing on either glucose or glycerol were harvested in mid-exponential phase by centrifugation at 7000 r.p.m., washed twice with and resuspended in ice-cold distilled water. Respiration was monitored with a Clark oxygen electrode linked to a YSI model 5300 oxygen monitor connected to a flat-bed Kipp & Zonen recorder. The electrode was immersed in a water-jacketed 10 ml chamber kept at 25 °C and provided with magnetic stirring. The assay mixture was composed of 3 ml fresh medium supplemented with 2% glucose or glycerol with different salt concentrations, plus 0·5 ml cell suspension corresponding to a final biomass concentration in the assay of $7{\cdot}3 \pm 2{\cdot}0$ mg dry weight ml^{-1} for the assays in glucose medium and $11{\cdot}8 \pm 3{\cdot}0$ mg dry weight ml^{-1} for the assays in glycerol medium. Oxygen consumption was monitored for 1–2 min and the gradient was used to calculate the initial respiration rate for the biomass present in the assay. The specific respiration rate was estimated by dividing the respiration rate by the biomass present in the assay.

Fermentation rates at 25 ° C were determined by manometry (Umbreit *et al.*, 1964) using a Warburg constant volume respirometer (B. Braun model VL 166). Cells were harvested as described above, except that they were resuspended in fresh medium containing 2% glucose to a final concentration of $3{\cdot}0 \pm 0{\cdot}6$ mg dry weight ml^{-1}. Assays were performed immediately after resuspension. Ethanol production by glucose-fermenting cells was monitored enzymically with alcohol dehydrogenase from Boehringer Mannheim.

Preparation of cell-free extracts. To prepare cell-free extracts, 80 ml of a mid-exponential phase culture (OD_{640} ~0·8) was centrifuged at 7000 r.p.m. for 5 min and the cells were kept frozen at −20 °C. Cell extracts were obtained immediately before the enzyme assays by adding 0·5 ml buffer (10 mM triethanolamine, 1 mM DTT, 1 mM EDTA, pH 7·5; Blomberg & Adler, 1989) and ~1 g 0·5 mm glass beads to the frozen pellet followed by at least 15 cycles of vortexing for 1 min with 1 min intervals in ice. Glass beads were separated from extracts by decantating and centrifugation at 14000 r.p.m. for 15 min.

Enzyme assays. Activities of glycolytic enzymes were determined *in vitro* in cell-free extracts, using the methods of Maitra & Lobo (1971). The various buffers were supplemented with 0–750 mM NaCl or KCl. All assays were done at 25 °C and changes in NAD(H) or NADP(H) concentration were monitored by A_{340} using a Perkin-Elmer Lambda 2 UV/Vis spectrophotometer connected to a Epson II recording system. Protein concentrations were determined by the Lowry method as modified by Peterson (1977). Coupling enzymes and substrates were obtained from Sigma. In all cases it was checked that coupling enzymes were not in limiting amounts in the presence of salt.

Determination of intracellular Na^+ and K^+ concentrations. These were determined using a Pu 7000 ICP Spectrometer (Unicam). Cells were collected in mid-exponential phase and centrifuged. The pellet was separated into two parts. One followed a cycle of four washes in ice-cold ultrapure water and the other in water containing the same salt concentration as the growth medium, followed by a cycle of six washes in 4 g $MgCl_2$ l^{-1} at 4 °C. Cells were disrupted by incubation in 17 ml HNO_3 l^{-1} for 24 h. Samples were centrifuged and quickly filtered through 0·45 µm Millipore sterile filters prior to injection. Both $MgCl_2$ and HNO_3 solutions were prepared in ultrapure water and controlled as to ion content. Ultrapure water was obtained from a Permutit filtering device. Results were indexed, through dry weight determinations, to intracellular volume.

Intracellular volume measurement. This was determined as described by Lages & Lucas (1995) in the absence of salt and by incubating in salt solutions of the same molarity as that of the growth medium.

RESULTS

Growth parameters and biomass yields

Growth of *D. hansenii* on glucose or glycerol, in the presence of different NaCl or KCl concentrations, was monitored. In each assay, the maximum specific growth rate, μ_{max}, during exponential growth was determined. In addition, the duration of the lag phase, as well as the final biomass (dry weight) obtained in the stationary phase, and the total substrate consumed were determined. Biomass yields, Y, and specific substrate consumption rates, μ_s, were calculated. Results obtained are summarized in Tables 1 and 2 and illustrated in Fig. 1.

D. hansenii was able to grow in 3.5–4 M of either NaCl or KCl in mineral medium. The toxic effects of both salts were similar. The monitored growth parameters decreased with increasing salt concentrations, except for duration of the lag phase which increased with salt concentration. The pattern of salt inhibition was exponential above a minimum salt concentration, below which no pronounced effect was detectable. This biphasic pattern fitted the general equation found in the literature to describe mathematically the toxic effect of alkanols on yeast growth parameters, metabolic fluxes and membrane processes (van Uden, 1985, 1989). A transposition of that equation gives: $\mu_{max_{[salt]}} = \mu_{max_0} e^{\pm k(c-c_{min})}$, where c_{min} is the salt concentration below which its inhibitory effect was negligible, $\mu_{max_{[salt]}}$ is the maximum specific growth rate in an exponentially growing culture in the presence of a certain salt concentration, and μ_{max_0} is the maximum specific growth rate in the absence of salt. The evaluation of each salt toxic effect was thus performed by comparing values of k and c_{min} (Table 3). Exponential inhibition was not detectable below 1·5 and 2 M NaCl or KCl for, respectively, glucose- or glycerol-grown cells.

Respiration and fermentation fluxes

Cells of *D. hansenii* growing exponentially on glucose respired and fermented glucose simultaneously. Fermentation was monitored by manometry, by the production of CO_2, and confirmed by enzymic detection of ethanol. Specific respiration rates were measured both

Table 1. Growth parameters of *D. hansenii* IGC 2968 in mineral medium with 2% glucose in the presence of various salt concentrations

Numbers of replicate experiments are shown in parentheses. Standard deviations of means from less than three experiments are not presented.

Parameter	NaCl concentration (M)								
	0	0·5	1	1·5	2	2·5	3	3·5	4
μ_{max} (h^{-1})	0·21 ± 0·04 (7)	0·20 ± 0·05 (3)	0·17 ± 0·04 (5)	0·14 ± 0·03 (5)	0·08 ± 0·03 (6)	0·03 ± 0·01 (4)	0·02 (2)	0·01 (1)	0 (2)
Lag phase (h)	16 ± 7 (6)	16 ± 5 (5)	17 ± 5 (6)	24 ± 6 (5)	28 ± 5 (5)	56 ± 12 (4)	100 (2)	ND	—
Y (g g^{-1})	0·17 ± 0·01 (3)	0·17 ± 0·03 (2)	0·14 ± 0·01 (6)	0·13 ± 0·01 (3)	0·1 ± 0·02 (4)	0·11 (1)	0·09 (1)	0·08 (1)	—
μ_s (g h^{-1} g^{-1})	1·24	1·18	1·21	1·08	0·80	0·27	0·22	0·13	—
	KCl concentration (M)								
	0	0·5	1	1·5	2	2·5	3	3·5	4
μ_{max} (h^{-1})	0·21 ± 0·04 (7)	0·20 ± 0·04 (3)	0·19 ± 0·02 (3)	0·16 ± 0·02 (3)	0·09 ± 0·01 (3)	0·04 ± 0·01 (3)	0·03 ± 0·01 (3)	0·01 (1)	0·008 (1)
Lag phase (h)	16 ± 7 (6)	8 ± 2 (3)	12 ± 3 (3)	14 ± 3 (3)	15 ± 4 (3)	18 ± 5 (3)	31 ± 5 (3)	100 (1)	550 (1)
Y (g g^{-1})	0·17 ± 0·01 (3)	0·16 (2)	0·16 (2)	0·17 (2)	0·13 (2)	0·11 (2)	0·09 (2)	0·08 (1)	0·07 (1)
μ_s (g h^{-1} g^{-1})	1·24	1·25	1·20	0·94	0·69	0·36	0·33	0·13	0·11

ND, Not determinable due to the very low biomass yields attained.

Table 2. Growth parameters of *D. hansenii* IGC 2968 in mineral medium with 2% glycerol in the presence of various salt concentrations

Growth was not detected at concentrations of NaCl or KCl greater than 4 M. Numbers of experiments are shown in parentheses. Standard deviations of means from less than three experiments are not presented.

Parameter	NaCl concentration (M)								
	0	0·5	1	1·5	2	2·5	3	3·5	4
μ_{max} (h^{-1})	0·06 ± 0·005 (3)	0·04 (2)	0·08 (2)	0·07 (2)	0·06 (2)	0·03 (2)	0·02 ± 0·01 (3)	0·014 (1)	0·006 (1)
Lag phase (h)	21 (2)	12 (2)	23 (2)	31 (2)	48 (2)	57 (2)	83 (2)	210 (1)	250 (1)
Y (g g^{-1})	0·33 (2)	0·28 (2)	0·29 (2)	0·30 (2)	0·24 (2)	0·34 (2)	0·074 (2)	0·07 (1)	0·04 (1)
μ_s (g h^{-1} g^{-1})	0·18	0·14	0·28	0·23	0·25	0·09	0·27	0·20	0·15
	KCl concentration (M)								
	0	0·5	1	1·5	2	2·5	3	3·5	4
μ_{max} (h^{-1})	0·06 ± 0·005 (3)	0·054 (2)	0·07 (2)	0·06 (2)	0·05 (2)	0·03 ± 0·006 (3)	0·02 ± 0·006 (3)	0·007 (1)	0·005 (1)
Lag phase (h)	21 (2)	15 (2)	18 (2)	24 (2)	30 (2)	32 (2)	50 (2)	60 (1)	65 (1)
Y (g g^{-1})	0·33 (2)	0·23 (2)	0·29 (2)	0·25 (2)	0·21 (2)	0·11 (2)	0·08 (2)	0·032 (1)	0·03 (1)
μ_s (g h^{-1} g^{-1})	0·18	0·23	0·24	0·24	0·24	0·27	0·25	0·22	0·17

by oxygen electrode and by manometric techniques. The latter technique was used to enable the establishment of a correlation between respiration and fermentation rates, expressed in the same units, µl O_2 consumed or CO_2 released min^{-1} (g dry weight)$^{-1}$. Specific glucose respiration and fermentation rates correlated in a proportion of 80–20% in mid-exponential phase glucose-grown cells. The inhibitory effect of increasing concentrations of NaCl or KCl on either specific glucose respiration or fermentation rates was detectable at lower salt concentrations than observed for the growth parameters presented above. The changes observed, exemplified in Figs 2 and 3, respectively, fit the same mathematical reasoning explained above. The values of k and c_{min} for specific respiration and fermentation rates are presented in Table 3. Inhibition constant values did not diverge from those obtained for growth parameters, allowing the calculation of a mean value for all parameters studied in glucose- or glycerol-grown cells (Table 3). Nevertheless, k values calculated for Y were not included in this calculation since they represent an indirect experimental result.

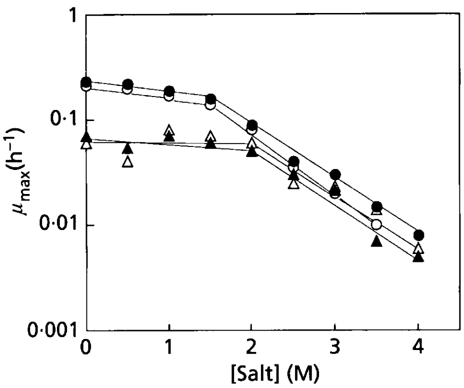

Fig. 1. Variation in maximum specific growth rate (μ_{max}) of *D. hansenii* grown in mineral medium with 2 % glucose (○,●) or glycerol (△,▲), with NaCl (○,△) or KCl (●,▲) concentration in the growth medium.

The toxic effect of either salt on respiratory flux was independent of the adaptation to the presence of either NaCl or KCl during growth on glucose. Cells grown on glucose in the presence of 0·5–3 M NaCl or KCl did not show a different inhibitory pattern on specific respiration rates from that described above, except when washes were performed in conditions isotonic to growth media, in which case a small generalized acquired resistance was observed (not shown). On the other hand, the effect of adaptation to salt during growth on

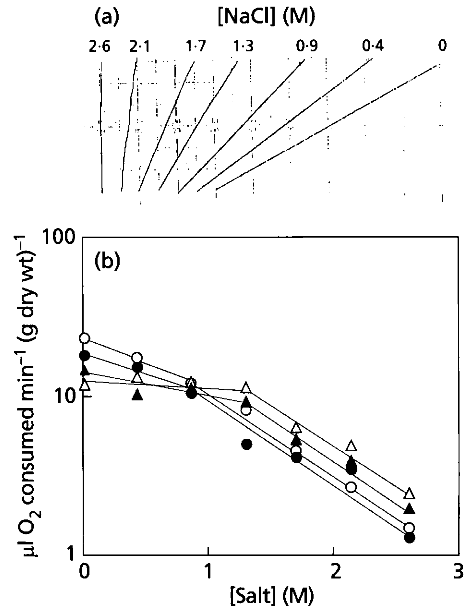

Fig. 2. (a) Example of a typical result obtained with the oxygen electrode measuring glucose respiration rates in the absence and in the presence of various NaCl concentrations in the assay buffer. Respiration rate in the absence of NaCl: 11·3 µl O_2 consumed min^{-1} (g dry weight)$^{-1}$. (b) Variation of glucose (○,●) or glycerol (△,▲) specific respiration rates with assay NaCl (○,△) or KCl (●,▲) concentrations of a culture of *D. hansenii* recovered respectively from mineral medium with 2 % glucose (○,●) or glycerol (△,▲).

Table 3. Inhibition constants (k) and salt minimum inhibitory concentrations (c_{min}) of all parameters under study

Calculations were performed using the mean values presented in Tables 1 and 2. Asterisked values were not included in calculation of means.

Parameter	k (M^{-1})		c_{min} (M)	
	NaCl	KCl	NaCl	KCl
Glucose				
μ_{max}	−1·3	−1·3	1·5	1·5
μ_s	−1·5	−0·6	1·5	1·5
Total biomass attained	−0·6	−1·2	1·5	1·5
Total glucose consumed	−0·6	−0·8	1·5	1·5
Y	−0·2*	−0·3*	0	1·5
Respiration rate	−1·3	−1·1	0·9	0·9
Fermentation rate	−1·1	−0·9	0·2	0·2
Mean ± SD	1·1 ± 0·4	1·0 ± 0·2		
Glycerol				
μ_{max}	−1·1	−1·2	2·0	2·0
Total biomass attained	−1·3	−1·7	2·0	2·0
Total glycerol consumed	−0·5	−0·5	0	0
Y	−1·0*	−1·0*	2·0	2·0
Respiration rate	−1·0	−1·2	1·3	1·3
Mean ± SD	1·0 ± 0·3	1·2 ± 0·4		

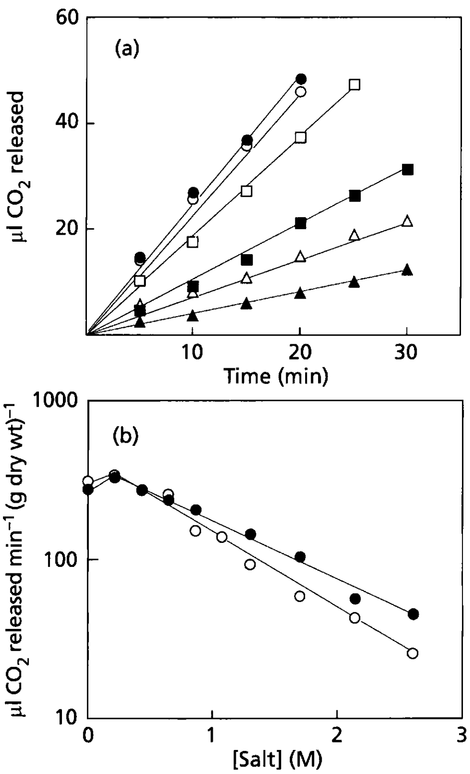

Fig. 3. (a) Example of a typical assay to measure specific fermentation rates in a Warburg respirometer, in the absence and in the presence of various NaCl concentrations (M) (0,○; 0·2,●; 0·4,□; 1·0,■; 1·3,△; 1·7,▲). (b) Variation of glucose-specific fermentation rate with assay NaCl (○) or KCl (●) concentration of a culture of *D. hansenii* IGC 2968 recovered from mineral medium with 2 % glucose.

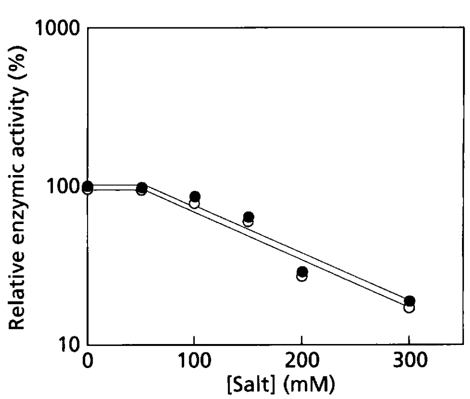

Fig. 4. Relative activity of glyceraldehyde-3-phosphate dehydrogenase with different NaCl (○) or KCl (●) concentrations, where 100 % = 0·4 U (mg total protein) $^{-1}$.

glycerol resulted in an acquired resistance of respiratory flux, translatable into an approximately constant value for glycerol-specific respiration rates in the presence of 0·5–3 M NaCl or KCl during assays for cell suspensions adapted during growth to salt concentrations $\geqslant$ 1·5 M (not shown).

Inhibition of glycolytic enzyme activity

In vitro assays of glyceraldehyde-3-phosphate dehydrogenase activity in the presence of NaCl or KCl were performed. A maximum specific activity of $0{\cdot}4 \pm 0{\cdot}1$ U (mg total protein)$^{-1}$ was measured at a substrate concentration well above saturation. All the values obtained in the presence of salt were indexed to this one in terms of relative activity (Fig. 4). An equally exponential toxic effect of both salts on the enzyme maximum specific activity was observed, but for lower salt concentrations than before. Taking this result into account, the glycolytic enzymes acting in glycolytic flux closer to glyceraldehyde 3-phosphate that did not require more than one coupling enzyme were assayed. The results are presented in Table 4.

Intracellular ion concentration

Considering that enzymes showed a much higher sensitivity to salt than did respiration and fermentation fluxes, intracellular concentrations of Na^+ and K^+ in cells grown in glucose with or without NaCl were determined (Fig. 5). To enable determination of concentrations, intracellular volumes for NaCl- or KCl-grown *D. hansenii* cells had to be determined. The value did not differ significantly from cells grown in glucose without salt: $0{\cdot}471 \pm 0{\cdot}110$ μl (mg dry weight)$^{-1}$ ($n = 40$). Intracellular potassium concentrations were generally higher than sodium concentrations, even in NaCl/-glucose-grown cells. No significant differences were obtained with different washing treatments for each sample (not shown). Cells cultivated at salt concentrations below 1–1·5 M NaCl or KCl showed an increase in intracellular concentrations of Na^+ or K^+ followed by an exponential decrease above these salt concentrations. Nevertheless the equation used above to express salt toxicity does not fit results since no 'plateau' of unchanged values in relation to control was observed.

DISCUSSION

From all the results presented, a general pattern of toxicity for either NaCl or KCl is apparent. Between each salt, no significant difference on general inhibitory effect could be found. This is well described by the kinetics of inhibition by both salts.

The generalized nature of the inhibition pattern on growth is in agreement with published results (Adler *et al.*, 1985; Larsson & Gustafsson, 1987). The values for the inhibition constant k and c_{min} are very similar for all growth parameters studied. At 3 M NaCl or KCl we registered a biomass yield on glucose of about 53 % of that in the absence of salt. In yet another strain, Prista & Madeira-Lopes (1995) registered a 60 % reduction in Y in the presence of approximately 3 M NaCl. In relation to the other parameters under study, the value found for the corresponding inhibition constant was smaller. This is understandable considering that Y is a relative unity. A striking difference was that growth yields on glycerol, obtained in the presence of salt concentrations above

Table 4. Inhibition constants (*k*) of glycolytic enzymes assayed *in vitro* in the presence of NaCl or KCl in the assay buffer

Enzyme	*k* (M^{-1})		Activity remaining at 250 mM (%)	
	NaCl	KCl	NaCl	KCl
Hexokinase	−1·6	−2·3	61·2	60·5
Glucose-6-P isomerase	−3·8	−3·3	58·0	61·3
Aldolase	−6·9	−4·1	15·0	45·5
Triose-P isomerase	−4·8	−4·0	30·6	32·8
Glyceraldehyde-3-P dehydrogenase	−6·8	−6·8	23·2	24·8
Phosphoglycerate kinase	−5·5	−4·5	58·4	61·2

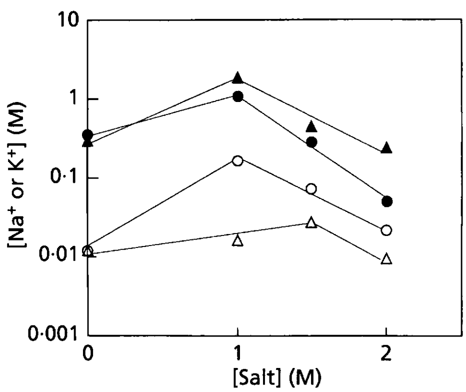

Fig. 5. Intracellular concentrations of Na^+ (○,△) and K^+ (●,▲) in NaCl (○,●) and KCl (△,▲) glucose-grown cells of *D. hansenii*. Results presented are means of 3 independent experiments.

2 M, decreased more steeply than that on glucose media. This is consistent with a double function of glycerol as carbon/energy source and as osmoprotectant.

Assuming that the effects observed on growth could be due to effects on main metabolic pathways, we proceeded to study glucose-specific respiration and fermentation rates and glycerol respiration rate. It should be pointed out that we confirmed the existence of simultaneous glucose respiration and fermentation. Other authors have diverged on this matter, and *D. hansenii* has been classified either as an exclusively (Gancedo & Serrano, 1989) or partially (Norkans, 1968; Barnett *et al.*, 1990) respiratory yeast. The acquired resistance of glycerol respiration observed due to the presence of salt during growth was not observed in the case of glucose respiration. This reinforced the idea, suggested above, that salt stress could be responsible for a metabolic diversion for glycerol production, on glucose media, or for glycerol retention, when this is the only carbon/energy source.

From the literature, the osmotolerance of *D. hansenii* is not only a consequence of its capacity to maintain a low intracellular salt concentration, but also of the fact that the enzymes responsible for glycerol production are not only very tolerant to salt, but are even stimulated by its presence (Norkans, 1968; Nilsson & Adler, 1990). Results obtained with glyceraldehyde-3-phosphate dehydrogenase pointed to an exponential toxic effect of either salt on the maximum specific activity of the enzyme, although for lower salt concentrations than before. Glycerol-3-phosphate dehydrogenase is stimulated by salt concentrations up to 150 mM (Nilsson & Adler, 1990). At the same concentrations of NaCl or KCl, glyceraldehyde-3-phosphate dehydrogenase was strongly inhibited. From results obtained with other representative glycolytic enzymes, we observed a lower sensitivity for those leading to fructose 6-phosphate (F6P), and a higher sensitivity after fructose 1,6-bisphosphate (F1,6BP) in the glycolytic pathway. In *Saccharomyces cerevisiae*, albeit using a different technical approach, Singh & Norton (1991) described the accumulation of phosphorylated glycolytic intermediates after salt shock, when F6P and F1,6BP reached the highest intracellular levels.

The measured intracellular ion concentrations were much higher than those at which enzyme sensitivity was determined. Intracellular sodium and potassium concentrations reached very high maximum values at external salt concentrations around 1 M. Nevertheless, at 1·5 M NaCl, intracellular sodium and potassium concentrations were lower, and similar to those published by Burke & Jennings (1990). The approximately constant ratio between K^+ and Na^+, regardless of salt concentration during growth, is suggestive of the activity of an efficient transport system capable of maintaining K^+/Na^+ homeostasis. Taking into consideration these results, and the difference in relative intrinsic sensitivity of glycolytic enzymes in relation to the stimulation of glycerol-3-phosphate dehydrogenase observed by Nilsson & Adler (1990), it can still be reasoned that there is a salt-triggered shift in glycolytic flux in the presence of salt stress. Nevertheless, caution should be exercised in the interpretation due to the danger in extrapolating results from *in vitro* enzyme sensitivity assays to *in vivo* conditions, especially due to the presence of Cl^- ions, whose cytoplasmic concentration is unknown. In addition, although the internal cation concentration appears to be much above the *in vitro* c_{min}, enzyme activity *in vivo* may be protected by cytoplasmic compartmentation, thereby decreasing the toxic effects of salt.

Sensitivity of glucose or glycerol respiration and fermentation to inhibition by salt translates into an inhibition of growth as a whole, but it is not, at the same time, matched quantitatively by the inhibition of the enzymes responsible for glycolytic flux, which were more salt-sensitive.

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