Appl Microbiol Biotechnol (1997) 47: 447–451

ORIGINAL PAPER

I. Krallish · H. Jeppsson · A. Rapoport · B. Hahn-Hägerdal

Effect of xylitol and trehalose on dry resistance of yeasts

Received: 9 July 1996 / Received revision: 29 October 1996 / Accepted: 2 November 1996

Abstract The effects of dehydration/rehydration on two strains of *Saccharomyces cerevisiae*: S600, a metabolically engineered xylose-utilising strain, and H158, the non-xylose-utilising host strain; and on the naturally xylose-utilising yeast *Pachysolen tannophilus* CBS 4044, were compared after glucose and xylose utilisation respectively. The yeast strains differed in their ability to excrete and accumulate intracellular xylitol. A high intracellular xylitol content before and after dehydration coincided with a higher viability after a dehydration/rehydration cycle. The intracellular trehalose content increased during dehydration in all three yeast strains, but this did not correspond to enhanced cell viability after dehydration/rehydration. The results are discussed in relation to the ability of xylitol and trehalose to structure water.

Introduction

Yeasts respond in different ways to dehydration: chromatin and mitochondrial DNA condensation has been observed, and the solubility of proteins, the activities of enzymes and the adenine nucleotide content are altered (Krallish et al. 1986, 1989; Beker and Rapoport 1987). An increase in unsaturated fatty acids (Zikmanis et al. 1982) and an accumulation of trehalose (Beker and Rapoport 1987; Wiemken 1990) have also been observed. The trehalose content correlated with the resistance of the yeast cells to extreme temperatures, dehydration/rehydration and freeze/thawing cycles (Hottiger et al. 1987; Wiemken 1990). Under conditions of reduced water activity, polyols accumulate in yeasts cells (Van Eck et al. 1989, 1993) and this has been suggested to increase their dry resistance (Rapoport and Beker 1984; Rapoport et al. 1988).

In the present study, the viability of the yeasts *Saccharomyces cerevisiae* S600, *S. cerevisiae* H158 and *Pachysolen tannophilus* CBS 4044 after dehydration/rehydration was compared in relation to the intracellular accumulation of xylitol and trehalose. The yeasts differ in xylitol formation, so that *S. cerevisiae* S600, a recombinant xylose-utilising strain derived from *S. cerevisiae* H158, and *P. tannophilus*, a natural xylose-utilising strain, form xylitol during xylose utilisation (Kötter et al. 1990; Kötter and Ciriacy 1993; Tantirungkij et al. 1993; Meinander et al. 1994; Walfridsson et al. 1995; Neirinck et al. 1985; Slininger et al. 1987), whereas none of the three yeasts form xylitol during glucose utilisation.

Materials and methods

Yeast strains and maintenance conditions

Saccharomyces cerevisiae H158, which originated from Gregg Payne (University of California, Berkely, Calif. USA) as *S. cerevisiae* GPY55-15Bα (leu2–3 leu2–112 ura3–52 trp1–289 his4–519 prb1 cir$^+$) was used as a host for transformation. The strain was maintained at 4 °C on agar plates containing (l^{-1}): 10 g yeast extract, 20 g Bacto-peptone, 18 g Bacto-agar and 20 g glucose. *Pachysolen tannophilus* CBS 4044 was maintained at 4 °C on the same medium but with glucose replaced by xylose. *S. cerevisiae* S600 (Walfridsson, personal communication), a strain derived from *S. cerevisiae* H158, contained a multicopy yeast expression vector with genes for xylose reductase (*XYL1*) (Hallborn et al. 1991) and xylitol dehydrogenase (*XYL2*) (Kötter et al. 1990). *S. cerevisiae* S600 was maintained on synthetic complete medium (Sherman et al. 1983) [0.67% Difco yeast nitrogen base without amino acids supplemented with adenine (13.5 mg l^{-1}), arginine (348 mg l^{-1}), aspartic acid (266 mg l^{-1}), histidine (58 mg l^{-1}), inositol (36 mg l^{-1}), isoleucine (525 mg l^{-1}), leucine (262 mg l^{-1}), lysine (91 mg l^{-1}), methionine (149 mg l^{-1}), phenylalanine (83 mg l^{-1}), serine

I. Krallish · A. Rapoport
Institute of Microbiology and Biotechnology,
Latvian University, LV-1586, Riga, Latvia

H. Jeppsson · B. Hahn-Hägerdal (✉)
Department of Applied Microbiology,
Centre for Chemistry and Chemical Engineering,
Lund Institute of Technology/Lund University,
P. O. Box 124, S-221 00 Lund, Sweden
Fax: + 46 46 2224203
e-mail: Barbel.Hahn-Hagerdal@tmb.lth.se

(105 mg l^{-1}), threonine (119 mg l^{-1}), tryptophan (82 mg l^{-1}), tyrosine (18 mg l^{-1}), uracil (22 mg l^{-1}) and valine (117 mg l^{-1}) with 20 g glucose l^{-1} as the carbon source and 18 g agar l^{-1}]. For selection of *S.cerevisiae* S600 leucine was omitted from the medium.

Cultivation conditions

S. cerevisiae H158 and *S. cerevisiae* S600 were grown in synthetic complete medium without *leucine* containing, in addition (l^{-1}), 0.8 g K_2HPO_4, 12.8 g KH_2PO_4, 20 g glucose and 20 g glucose in combination with 10 g xylose, respectively. The pH of the medium was adjusted to 5.5. The cultivation medium for *P. tannophilus* CBS 4044 consisted of (l^{-1}) 10 g yeast extract, 20 g Bacto-peptone and 20 g xylose or glucose. The pH of the medium was adjusted to 6.5. The sugar solutions were autoclaved separately and the amino acid solution was filtered under sterile conditions. Yeast cells from an agar plate were transferred to 250 ml appropriate medium and the yeast cells were incubated for 24 h at 30 °C, in a 500-ml flask at 150 rpm in a water rotary shaker. A 10-ml sample of inoculum was transferred to 1-l flasks containing 500 ml appropriate medium and incubated under the same conditions as above until the yeast cells had reached the stationary phase of growth.

Dehydration of yeast cells

The cells were harvested by centrifugation (4000 g, 10 min, 4°C; Beckman J2-21), washed twice with distilled water and filtered using a membrane filter (pore size 0.45 μm, Schleicher & Schüll, Dassel, Germany). The thin layer of biomass collected on the membrane filter was air-dried at 30 °C until 8%–12% of the residual moisture remained, usually 14–16 h.

Rehydration of yeast cells and viability measurement

Rehydration of dried yeast cells was performed at room temperature by suspending 30–50 mg dry weight of cells in 5 ml sterile distilled water for 10 min. The number of living cells was determined using a fluorescence microscopy method (Rapoport and Meysel 1985). The yeast cell suspension was mixed with an equal volume of fluorochrome primuline solution (1 mg ml^{-1}), stained for 3–5 min and then examined with fluorescence microscopy. In living cells only the cell wall is fluorescent while dead cells have a bright yellow-green fluorescence.

Preparation of cell extract for analysis of intracellular polyol content

Yeast biomass was suspended in 0.1 M potassium phosphate buffer (pH 7.5) and the cells were immediately broken using glass beads during intensive shaking for 20 min at 4 °C. The cell debris was removed by centrifugation (15 000 *g*, 20 min, 4 °C, Beckman J2-21). The cell-free extracts were collected and stored at −80 °C until analysed.

Analytical methods

Growth was monitored by absorbance measurements at 620 nm. For the dry weight of the biomass (g l^{-1}) 5 ml yeast suspension was filtered through a membrane filter (pore size 0.45 μm, Schleicher & Schüll, Dassel, Germany), washed with 10 ml distilled water and dried in a microwave oven for 10 min at full power. For the rehydrated biomass, the dry weight and the residual moisture content were determined after drying in a conventional oven at 105 °C until constant weight was observed.

Glucose, xylose, xylitol, glycerol, acetic acid and ethanol were analysed with column liquid chromatography (CLC). A Varian CLC system equipped with one or two Aminex HPX 87-H columns (each 300 mm × 7.8 mm) coupled in series (BioRad Laboratories, Richmond, Calif.) was used at 45 °C with a Tecator 590 differential refractive index detector (Höganäs, Sweden). The mobile phase was 5 mM H_2SO_4 administered at a flow rate of 0.6 ml min^{-1} and 0.5 ml min^{-1} respectively. The two-column system was used for the separation of sugar alcohols (Jeppsson et al. 1995)

The intracellular xylitol content in the cell-free extract was determined enzymatically using sorbitol dehydrogenase, as described by Bässler (1974) and expressed as μg mg^{-1} dry weight.

Trehalose was assayed by the antron method (Stewart 1975) in acid extracts prepared by mixing a biomass sample (50 mg dry weight) with 5 ml 0.5 M trichloroacetic acid and incubating at room temperature for 30 min with occasional shaking (Trevelyan and Harrison 1956). The samples were centrifuged (2000 *g*, 10 min), the extraction was repeated twice and the supernatants were combined. The extracts were collected and stored at −80 °C until analysed. The intracellular trehalose content was expressed as μg mg^{-1} dry weight.

Reproducibility of the results

All experiments were repeated at least three times. Representative results are reported.

Results

The concentration of extracellular metabolites, and the intracellular glycerol and trehalose content

Substrate consumption and product formation during fermentation of glucose and xylose, respectively, with *S. cerevisiae* H158, *S. cerevisiae* S600 and *P. tannophilus* CBS 4044 were first determined. *S. cerevisiae* H158 can only ferment glucose since it lacks the xylose-metabolising enzymes xylose reductase and xylitol dehydrogenase (Hallborn et al. 1991). Xylose fermentation with the recombinant xylose-utilising *S. cerevisiae* S600 was carried out in the presence of glucose since this had earlier been observed to be more efficient (Walfridsson et al. 1995). A representative fermentation of a glucose-containing medium with *S.cerevisiae* H158 is shown in Fig. 1. During glucose consumption, cell mass and

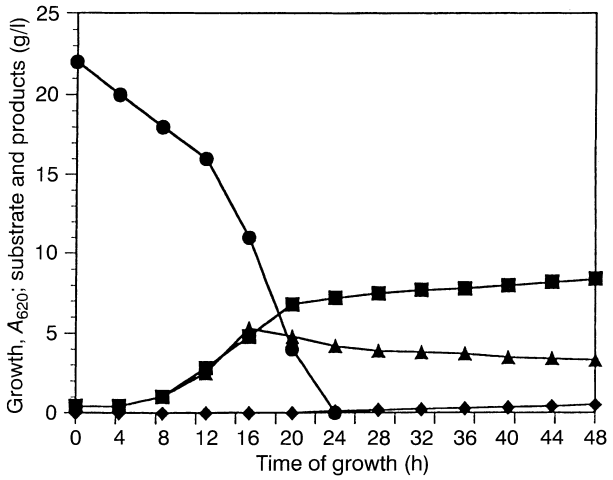

Fig. 1 Fermentation of glucose by *Saccharomyces cerevisiae* H 158. ■ Biomass as absorbance at 620 nm, ● glucose, ▲ ethanol, ◆ glycerol

ethanol were formed and, at the very end of the fermentation, small amounts of glycerol were observed in the medium. The pattern was similar for the three investigated strains fermenting the two carbon sources with some difference in the rate of substrate consumption and formation of products. Table 1 summarises the concentrations of extracellular metabolites at the end of the fermentations. When glucose was fermented by *S. cerevisiae* H158 and *P. tannophilus* CBS 4044, glycerol, ethanol and acetic acid were formed, whereas when *S. cerevisiae* S600 and *P. tannophilus* CBS 4044 fermented a xylose-containing medium, xylitol was formed in addition to these products. *S. cerevisiae* S600 formed about three times more xylitol than glycerol. With *P. tannophilus* CBS 4044 no glycerol formation was detected.

At the end of the fermentation the cells were harvested and the intracellular xylitol and trehalose contents were determined before dehydration (Fig. 2). In cells of *S. cerevisiae* S600 derived from a glucose/xylose mixture the intracellular xylitol content was 1.4 times higher than cells of *S. cerevisiae* H158 derived from glucose (75 μg mg^{-1} dry weight and 53 μg mg^{-1} dry weight, respectively). Similarly, a higher intracellular xylitol content was found for xylose-derived cells of *P. tannophilus* CBS 4044 compared with glucose-derived cells (64 μg mg^{-1} dry weight and 40 μg mg^{-1} dry weight, respectively). The intracellular trehalose content was lower in xylose-derived cells of *S. cerevisiae* S600 compared with glucose-derived cells of *S. cerevisiae* H158 (50 μg mg^{-1} dry weight and 80 μg mg^{-1} dry weight, respectively). The same was found when the trehalose contents of xylose-derived and glucose-derived cells of *P. tannophilus* CBS 4044 were compared (39 μg mg^{-1} dry weight and 127 μg mg^{-1} dry weight, respectively). The intracellular trehalose content for *P. tannophilus* CBS 4044 cells represented both the lowest and the highest values obtained.

The intracellular content of xylitol and trehalose after dehydration

After dehydration the intracellular xylitol and trehalose contents in the yeast cells were determined (Fig. 2). In glucose-derived *S. cerevisiae* H158 the intracellular xylitol content increased marginally from 53 μg mg^{-1} dry weight to 67 μg mg^{-1} dry weight, whereas the trehalose content by almost a factor of two from 80 μg mg^{-1} dry weight to 151 μg mg^{-1} dry weight after dehydration. In contrast, both the xylitol and the trehalose contents in xylose-derived cells of *S. cerevisiae* S600 increased approximately by a factor of two from 75 μg mg^{-1} dry weight to 154 μg mg^{-1} dry weight and from 50 μ g mg^{-1} dry weight to 92 μg mg^{-1} dry weight, respectively. However, in these cells the trehalose content was still only 2/3 of the content in the glucose-derived cells of *S. cerevisiae* H158 after dehydration. Similarly, in both glucose- and xylose-derived cells of *P. tannophilus* CBS 4044, the intracellular xylitol content increased by a factor of two (from 40 μg mg^{-1} dry weight to 88 μg mg^{-1} dry weight and from 64 μg mg^{-1} dry weight to 110 μg mg^{-1} dry weight, respectively). On the other hand, dehydration only marginally changed the intracellular content of trehalose in these cells.

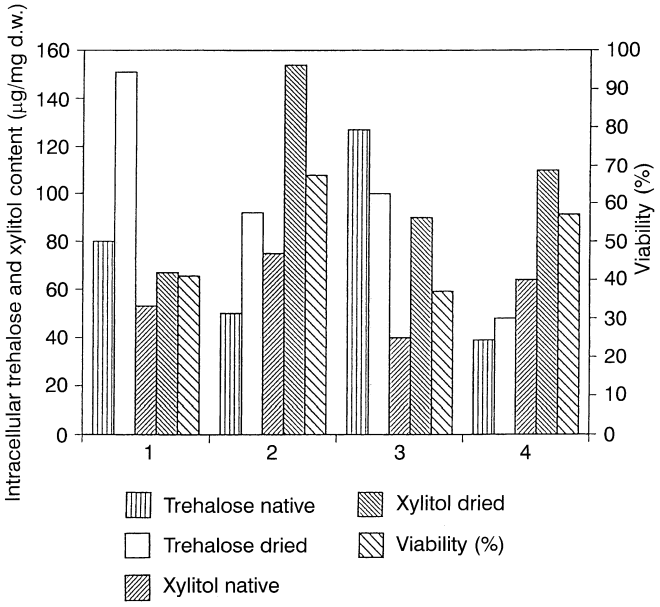

Fig. 2 Intracellular trehalose and xylitol content in yeast cells before and after dehydration and viability after a dehydration/rehydration cycle. *1 S. cerevisiae* H 158 grown on glucose; *2 S. cerevisiae* S 600 grown on glucose+ xylose; *3 P. tannophilus* CBS 4044 grown on gluclose; *4 P. tannophilus* CBS 4044 grown on xylose

Viability of yeast cells after dehydration/rehydration

The viability of the yeast cells was determined immediately after the dried cells had been resuspended in dis-

Table 1 Extracellular substrate and product concentrations after completed fermentation. *ND* not detectable, *NA* not applicable

Yeast strain	Carbon source	Extracellular metabolites (g/l)					
		Glucose	Xylose	Xylitol	Glycerol	Ethanol	Acetic acid
Saccharomyces cerevisiae H158	Glucose	ND	NA	ND	0.5	3.3	1.2
Saccharomyces cerevisiae S600	Glucose+xylose	ND	3.4	3.8	1.2	4.3	0.3
Pachysolen tannophilus CBS 4044	Glucose	0.52	NA	ND	0.1	4.5	0.1
	Xylose	ND	2.4	0.5	ND	0.3	0.4

tilled water at room temperature. Under these conditions, self-protective intracellular reactions do not occur (Beker and Rapoport 1987). The viability of the xylose-derived cells of *S. cerevisiae* S600 and of *P. tannophilus* CBS 4044 was 68% and 57% respectively, compared with 41% and 37% for glucose-derived cells of *S. cerevisiae* H158 and of *P. tannophilus* CBS 4044 respectively. The viability of the differently derived yeast cells correlated positively with the intracellular xylitol content both before and after dehydration (Fig. 2). No correlation was found between the viability and the intracellular trehalose content, either before or after dehydration, nor was there any correlation between the viability and the sum of the xylitol and trehalose contents before or after dehydration.

Discussion

The present study showed that the intracellular accumulation of xylitol can enhance the dry resistance of yeast cells as determined by the viability after a dehydration/rehydration cycle. On the other hand, the intracellular accumulation of trehalose had no such effect under the experimental conditions chosen in the present investigation.

Compatible solutes, such as glycerol, sorbitol, glycol, arabitol, betaine and xylitol, accumulate intracellularly to maintain the intracellular osmotic pressure in salt-induced stress (Adler et al.1985; Albertyn et al. 1994; Blomberg and Adler 1992; Kets et al. 1996). These solutes can also increase the thermostability of enzymes (Ye and Combes 1991; Erarslan 1995), which has been suggested to be due to direct interactions between enzymes and polyols (Erarslan 1995), to indirect action on the water structure (Hägerdal and Martens 1976; Hägerdal 1978; Kelkar and Deshpande 1991) and to a combination of both (Hahn-Hägerdal 1986; Ye and Combes 1991). The protective function of trehalose has been interpreted similarly as the function of compatible solutes: through indirect interactions with water (Crowe et al. 1984a; b, 1985, 1987; Rudolph et al. 1986; Carpenter and Crowe 1989; Wiemken 1990) and through direct interactions with macromolecular structures such as membranes (Crowe et al.1986; Leslie et al. 1994).

When xylitol accumulates intracellularly it may preserve cellular functions during dehydration/rehydration by interacting with the macromolecules, but probably to a greater extent, by structuring intracellular water so that it is not available for the denaturation of macromolecular structures such as enzymes and membranes (Hägerdal and Martens 1976; Hägerdal 1978). This interpretation is consistent with the present observation that there was no correlation between the accumulation of intracellular trehalose and viability. Trehalose, having more than twice the molecular mass (342 Da) of xylitol (150 Da), has to be present at a higher concentration than xylitol to structure water to the same extent (Norrish 1966; Chirife and Ferro Fontan 1980; Andersson and Hahn-Hägerdal 1987). In all cases in the present study, the intracellular content of trehalose was in the same range as the content of xylitol. High levels of trehalose (up to 20%–35% dry weight) stabilise intracellular membranes in anhydrobiotic organisms, which are capable of surviving complete dehydration (Crowe et al. 1984a). However, intracellular levels of trehalose around 10% of the dry weight – the range observed in the present investigation – were not sufficient to maintain the viability of yeast cells freeze/thawing cycles (Gélinas et al. 1989).

When the yeast cells are air-dried at 30 °C, slow dehydration at physiological temperatures permits xylitol and trehalose synthesis during the initial dehydration phase, when sufficient free water is available for metabolic processes to proceed. Xylitol and trehalose can be synthesised from metabolites of the glycolytic and the pentose phosphate pathways and from storage carbohydrates such as glycogen, so that the intracellular glycogen content decreases during dehydration of *S. cerevisiae* (Beker and Rapoport 1987). The present study indicates that, when a xylose-utilising pathway is present, the yeasts *S. cerevisiae* and *P. tannophilus* preferentially synthesise xylitol when exposed to dehydration.

Acknowledgements This study was supported by NUTEK (the Swedish National Board for Industrial and Technical Development), NFR (the Swedish Natural Science Research Council). Dr. Irina Krallish acknowledges the support of the Swedish Institute.

References

Adler L, Blomberg A, Nilsson A (1985) Glycerol metabolism and osmoregulation in the salt-tolerant yeast *Debaryomyces hansenii.* J Bacteriol 162: 300–306

Albertyn J, Hohmann S, Prior BA (1994) Characterization of the osmotic-stress response in *Saccharomyces cerevisiae*: osmotic stress and glucose repression regulate glycerol-3-phosphate dehydrogenase independently. Curr Genet 25: 12–18

Andersson E, Hahn-Hägerdal B (1987) Enzyme action in polymer and salt solutions. I. Stability of penicillin acylase in poly(ethylene glycol) and potassium phosphate solutions in relation to water activity. Biochim Biophys Acta 912: 317–324

Bässler K-H (1974) Xylitol. In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol 3. Academic Press, New York, San Francisco, London, pp 1381–1384

Beker M, Rapoport A (1987) Conservation of yeasts by dehydration. Adv Biochem Eng Biotechnol 35: 127–171

Blomberg A, Adler L (1992) Physiology of osmotolerance in fungi. Adv Microbiol Physiol 33: 145-212

Carpenter JF, Crowe JH (1989) An infrared spectroscopic study of the interaction of carbohydrates with dried proteins. Biochemistry 28: 3916–3922

Chirife J, Fontan CF (1980) A study of the water activity lowering behavior of polyethylene glycols in the intermediate moisture range. J Food Sci 45: 1717–1719

Crowe JH, Crowe LM, Chapman D (1984a) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. Science 223: 701–703

Crowe JH, Whittam MA, Chapman D, Crowe LM (1984b) Interactions of phospholipid monolayers with carbohydrates. Biochim Biophys Acta 769: 151–159

Crowe LM, Crowe JH, Chapman D (1985) Interaction of carbohydrates with dry dipalmitoylphosphatidylcholine. Arch Biochim Biophys 236: 289–296

Crowe LM, Womersley C, Crowe JH, Reid D, Appel L, Rudolph A (1986) Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim Biophys Acta 861: 131–140

Crowe JH, Crowe LM, Carpenter JF, Aurell Wistrom C (1987) Stabilization of dry phospholipid bilayers and proteins by sugars. Biochem J 242 : 1–10

Erarslan A (1995) The effect of polyol compounds on the thermostability of penicillin G acylase from a mutant of *Escherichia coli* ATCC 11105. Process Biochem 30: 133–139

Gélinas P, Fiset G, LeDuy A, Goulet J (1989) Effect of growth conditions and trehalose content on cryotolerance of bakers' yeast in frozen doughs. Appl Environ Microbiol 55: 2453–2459

Hägerdal B (1978) Stability of myoglobin to acetone treatment characterised by differential scanning calorimetry and resolubility in water. J Food Sci 43: 22–26

Hägerdal B, Martens H (1976) Influence of water content on the stability of myoglobin to heat treatment. J Food Sci 41: 933–937

Hahn-Hägerdal B (1986) Water actvity – a possible external regulator in biotechnical processes. Enzyme Microb Technol 8: 322–327

Hallborn J, Walfridsson M, Airaksinen U, Ojamo H, Hahn-Hägerdal B, Penttilä M, Keränen S (1991) Xylitol production by recombinant *Saccharomyces cerevisiae.* Biotechnology 9: 1090–1095

Hottiger T, Boller T, Wiemken A (1987) Rapid changes of heat and dessication tolerance correlated with changes of trehalose content in *Saccharomyces cerevisiae* cells subjected to temperature shifts. FEBS Lett 220: 113–115

Jeppsson H, Alexander NJ, Hahn-Hägerdal B (1995) Existence of cyanide-insensitive respiration in the yeast *Pichia stipitis* and its possible influence on product formation during xylose utilisation. Appl Environ Microbiol 61: 2596–2600

Kelkar HS, Deshpande MV (1991) Effect of polyols on the thermostability of pullulan-hydrolysing activity from *Sclerotium rolfsii* . Biotechnol Lett 13: 901–906

Kets EPW, Teunissen PJM, Bont JAM de (1996) Effect of compatible solutes on survival of lactic acid bacteria subjected to drying. Appl Environ Microbiol 62: 259–261

Kötter P, Amore R, Hollenberg CP, Ciriacy M (1990) Isolation and characterization of the *Pichia stipitis* xylitol dehydrogenase gene *XYL2* and construction of a xylose-utilizing *Saccharomyces cerevisiae* transformant. Curr Gen 18: 493–500

Kötter P, Ciriacy M (1993) Xylose fermentation by *Saccharomyces cerevisiae.* Appl Microbiol Biotechnol 38: 776–783

Krallish IL, Damberga BE, Beker MJ (1986) The effect of yeasts dehydration on the activity of a number of enzymes of cell energetic metabolism. Appl Microbiol Biotechnol 23: 482–486

Krallish IL, Damberga BE, Beker MJ (1989) State of adenosine phosphates during dehydration of yeast. Appl Microbiol Biotechnol 31: 194–199

Leslie SB, Teter SA, Crowe LM, Crowe JH (1994) Trehalose lowers membrane phase transitions in dry yeast cells. Biochim Biophys Acta 1192: 7–13

Meinander N, Hallborn J, Keränen S, Ojamo H, Penttilä M, Walfridsson M, Hahn-Hägerdal B (1994) In: Alberghina L, Frontali L, Sensi P (eds) Proceedings of the 6th European Congress on Biotechnology Elsevier Science Amsterdam Oxford New York, pp 1143–1146

Neirinck LG, Tsai CS, Labelle JL, Schneider H (1985) Xylitol as a carbon source for growth and ethanol production by *Pachysolen tannophilus*. Can J Microbiol 31: 451–455

Norrish RS (1966) An equation for the activity coefficients and equilibrium relative humidities of water in confectionery syrups. J Food Technol 1: 25–39

Rapoport AI, Beker MJ (1984) Effect of sucrose and lactose on resistance of the yeast *Saccharomyces cerevisiae* to dehydration. Microbiology (NY) 52: 556–559

Rapoport AI, Meysel MN (1985) Survival rates of yeast organisms after dehydration as determined by fluorescence microscopy. Microbiology (NY) 54: 53–55

Rapoport AI, Puzyrevskaya OM, Saubenova MG (1988) Polyols and resistance of yeasts to dehydration. Microbiology (NY) 57: 269–271

Rudolph AS, Crowe JH, Crowe LM (1986) Effects of three stabilizing agents – proline, betaine, and trehalose on membrane phospholipids. Arch Biochim Biophys 245: 134–143

Sherman F, Fink G, Hicks JB (1983) Methods in yeast genetics. A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York

Slininger PJ, Bolen PL, Kurtzman CP (1987) *Pachysolen tannophilus*: properties and process considerations for ethanol production from D-xylose. Enzyme Microbiol Technol 9: 5–15

Stewart PR (1975) Analytical methods for yeasts. Methods Cell Biol. 12: 111–147

Tantirungkij M, Nakashima N, Seki T, Yoshida T (1993) Construction of xylose – assimilating *Saccharomyces cerevisiae*. J Ferment Bioeng 75: 83–88

Trevelyan WE, Harrison IS (1956) Studies on yeast metabolism. 7. Yeast carbohydrate fractions. Separation from nucleic acid, analysis and behaviour during anaerobic fermentation. Biochem J 63: 23–33

Van Eck JH, Prior BA, Brandt EV (1989) Accumulation of polyhydroxy alcohols by *Hansenula anomala* in response to water stress. J Gen Microbiol 135: 3505–3513

Van Eck JH, Prior BA, Brandt EV (1993) The water relations of growth and polyhydroxy alcohol production by ascomycetous yeasts. J Gen Microbiol 139: 1047–1054

Walfridsson M, Hallborn J, Penttilä M, Keränen S, Hahn-Hägerdal B (1995) Xylose-metabolizing *Saccharomyces cerevisiae* strains overexpressing the *TKL1* and *TAL1* genes encoding the pentose phosphate pathway enzymes transketolase and transaldolase. Appl Environ Microbiol 61: 4184–4190

Wiemken A (1990) Trehalose in yeast, stress protectant rather than reserve carbohydrate. Antonie van Leeuwenhoek 58: 209–217

Ye W-N, Combes D (1991) Effect of glucose oxidase concentration on its thermostability in the presence of polyols. Biotechnol Lett 13: 421–426

Zikmanis PB, Auzina LP, Auzane SI, Beker MJ (1982) Relationship between the fatty acid composition of lipids and the viability of dried yeast *Saccharomyces cerevisiae*. Eur J Appl Microbiol Biotechnol 15: 100–103