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Accumulation of trehalose in *Saccharomyces cerevisiae* growing on maltose is dependent on the *TPS1* gene encoding the UDPglucose-linked trehalose synthase

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Abstract When yeast strains were cultivated on maltose, the synthesis of trehalose already started in the exponential phase of growth, well before exhaustion of the sugar from the medium. This active pattern of trehalose accumulation was also observed in a maltose constitutive mutant strain growing on glucose. However, this accumulation was completely prevented by deletion of the *TPS1* gene coding for the catalytic subunit of the UDPglucose-linked trehalose-6-phosphate synthase, indicating that no alternative pathway for trehalose synthesis exists in yeast. The active pattern of trehalose accumulation seems to be consistent with the finding that trehalose-6-phosphate synthase is more active in strains growing on maltose than on glucose.

Key words: Trehalose; Maltose; Trehalose synthase; Trehalase; *Saccharomyces cerevisiae*

1. Introduction

Trehalose is a non-reducing disaccharide widely found in nature. In the yeast *Saccharomyces cerevisiae* its accumulation occurs under conditions of restricted growth [1], or upon various stress conditions [2]. In 1958, Cabib and Leloir [3] identified for the first time two enzymatic activities leading to the formation of trehalose from UDP-glucose (UDP-Glc) and glucose-6-phosphate (Glc6P). The two enzymes catalyzing these reactions, trehalose-6-phosphate synthase (T6Psynthase) and trehalose-6-phosphate phosphatase (T6Pphosphatase) were thereafter shown to form a multifunctional protein complex [4,5]. Upon purification, this protein complex was resolved into three polypeptides of 56, 102 and 123 kDa [5,6] encoded respectively by the genes *TPS1* (=*FDP1*, *CIF1*, *GGS1*, *BYP1*, *TSS1*) [6–8], *TPS2* [9] and *TPS3* [8]. It has been shown that *TPS1* and *TPS2* code for the catalytic subunit of T6Psynthase and T6P phosphatase, respectively, while *TPS3* encodes a subunit which apparently confers the integrity to the protein complex [9]. Deletion of the *TPS1* gene causes an inability to grow on glucose and on other readily fermentable carbon source, but not on maltose and galactose, as well as a number of other biochemical features including a rapid depletion of ATP, an hyperaccumulation of hexose phosphates and a lack of cAMP increase upon glucose addition [10]. These pleiotropic effects suggested a regulatory role of the protein encoded by *TPS1* in the glycolytic flux. An apparent coherent answer to this question was given by Blasquez et al. [11] who reported a strong inhibitory effect of trehalose-6-phosphate (T6P), the product of the reaction catalyzed by T6P synthase, on the major glucose-phosphorylating activity in yeast, namely hexokinase II. In confirmation of the essential feed-back inhibition of hexokinase by T6P as a control of glycolytic flux, extragenic suppressors of *tps1* mutants were found to contain reduced activity of hexokinase II [12].

Besides this trehalose pathway (referred to as the classical pathway), Panek and coworkers [13] suggested the existence of an alternative trehalose synthesis pathway which is specifically linked to maltose utilization. Their arguments in favor of this alternative pathway were the following: (i) strains with *MAL* genes in either the inducible or constitutive state accumulate trehalose during exponential growth on maltose, and this accumulation still occurred even after introduction of a *sst1* mutation confering no detectable in vitro T6P synthase activity [14,15]. It should be stressed that the *sst1* mutants are partial revertants of *fdp1* (allelic to *tps1*) mutants able to grow on glucose but not on fructose [16]; (ii) strains harbouring one of the five *MAL* genes in the constitutive state exhibited a very active trehalose accumulation (called TAC^+ phenotype) during late logarithmic phase of growth on glucose, and this phenotype was apparently not affected upon introduction in these strains of the *sst1* mutation [16]. This TAC^+ phenotype was, however, absent in maltose-inducible (*MAL*) and nonfermenting maltose (mal^-) strains growing on glucose. Taking together, these results were interpreted by invokating the existence of a modulator encoded by the *MAL* locus which induces a second enzyme system for trehalose synthesis [13,16]. Later on, they identified an ADP-glucose dependent trehalose-6-phosphate synthase in both wild type and *fdp1* mutants (allelic to *tps1*) strains [17], and suggested that the synthesis of trehalose in this mutant was due to this latter activity. However, they did not show whether a relationship existed between the activity of this new enzyme and the expression of *MAL* gene.

This present work was undertaken in order to further elucidate the relationship between maltose utilization and trehalose metabolism in *MAL* inducible and *MAL* constitutive strains bearing a wild type and a disrupted *TPS1* gene and to clarify the TAC^+ phenotype associated with the *MAL* constitutive trait.

2. Materials and methods

2.1. Reagents

Restrictions enzymes were from Gibco BRL (Life Technology, Eragny, France). Auxiliary enzymes and biochemicals were purchased from Boehringer Mannheim (Meylan, France) or Sigma (Sigma Chimie, Saint Quentin, France). Chemicals were from Merck

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(Darmstadt, Germany). Random priming kit for either non-radioactive labelling (ECL kit) or radioactive kit and [α-^{32}P]dATP for Southern blot analysis were purchased from Amersham (Les Ulis, France).

2.2. Strains, plasmids and culture conditions

The *S. cerevisiae* strains KT1113 (and its congenic KT1112 with the opposite mating type) *Matα ura3-52 leu2,3-112 his3-Δ200 mal GAL TPS1*, W303-1B, *Matα ade2-1 his3-11,15 ura3-1 leu2-3,112 trp1-1 GAL mal TPS1* [18]; 1403-7A, *Mata ura3-52 trp1 suc gal MAL4-C TPS1* (Berkeley Stock Center) and DFY1, *Mata lys1 MAL SUC MAL GAL TPS1* (D. Fraenkel) were used as 'wild type'. To construct strain TPY8 (*Matα ura3-52 leu2-3,112 his3-Δ200 lys1GAL MAL TPS1*), strain KT1113 and DFY1 were crossed and one of the spore isolated from tetrads dissected with the indicated genotype was selected for further study. Strain TPY9 (*Matα ura3-52, his3-Δ200 leu2-3,112 MAL4-C GAL TPS1*) was a spore isolated from the cross between KT1113 and 1403-7A strains. Mating, sporulation and tetrads dissection were performed according to standard protocol [19].

Deletion of *TPS1* in strains KT1112, W303-1B, TPY8 and TPY9 were done by digestion of the pMR19 plasmid (kindly given by Dr. C. Gancedo, CSIC, Madrid) with *SphI* and integrated into *TPS1* locus by the one-step disruption protocol [20]. The *tps1* deletion mutants isolated on galactose minimum plate as described by Gonzalez et al. [10] and confirmed by Southern blot for correct integration had in our hands no T6P synthase activity. The plasmid pRS315/MAL63 containing the regulatory sequence of the *MAL6* locus was a kind gift of Dr. C. Michels (Dept of Biology, Queens College, New York) and was used to allow *mal*$^-$ strains to grow on maltose. Yeast transformations were performed by the lithium acetate method of Schiestl and Gietz [21].

Yeast cells were grown at 30°C on either rich medium (1% yeast extract, 2% Bacto-peptone and the adequate carbon source at the final concentration of 2%) or on minimal medium (0.67% yeast nitrogen base w/o amino acids, and the appropriate auxotrophic requirements and carbon source at the final concentration of 2% final).

2.3. Biochemical assays

Extracts were obtained by shaking on a vortex 100 mg wet cells in 0.5 ml of an ice-cold HEPES buffer 25 mM, pH 7.1 containing phenylmethyl sulfonyl fluoride (PMSF) 1 mM, EDTA 2 mM and KCl 100 mM, with 1 g glass beads (0.5 mm diameter) for 6 periods of 30 s, with 30 s intervals in ice after each period. The extracts were centrifuged for 5 min at 700 × *g* and the supernatant were centrifuged at 10,000 × *g* for 15 min. This second supernatant was used for enzyme assays. T6P synthase was assayed by the formation of UDP formed as described by Vandercammen et al. [4], except that the temperature of incubation was 45°C, in order to reduce to nil the activity of glycogen synthase which also produced UDP (unpublished data). Trehalase [22] and total alpha-glucosidase [23] were assayed by published procedures.

For trehalose assay, the cells (10–20 mg dry weight) were quickly collected by filtration, washed twice with ice-cold water. The filtered yeasts were dropped in either 3 ml of boiling ethanol for 10 min or in 0.5 ml Na_2CO_3 for 30 min at 80°C. The ethanolic suspension was lyophilized, the residue was resuspended in 2 ml of 25 mM MES/KOH, pH 6.5 and the supernatant was used for metabolites determination. Unless otherwise stated, trehalose was determined by the method of Vandercammen et al. [24] except that commercial pig kidneys trehalase (Sigma) was used instead of partially purified rabbit kidney enzyme.

2.4. Other methods

Cell growth was monitored by direct counting with an haemocytometer. Residual glucose, galactose and maltose in the culture medium were measured by specific methods [25]. Protein was measured by the method of Bradford [26] using bovine serum albumin as a standard. Results shown are representative of at least two different experiments which yielded similar results.

3. Results and discussion

3.1. Pattern of trehalose accumulation on glucose and on maltose

In this work, the patterns of trehalose accumulation have been monitored during growth on glucose or on maltose in a *mal*$^-$ strain (KT1113), a *MAL* (inducible) strain (TPY8) and a

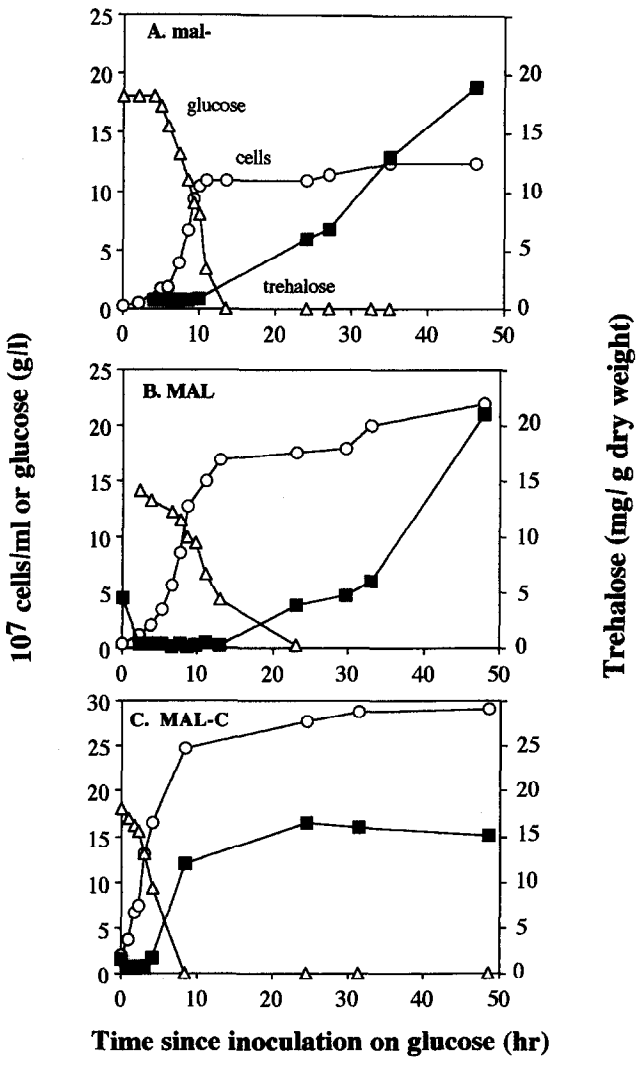

Fig. 1. Trehalose accumulation during growth of *S. cerevisiae* strains KT1112 (*mal*$^-$), TPY8 (*MAL*) and TPY9 (*MAL-C*) on glucose. The terms mal$^-$, MAL and MAL-C refer, respectively, to a strain unable to express *MAL* genes, to a strain which induces *MAL* genes in response to maltose, and to a strain which contains constitutive *MAL* genes.

MAL4-C (TPY9) strain bearing a dominant constitutive, glucose-repression insensitive allele of *MAL4* locus (TPY3). This constitutive trait has been shown to be due to an alteration in the *MAL43* gene which encodes a trans-activating factor of the *MAL* genes [27]. In regard to the experiments of growth on glucose, we found, in agreement with previous works [1,14,28], that both *mal*$^-$ and *MAL* strains accumulated trehalose at a similar rate of about 1 mg/h/g of dry cells during stationary phase of growth when all the glucose from the medium has been consumed (Fig. 1A and B). In contrast, a *MAL4-C* strain started to accumulate trehalose late in the exponential growth on glucose and at a rate of about 3.5 mg/h/g dry cells (Fig. 1C). The property to actively accumulate trehalose before exhaustion of glucose was likely associated to the constitutive expression of *MAL* genes as it was observed in another yeast strain (W303-1B) in which the *MAL4-C* allele was introduced by a genetic cross with 1403-7A and in a *MAL2-C* strain (results not shown). In contrast, none of the *mal*$^-$ and *MAL*-inducible

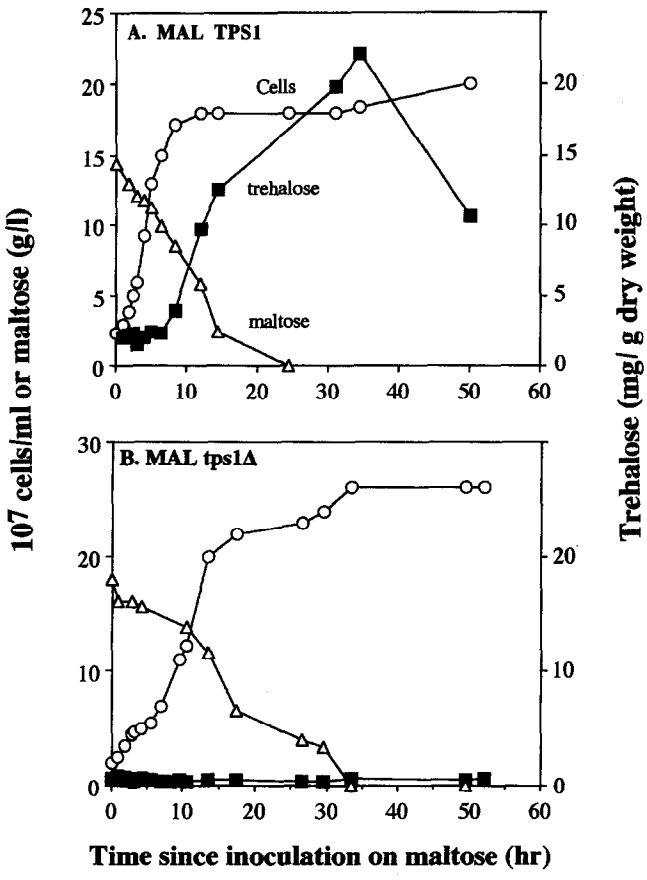

Fig. 2. Trehalose levels during growth of *MAL* strains carrying an intact copy (*MAL TPS1*) or a disrupted *TPS1* gene (*MAL tpsΔ1*) on maltose. The maltose medium was inoculated with the strains previously grown on a 2% galactose rich medium.

strains tested showed this behaviour, irrespective of their genetic background (results not shown). Hence, it can be concluded that, in confirmation to previous works from Panek's group [13], the constitutive expression of a *MAL* gene speeds up the synthesis of trehalose during growth on glucose.

Trehalose levels were then determined during the growth of a *MAL*-inducible (TPY8) and of a *MAL4-C* (TPY3) strains on maltose, using as an inoculum, cells grown on a galactose medium. The pattern of trehalose metabolism in a *MAL*-inducible strain is shown on Fig. 2A. Trehalose was already detectable although it was very low (less than 1 mg/g dry cells) in the early log phase of growth. It accumulated during the exponential phase at a rate of about 1 mg/h/g dry cells, and reached a maximum when all the maltose from the medium was fermented. A similar metabolic profile of trehalose on maltose was obtained in *mal*$^-$ strain (e.g. KT113 and W303-1A) transformed with the plasmid pRS315/MAL63 containing the regulatory *MAL63* gene which encodes the *trans*-activating factor of the *MAL6* locus (results not shown). We found, however, some differences in a *MAL4-C* strain. Upon inoculation in the maltose medium, trehalose initially present in the inoculated cells, was not degraded, as usually observed ([1,28] and see Fig. 1), but it was maintained at an almost constant level during the first part of the logarithmic growth. This indicated that, even if trehalase was not active under this condition, the enzymic system for trehalose synthesis had to operate in order to compensate for the dilution of initial trehalose by cellular growth. Net accumulation of trehalose then occurred during late exponential phase of growth until exhaustion of maltose from the medium. As a conclusion, the growth of cells on maltose is permissible for trehalose accumulation, and this effect could result from the fact that this sugar has a lower repressible effect than glucose on various sugar sensitive metabolic activities including trehalose synthesis [28,29]. To test this hypothesis, similar experiments were carried out using galactose, a lower repressible sugar than glucose, as a carbon source. We found, as in the case with maltose, that trehalose accumulated in late exponential phase well before galactose was exhausted (results not shown).

3.2. *Synthesis of trehalose on maltose depends on T6Psynthase encoded by* TPS1

Work from Panek's group indicated that mutants with no detectable UDPGlc-dependent T6P synthase activity as measured in vitro were able to accumulate trehalose during growth on maltose, but not on glucose unless they harboured a *MAL*-constitutive locus. We therefore tested more directly this observation by disrupting the *TPS1* gene in both *MAL* and *MAL-C* strains. The deletion of this gene completely eliminated the UDPGlc-dependent T6P synthase (not shown) and resulted in an inability of the mutants to accumulate trehalose during

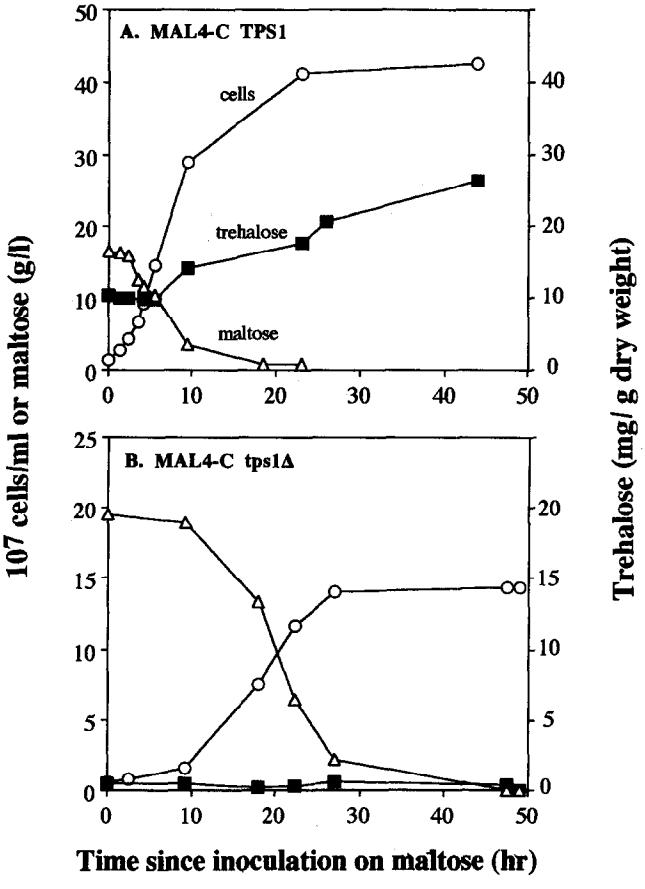

Fig. 3. Trehalose levels during growth of a *MAL4-C* strain carrying an intact copy (*MAL-C TPS1*) or a disrupted *TPS1* gene (*MAL-C tps1Δ*) on maltose. The maltose medium was inoculated with the strains previously grown on 2% galactose rich medium.

Table 1
Trehalose contents in TPY8 and TPY9 strains growing on maltose as assayed by the anthrone method as described in [1] or by the glucose oxydase method after incubation with commercial trehalase [24]

Strain	Exponential phase ($\pm 2 \cdot 10^7$ cells/ml)		Stationary phase ($\pm 2 \cdot 10^8$ cells/ml)	
	Trehalase digestion	Anthrone	Trehalase digestion	Anthrone
TPY8 (*MAL*)	1.9 ± 0.30	8.0 ± 1.50	11.70 ± 1.60	15 ± 2.30
TPY9 (*MAL4-C*)	5.40 ± 0.70	8.5 ± 1.50	15.5 ± 2.0	20 ± 3.0

The data presented are the mean ± S.E.M. of three independent experiments. The values are expressed in mg glucose equivalent per g dry weight.

growth on maltose (Figs. 2B and 3B) and on galactose (not shown), as well as during a heat treatment of exponentially growing cells on maltose (not shown). These results clearly indicated that the accumulation of trehalose on maltose required a functional *TPS1* gene. Hence, our data are at variance with those from Panek's group who reported a synthesis of trehalose in yeast bearing *cif1* or *fdp1* mutants [14,16,17] which were shown later to be mutations in the same *TPS1* gene [6–9]. In search for an explanation to this discrepancy, one could suggest that the *cif1* and *fdp1* mutants used by these authors had still a very low T6P synthase activity, and that they only detected trehalose on maltose but not on glucose because they did their measurement in a too short period (2–3 h) after entrance of cells into stationary phase on glucose. Another reason for this discrepancy could lie in the assay of trehalose by the anthrone method. As shown in Table 1, this chemical method gave values of trehalose which were 40 to 200% higher than those obtained by a specific enzymatic assay based on hydrolysis of trehalose into glucose by trehalase [24]. Moreover, overestimations by the anthrone method were the highest with exponential phase cells. It was found that 30 to 50% of this anthrone-reacting material corresponded to residual maltose which has been probably not removed by the extensive washings of the filters. Our results also indicate that, even if an ADP-glucose-dependent T6P synthase exists in yeast [17], its role in trehalose synthesis in vivo would be negligible.

Table 2
Activity of T6P synthase in *TPY8* and *TPY9* strains during exponential and stationary phase of growth on glucose and maltose

Strain	Glucose medium		Maltose medium	
	Exponential phase ($\pm 2 \cdot 10^7$ cells/ml)	Stationary phase ($\pm 2 \cdot 10^8$ cells/ml)	Exponential phase ($\pm 2 \cdot 10^7$ cells/ml)	Stationary phase ($\pm 3 \cdot 10^8$ cells/ml)
TPY8 (*MAL*)	15 ± 5.0	50 ± 10	45 ± 5.0	80 ± 20.0
TPY9 (*MAL4-C*)	40 ± 8.0	160 ± 15	90 ± 10	200 ± 40.0

Data are the mean ± S.E.M. of two independent experiments. The activity of T6P synthase is expressed in nmol UDP formed/min/mg protein.

3.3. *T6P synthase and trehalase activity during growth on glucose and maltose*

Since the above results precluded the existence of an alternative trehalose pathway linked to maltose utilization, it remained to understand how strains was able to accumulate trehalose during exponential growth on maltose, while this accumulation was delayed until the stationary phase of growth on glucose. As the steady-state concentration of trehalose is controlled by the rate of synthesis mediated by T6P synthase and the rate of degradation dependent on neutral trehalase [29,30], the activities of these two enzymes were measured in yeast growing on maltose and on glucose. While no significant difference in trehalase activity between glucose and maltose conditions were observed, data from Table 2 shows that T6P synthase was 2–3 more active in cells growing on maltose than on glucose. Since it is known that T6P synthase is sensitive to catabolite repression [6,8,29], this result suggests that this enzyme is less repressed by maltose. Results of this table also show that the *MAL4-C* strain bearing maltose constitutive genes contained 2- to 3-fold more T6P synthase than the *MAL* strain, suggesting a positive effect of *MAL* gene on the synthesis and/or activity of this enzyme. This result will require further investigation. As a conclusion, the accumulation of trehalose during growth on maltose could be a consequence of the well-known effect of this sugar to exert a lower catabolite repression than glucose. In favour of this idea, Blasquez and Gancedo [12] have found that mutants with reduced hexokinase II whose phosphorylation activity seems to be correlated with the level of glucose repression [31], accumulated trehalose during exponential phase of growth on glucose. Furthermore, the higher efficiency of *MAL4-C* strains to accumulate trehalose on glucose may be a consequence of this strain to contain more T6P synthase activity than the *mal*$^-$ and *MAL* strains.

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References

[1] Lillie, S.H. and Pringle, J.R. (1980) J. Bacteriol. 141, 1384–1394.
[2] Wiemken, A. (1990) Antonie Van Leeuwenhoek 58, 209–217.
[3] Cabib, E. and Leloir, L.F. (1958) J. Biol. Chem. 231, 259–275.
[4] Vandercammen, A., François, J. and Hers, H.-G. (1989) Eur. J. Biochem. 182, 613–620.
[5] Londesborough, J. and Vuorio, O. (1991) J. Gen. Microbiol. 137, 323–330.
[6] Bell, W., Klaassen, P., Ohnacker, M., Boller, T., Herweijer, M., Schoppink, P., Van Der Zee, P. and Wiemken, A. (1992) Eur. J. Biochem. 209, 951–959.
[7] Van Aelst, L., Hohmann, S., Bulaya, B., de Koning, W. et al. (1992) Mol. Microbiol. 8, 927–943.
[8] Vuorio, O., Kalkkinen, N. and Londesborough, J. (1993) Eur. J. Biochem. 216, 849–861.
[9] De Virgilio, C., Bürckert, N., Bell, W., Jenö, P., Boller, T. and Wiemken, A. (1993) Eur. J. Biochem. 212, 315–323.
[10] Gonzales, M.I., Stuck, R., Blasquez, M.A., Feldmann, H. and Gancedo, C. (1992) Yeast 8, 183–192.
[11] Blasquez, M.A., Lagunas, R., Gancedo, C. and Gancedo, J.M. (1993) FEBS Lett. 329, 51–54.
[12] Blasquez, M.A. and Gancedo, C. (1994) Curr. Genet. 25, 89–94.
[13] Panek, A. (1990) J. Biotechnol. 3, 121–130.

[14] Panek, A.D., Sampaio, A.L., Braz, G.C. and Mattoon, J.R. (1979) Cell. Mol. Biol. 25, 334–354.
[15] De Oliveira, D.E., Arrese, M., Kidane, G., Panek, A.D. and Mattoon, J.R. (1986) Curr. Genet. 11, 97–106.
[16] Operti, M.S., Oliveira, D.E., Freitas-Valle, A.B., Oestreicher, E.G., Mattoon, J.R. and Panek, A.D. (1982) Curr. Genet. 5, 69–76.
[17] Paschoalin, V.M.F., Silva, J.T. and Panek, A.D. (1989) Curr. Genet. 16, 81–87.
[18] Thomas, B.J. and Rothstein, R. (1989) Cell 56, 619–630.
[19] Rose, M.D., Winston, F. and Hieter, P. (1990) Methods in Yeast Genetics: A Manual Course Book, Cold Spring Harbor Laboratory Press, NY.
[20] Rothstein, R. (1983) Methods Enzymol. 101, 202–211.
[21] Shiestl, R.H. and Gietz, D.R. (1989) Curr. Genet. 16, 339–346.
[22] François, J., Van Schaftingen, E. and Hers, H.-G. (1984) Eur. J. Biochem. 145, 187–193.
[23] Zimmermann, F.K., Kaufmann, I., Rasenberger, H. and Haußmann, P. (1977) Mol. Gen. Genet. 151, 95–103.
[24] Vandercammen, A., François, J., Torres, B.B., Maia, J.C.C. and Hers, H.-G. (1990) J. Gen. Microbiol. 136, 137–146.
[25] Bergmeyer, H.U. (1983) Methods in Enzymatic Analysis, Verlag Chemie, Weinheim, Germany.
[26] Bradford, M.A. (1976) Anal. Biochem. 72, 248–254.
[27] Charron, M.J. and Michiels, C.A. (1987) Genetics 116, 23–31.
[28] Gancedo, J.M. (1992) Eur. J. Biochem. 206, 297–313.
[29] François, J., Neves, M.J. and Hers, H.-G. (1991) Yeast 7, 575–587.
[30] Kopp, M., Muller, H. and Holzer, H. (1993) J. Biol. Chem. 268, 4766–4774.
[31] Ma, H., Bloom, L.M., Zhu, Z., Walsh, C.T. and Botstein, D. (1989) Mol. Cell. Biol. 9, 5643–5651.