APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1995, p. 109–115 Vol. 61, No. 1
0099-2240/95/$04.00+0
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Differential Importance of Trehalose in Stress Resistance in Fermenting and Nonfermenting *Saccharomyces cerevisiae* Cells

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Received 19 July 1994/Accepted 29 October 1994

The trehalose content in laboratory and industrial baker's yeast is widely believed to be a major determinant of stress resistance. Fresh and dried baker's yeast is cultured to obtain a trehalose content of more than 10% of the dry weight. Initiation of fermentation, e.g., during dough preparation, is associated with a rapid loss of stress resistance and a rapid mobilization of trehalose. Using specific *Saccharomyces cerevisiae* mutants affected in trehalose metabolism, we confirm the correlation between trehalose content and stress resistance but only in the absence of fermentation. We demonstrate that both phenomena can be dissociated clearly once the cells initiate fermentation. This was accomplished both for cells with moderate trehalose levels grown under laboratory conditions and for cells with trehalose contents higher than 10% obtained under pilot-scale conditions. Retention of a high trehalose level during fermentation also does not prevent the loss of fermentation capacity during preparation of frozen doughs. Although higher trehalose levels are always correlated with higher stress resistance before the addition of fermentable sugar, our results show that the initiation of fermentation causes the disappearance of any other factor(s) required for the maintenance of stress resistance, even in the presence of a high trehalose content.

The disaccharide trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) has a widespread occurrence in nature and is present in particularly large quantities in many survival forms of fungi, nematodes, insects, and plants, such as stationary-phase cells, spores, cysts, sclerotia, and dried desert resurrection plants (10, 15, 52, 58, 60). For a long time, the main role of trehalose in fungi was considered to be as a storage compound (15, 52). In recent years, however, much attention has been drawn to a possible function of trehalose as a stress protectant, mainly based on the remarkable stress-protection properties of trehalose in vitro and on the strong correlation between trehalose content and stress resistance in vivo (58, 60).

After the first clear-cut report by Crowe et al. (9), many papers documenting the impressive and specific protective effect of trehalose against stress treatments of biological structures in vitro (e.g., references 6, 8, and 44) have been published. The protective effect of trehalose is not well understood at the molecular level. Two hypotheses (the water replacement hypothesis and the glass transition hypothesis) have been proposed, but there is no straightforward explanation, particularly for the superiority of trehalose in conferring stress protection compared with that afforded by molecules with structures similar to that of trehalose (3, 5, 6, 8, 22, 31, 45). In the yeast *Saccharomyces cerevisiae*, a strong correlation between trehalose content and stress resistance has been demonstrated in different strains, in a variety of growth conditions, during sublethal heat treatment and other stress conditions, and in mutants affected in the Ras-adenylate cyclase pathway (1, 18, 21, 24, 27–29, 34).

The trehalose content of commercial baker's yeast is widely believed to be a critical parameter for its stress resistance, and this has received a lot of attention, particularly with respect to the production of "instant active dry yeast" (40, 54). Over the years, the culture conditions of commercial baker's yeast have been optimized in order to obtain a high trehalose content. At present, trehalose levels of 15 to 20% of the dry weight are common, with 10% being considered a critical threshold (18, 54). This is much higher than the levels found in laboratory strains grown in batch culture, which are at most 4 to 5% of the dry weight.

For the preservation of yeast viability in frozen doughs, a high trehalose content is also considered crucial. Initiation of fermentation upon the mixing of nutrients with yeast cells during dough preparation triggers a rapid mobilization of trehalose and a rapid loss of freeze resistance. To minimize both processes, the preparation of frozen doughs is generally carried out at a reduced temperature. Preservation of freeze resistance by the elimination or reduction of trehalose mobilization through the genetic engineering of the genes of trehalose metabolism has been claimed in recent patent applications (14, 33).

Yeast cells growing on a rapidly fermented sugar, such as glucose, have little stress resistance and also a very low trehalose level. On the other hand, stationary-phase cells and cells grown on nonfermentable carbon sources have both a high stress resistance and a high trehalose content. The addition of a glucose-containing medium to such cells triggers rapid mobilization of trehalose and rapid loss of stress resistance (16, 17, 32, 49, 52). This fermentation-induced loss of stress resistance is of great importance for the production of frozen doughs with commercial baker's yeast. Whereas the stress resistance of commercial yeast cells is very high before contact with nutrients, mixing of the yeast cells with the flour results in the rapid mobilization of trehalose and a rapid loss of stress resistance, notably freezing resistance. This necessitates special production conditions for frozen doughs in order to maximize the preservation of the leavening power of the yeast in the dough during freeze storage (43).

A major problem in assessing the importance of trehalose for stress resistance up to now was the lack of mutants specifically affected in trehalose metabolism. Recently, several genes

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TABLE 1. Strains used in this study

Strain	Genotype
YSH6.36-3B (wild type)[a]	α *leu2-3,112 ura3-52 trp1-92*
YSH396	α *leu2-3,112 ura3-52 trp1-92 nth1*Δ::*URA3*
YPVD640	α *leu2-3,112 ura3-52 trp1-92 nth1*Δ::*LEU2*
YSH292	α *leu2-3,112 ura3-52 trp1-92 ggs1/tps1*Δ::*TRP1*
YPVD819	α *leu2-3,112 ura3-52 trp1-92 PDC1prom-GGS1/TPS1*
YPVD613	**a**/α *leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1-92/trp1-92*
YPVD609	**a**/α *leu2-3,112/leu2-3,112 ura3-52/ura3-52 trp1-92/trp1-92 nth1*Δ::*LEU2/nth1*Δ::*LEU2*

[a] Descendant of diploid wild-type strain M5 (48).

involved in trehalose biosynthesis and trehalose hydrolysis have been cloned. Biosynthetic genes include *GGS1/TPS1*, encoding trehalose-6-phosphate synthase (2, 20, 37, 55, 59), *TPS2*, encoding trehalose-6-phosphate phosphatase (11), and *TSL1* (59) and *TPS3* (36), probably encoding a regulatory subunit of the trehalose synthase complex. In addition, the *NTH1* gene encoding neutral trehalase has also been cloned (30). The availability of genes of trehalose metabolism should now in principle allow more specific manipulation of the trehalose content of yeast cells in order to investigate its relevance for stress resistance: *ggs1/tps1* mutants are completely devoid of trehalose, while *nth1* mutants display strongly reduced mobilization of trehalose.

An unexpected complication with the *ggs1/tps1* mutants is that they not only lack the capacity to synthesize trehalose but display a complex pleiotropic phenotype. They are unable to grow on rapidly fermentable sugars like glucose and fructose, are deficient in many glucose-induced regulatory phenomena, and display a sporulation defect and an aberrant control of glycogen metabolism (20, 25, 55–57). The molecular basis of this complex phenotype is not well understood. We have shown previously that deletion of the *HXK2* gene encoding the most active hexokinase isoenzyme restores both growth on glucose and the glucose-induced regulatory phenomena in a *ggs1/tps1*Δ mutant, but not the trehalose level (26). Therefore, for the present paper, we have used strains with either only a deletion of the *GGS1/TPS1* gene or an additional deletion of the *HXK2* gene. Both strains completely lack trehalose. In addition, we have used a strain which has a deletion of the *NTH1* gene and which displays a higher initial trehalose level and a very slow mobilization of trehalose during the initiation of fermentation and also a strain which overexpresses *GGS1/TPS1* and which displays a higher initial trehalose level but has a trehalose mobilization rate similar to that of the wild-type strain. In addition to haploid strains grown under standard laboratory conditions in batch culture, we have also used the corresponding diploid strains grown under industrial pilot-scale conditions in order to obtain cells with a trehalose content higher than 10%. Our results confirm the correlation between trehalose content and stress resistance, but only for nonfermenting cells. Once the cells start to ferment, this correlation is lost. The mobilization of trehalose during the initiation of fermentation is not responsible for the loss of stress resistance, and maintenance of a high trehalose level does not suffice to prevent loss of stress resistance under these conditions.

MATERIALS AND METHODS

***S. cerevisiae* strains.** The isogenic strains used in this study are listed in Table 1.

The *nth1*Δ and *ggs1/tps1*Δ strains were constructed by precise deletion of the complete *NTH1* or *GGS1/TPS1* open reading frame by inverse PCR. For overexpression of *GGS1/TPS1*, the coding region was cloned under the control of the constitutive *PDC1* promoter on an integrating plasmid (Ylplac211, marker *URA3*) (19). Subsequently, the plasmid was integrated into the *ura3-52* genomic allele. A detailed description of these constructions will be published elsewhere (57a). The strains YPVD613 and YPVD609 were obtained by diploidization of strains YSH6.36-3B and YSH396, respectively. These strains were diploidized by transformation with a centromeric vector (YCp50, marker *URA3*) carrying the *HO* gene (kindly provided by P. Linder and M. Hall, Biozentrum Basel) (23). The single-cell clones of the transformants were checked for their ploidy by flow cytometry with propidium iodide as the DNA stain (41). The diploids were then streaked out on medium with fluoro-orotic acid (50) to select for plasmid loss, which was confirmed by replica plating the growing colonies onto uracil-free medium.

Culture and incubation conditions. Yeast cells were grown in YPglycerol (YPG) (1% yeast extract, 2% Bacto Peptone, 3% glycerol) to exponential growth phase or in YPglucose (1% yeast extract, 2% Bacto Peptone, 2% glucose) to stationary phase at 30°C in shake flasks in a gyratory incubator. The cells were harvested, washed, and resuspended in YPG. Subsequently, they were preincubated for 45 min at 30°C, after which glucose was added to a final concentration of 100 mM. Samples were taken just before and at 30, 60, and 90 min after the addition of the glucose.

Determination of trehalose content and heat and freeze resistance. Trehalose levels were assayed with *Humicola* trehalase, glucose oxidase, and peroxidase according to the method of Neves et al. (38). For determinations of heat and freeze resistance, the cells were washed with ice-cold water and resuspended in ice-cold YPG. After dilution with YPG to an optical density at 600 nm of 1, 0.5 ml of the cell suspension was incubated at 52°C for either 5, 10, or 20 min for determination of heat resistance and stored at −20°C for 4 days for determination of freeze resistance. Non-heat-shocked cells served as a control. After heat shock, the cells were quickly cooled on ice and diluted 10^3 and 10^4 times. A sample (0.1 ml) of each dilution was plated on YPglucose plates or on YPG plates in the case of the *ggs1/tps1*Δ strain (which is unable to grow on glucose). Survival after heat or freeze shock was estimated by counting the number of colonies after 2 days of growth at 30°C. Survival rates are expressed as percentages of the number of colonies relative to the number of colonies in the nonstressed controls.

Pilot-scale conditions. Diploid wild-type and *nth1*Δ strains were grown on beet molasses supplemented with 2.5% yeast extract, 0.25% tryptophan, and 1.7% leucine (wt/wt of molasses at 50% sucrose). The culture was performed in a 20-liter fermentor (10-liter medium volume) according to standard fed-batch procedures (42). The average (mass) multiplication coefficient was 1.05 h^{-1}. The temperature was maintained between 30 and 34°C, and the pH was regulated between 4.5 and 6.

Determination of gassing power. The gassing power was determined in a fermentometer as described by Burrows and Harrison (4). The results were expressed as the volume (in milliliters) of gas produced in 2 h at 30°C. The initial gassing power of the two diploid laboratory strains, as determined for compressed yeast cells, was the same for the wild type and for the *nth1*Δ strain (27 ml of CO_2 produced in 2 h at 30°C starting from 20 g of flour and 160 mg of yeast cells [dry weight]).

Frozen dough experiments. Because of the low gassing power of the laboratory strains compared with that of the industrial strains, a dough with 10% compressed yeast cells (with about 30% dry matter) was prepared in order to obtain significant fermentative activity initially in the dough. The formulation of the dough was as follows: wheat flour, 100 g; water, 61 g; NaCl, 2 g; fats, 0.7 g; sucrose, 5 g; and compressed yeast, 10 g. The dough was prepared within a time period of 15 min. The initial temperatures of the flour and the water were −9.5°C and +1°C, respectively. The temperature of the dough at the end of the mixing process was 17°C. The dough was divided into small pieces of 20 g and immediately stored at −20°C. The gassing power for different storage periods was determined as described above. For this purpose, the frozen doughs were allowed to thaw beforehand for 2 h at +4°C.

Reproducibility of the results. All experiments were repeated at least twice. Representative results are shown.

RESULTS

Trehalose content and stress resistance in cells grown under laboratory conditions. In a first series of experiments, we used cells grown under laboratory conditions. The correlation between trehalose and stress resistance before and after the addition of glucose to cells was investigated in cells grown on YPglucose to stationary phase (strains: wild type, *ggs1/tps1 hxk2*, *hxk2*, and *nth1*) (Fig. 1) and in cells grown on YPG to exponential phase (strains: wild type, *ggs1/tps1*, *nth1*, and wild type with *GGS1/TPS1* overexpression) (Fig. 2). A strain with a deletion of the *NTH1* gene encoding the neutral trehalase displayed a higher initial trehalose level and a much slower

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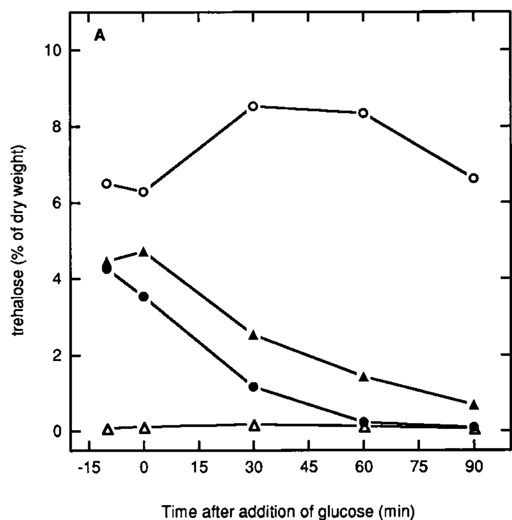


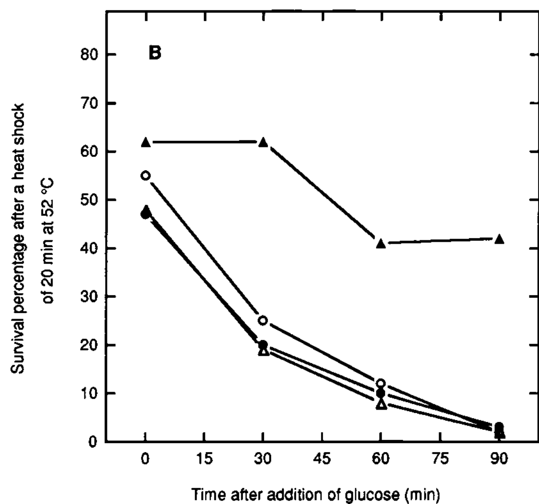
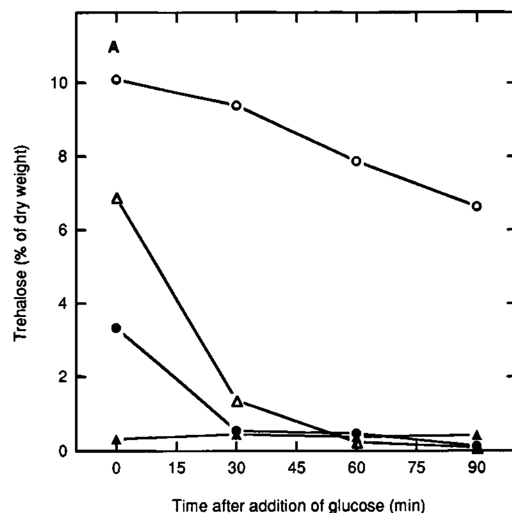
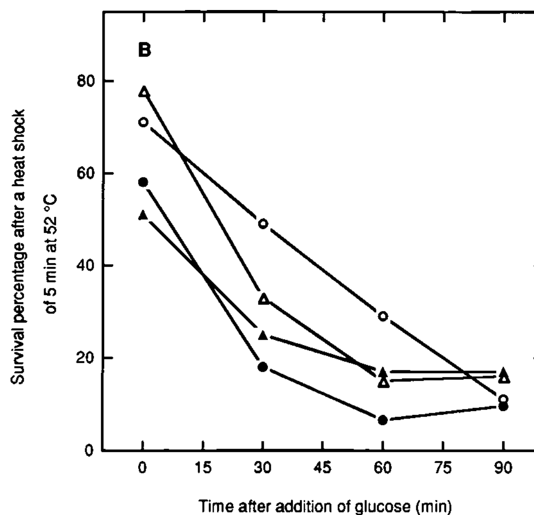


FIG. 1. Trehalose content (A) and heat resistance (B) before and after the addition of glucose to stationary-phase yeast cells. The cells were grown to stationary phase on YPglucose. ●, wild type (YSH6.36.-3B); ○, *nth1*Δ; ▲, *hxk2*Δ; △, *ggs1/tps1*Δ *hxk2*Δ. Heat resistance was determined as survival percentages after a heat shock of 20 min at 52°C.

glucose-induced mobilization of trehalose (Fig. 1A and 2A). The slow disappearance of trehalose might be due to dilution by growth and/or hydrolysis by the acid trehalase in the vacuole. Another possible explanation might be the presence of a homolog of *NTH1*. Such a homolog has recently been discovered in the European Yeast Genome Sequencing project. The predicted amino acid sequence of the *NTH2* gene product is 77% identical with that of *NTH1* (62). The function of *NTH2* is unclear. A strain with overexpression of *GGS1/TPS1* also had a higher initial trehalose level, but its trehalose mobilization was as rapid as that in the wild-type strain (Fig. 2A). In both the *ggs1/tps1*Δ and the *ggs1/tps1*Δ *hxk2*Δ strains, the trehalose contents are very low (Fig. 1A and 2A) as reported previously (26, 55). The *hxk2*Δ strain had a trehalose content similar to that in the wild-type strain.

Before the addition of glucose, all strains, including the *ggs1/tps1*Δ strains, showed a higher heat resistance than after 90 min of incubation with glucose (Fig. 1B, 2B, and 2C). In the strains with higher trehalose levels, however, a higher heat resistance was observed (Fig. 1B and 2C). Upon the addition of





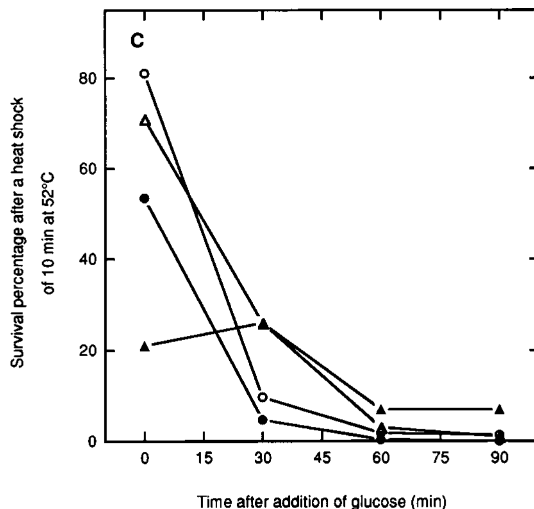


FIG. 2. Trehalose content and heat resistance before and after the addition of glucose to exponential-phase glycerol-grown yeast cells. (A) Trehalose content. (B) Heat resistance as measured by survival percentages after a heat shock of 5 min at 52°C. (C) Heat resistance as measured by survival percentages after a heat shock of 10 min at 52°C. ●, wild type (YSH6.36.-3B); ○, *nth1*Δ; ▲, *ggs1/tps1*Δ; △, wild type + YIp*PDC1*prom-*GGS1/TPS1*.

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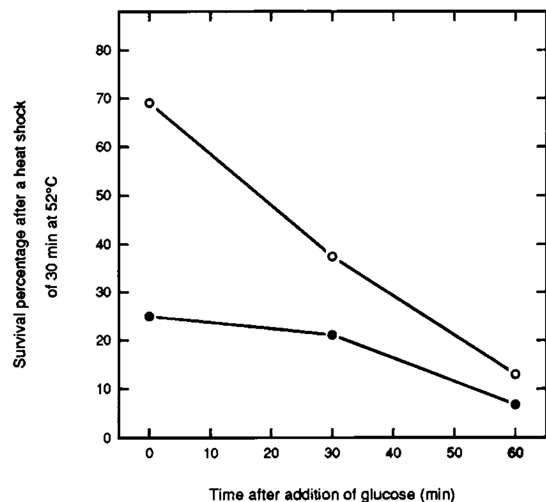

FIG. 3. Heat resistance before and after the addition of glucose to cells of diploid strains obtained from pilot-scale cultures. ●, diploid wild type (M5); ○, *nth1*Δ/*nth1*Δ diploid.

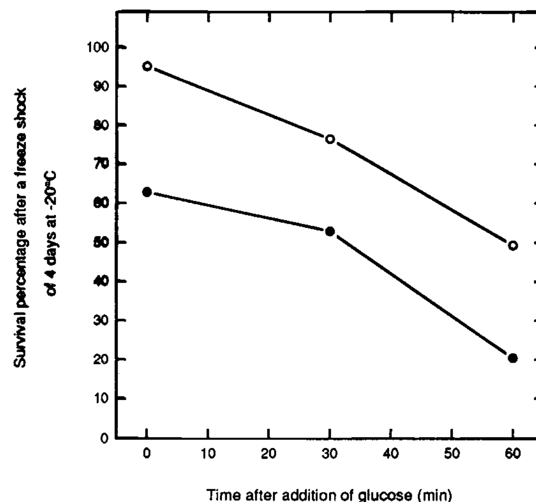

FIG. 4. Freeze resistance before and after the addition of glucose to cells of diploid strains obtained from pilot-scale cultures. ●, diploid wild type (M5); ○, *nth1*Δ/*nth1*Δ diploid.

glucose, heat resistance rapidly disappeared to very low levels in all strains (Fig. 1B, 2B, and 2C) and there was no significant correlation with the residual trehalose level (Fig. 1A and 2A). Similar results have been obtained when yeast cells were freeze shocked at −20°C (results not shown). There was also no correlation between the level of trehalose and freeze resistance during the initiation of fermentation. In general, the loss of freeze resistance was slower than the loss of heat resistance (results not shown). Surprisingly, the *hxk2*Δ control strain showed a slower loss of glucose-induced stress resistance compared with the losses for other strains. This does not appear to be related to the deficiency in glucose repression or to slower resumption of fermentation or growth caused by the *hxk2*Δ mutation since the *hxk2*Δ *ggs1/tps1*Δ strain did not show this property.

Trehalose content and stress resistance in cells grown under pilot-scale conditions. In a second series of experiments, we investigated the correlation between trehalose content and stress resistance in cells grown under pilot-scale conditions, since cells grown under such conditions have a much higher initial trehalose level than cells grown under laboratory conditions. Because of the slow growth rate of haploid laboratory strains under pilot-scale conditions, we diploidized the two most relevant strains (wild type and *nth1*Δ) in order to obtain more vigorous strains with faster growth rates. This was indeed the case (results not shown). The two diploid strains obtained were grown in a 20-liter fermentor (see Materials and Methods).

The trehalose level in the diploid wild-type strain grown under pilot-scale conditions was 8.8% of the dry weight (i.e., about twofold higher than that of the isogenic haploid strain grown under laboratory conditions). The diploid *nth1*Δ strain grown under pilot-scale conditions had a trehalose content of 11.7% of the dry weight. In the wild-type strain, which began with a trehalose content of 8.8%, the addition of glucose caused a nearly complete mobilization of trehalose after 60 min (content of 1.6%). In the *nth1*Δ strain, on the other hand, the trehalose content 60 min after the addition of glucose was still higher than 10% (10.6%) of the dry weight. Heat resistance (Fig. 3) and freeze resistance (Fig. 4) before the addition of glucose were much higher in the *nth1*Δ strain than in the wild-type strain, confirming the positive correlation between trehalose content and stress resistance in the absence of fermentation. After the addition of glucose, however, both heat and freeze resistance diminished greatly in both strains (Fig. 3 and 4). In the *nth1*Δ strain, heat resistance 60 min after the addition of glucose was already similar to that in the wild-type strain in spite of a trehalose level which was still higher than 10% of the dry weight (Fig. 3).

Loss of gassing power in frozen doughs. We measured the loss of the fermentation power of the yeast present in frozen doughs as a function of storage time at −20°C. The doughs were made either with the diploid wild-type strain or with the diploid homozygous Δ*nth1* strain grown under pilot-scale conditions. The original doughs made with the two strains were divided into small aliquots that were stored at −20°C for up to 60 days. After the doughs were thawed, fermentation power was measured as described in Materials and Methods. The two values for each strain at time 0 represent the fermentation power of an aliquot of dough that was not frozen and another aliquot that was frozen and immediately thawed again. The results shown in Fig. 5 indicate that the freezing of the dough itself has only a minor influence on the fermentation power of the yeast. Prolonged storage of the dough at −20°C, however, causes a gradual loss of fermentation power. In the *nth1*Δ strain, which had a significantly higher initial trehalose level, the loss of fermentation power as a function of storage time at −20°C is not improved by comparison with that of the control strain (Fig. 5).

DISCUSSION

Previous research on the possible importance of trehalose as a stress protectant in yeast has focused nearly exclusively on the question of whether trehalose does or does not act as a stress protectant (58, 60). Wiemken strongly favors the idea that trehalose functions as a stress protectant. He even proposed that this would be its sole function, dismissing the previous notion of a function as a storage carbohydrate (60). This argument focuses on conditions in which a correlation between trehalose content and stress resistance is observed: in stationary-phase cells and cells grown on nonfermentable carbon sources, during sublethal heat treatment and other sublethal stress conditions, and in mutants of the Ras-adenylate cyclase

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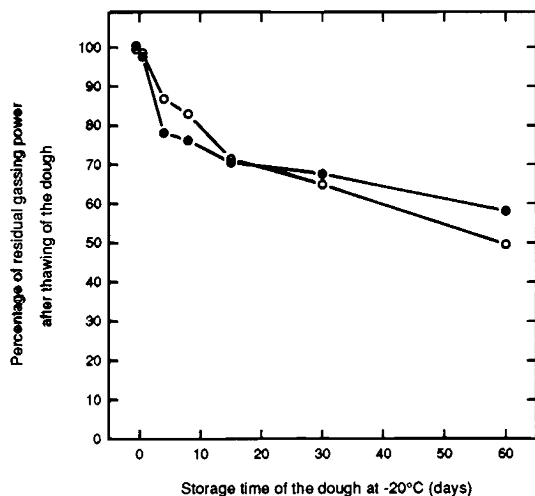

FIG. 5. Loss of gassing power in frozen doughs stored for different periods of time at −20°C and made with yeast cells of diploid strains obtained from pilot-scale cultures. The doughs were thawed for 2 h before determination of the gassing power. ●, diploid wild type (M5); ○, *nth1*Δ/*nth1*Δ diploid. The two values at time 0 represent the gassing power of unfrozen dough and that of dough which was immediately thawed again after freezing. Residual gassing power in the frozen doughs for different time periods of storage is expressed as percentages of the gassing power of the yeast cells in unfrozen dough.

pathway (1, 18, 21, 24, 27–29, 34). All these conditions and mutations, however, also affect other characteristics, in particular the synthesis of heat shock proteins, which are also involved in stress resistance (35, 46, 47). Conflicting conclusions about the importance of trehalose and heat shock proteins for stress resistance have been drawn from experiments with organisms with mutated heat shock proteins (13, 61).

Recently, mutants with mutated trehalose biosynthesis have been used by De Virgilio et al. (12) and by Nwaka et al. (39) to study the role of trehalose in the acquisition of thermotolerance during sublethal heat treatment. In both studies, the authors limited themselves mainly to induced thermotolerance, while in our work we have concentrated on intrinsic thermotolerance. De Virgilio et al. (12) reported that a *ggs1*/*tps1* mutant still acquired thermotolerance, but to a lesser degree than the wild-type strain. They also pointed, however, to the pleiotropic phenotype of *ggs1*/*tps1* mutants, which makes interpretation of this result difficult. A similar result obtained with a trehalose-6-phosphate phosphatase mutant is even more problematic because of the very large hyperaccumulation of trehalose-6-phosphate during sublethal heat treatment, which is toxic for the cells. Finally, thermotolerance dropped faster in a wild-type strain than in a *nth1*Δ mutant after a return to normal temperature, but this was only measured at one single time point and the difference was relatively small compared with the large difference in trehalose content. Most likely, at later time points, the difference in thermotolerance would in our view have disappeared completely in spite of the remaining large difference in trehalose content. Recent results obtained by Nwaka et al. confirm that after a return to normal temperatures following a sublethal heat treatment, induced heat resistance drops much faster than the trehalose level (39). Hence, from these results, it appears that trehalose is not essential for acquired thermotolerance during sublethal heat treatment and that it is unable to maintain thermotolerance in the absence of another factor(s) induced during sublethal heat treatment. On the other hand, its accumulation might enhance the degree of acquired thermotolerance.

Our results also indicate that a possible role of trehalose as a stress protectant depends on the conditions. In stationary-phase cells and cells growing on nonfermentable carbon sources, there is a clear correlation between the trehalose level and stress resistance. However, it also has to be emphasized that in the complete absence of trehalose, such cells are much more stress resistant than fermenting cells. Hence, there must be additional factors besides trehalose which contribute in an important way to the higher stress resistance of nonfermenting cells. Induction of heat resistance without any effect on the trehalose level has also been observed after yeast cells are treated with a plant cytokinin, confirming that other factors besides trehalose are able to enhance stress resistance (7). The initiation of fermentation is associated with a rapid loss of stress resistance which is clearly not due to the mobilization of trehalose. Also, in cells devoid of trehalose, there is a rapid loss of stress resistance after the addition of glucose. This indicates that glucose causes the rapid disappearance of the additional stress-protecting factors. The absence of trehalose mobilization in the *nth1*Δ strain is unable to abolish the rapid loss of stress resistance. This confirms that the loss of stress resistance is not a consequence of the mobilization of trehalose. Even more importantly, it also shows that a high trehalose level of more than 10% of the dry weight is unable to protect against a stress treatment in the absence of the other factor(s) whose disappearance was triggered by glucose. Our results therefore indicate that in stationary-phase cells and cells growing on nonfermentable carbon sources, a specific factor(s) is present which is absent in fermenting cells and which is indispensable for high stress resistance. Only when this factor(s) is present, is trehalose able to give a further improvement in stress resistance. Such a further improvement might also be obtained in fermenting cells if the disappearance of the other factor(s) required for stress resistance could in some way be prevented.

This conclusion also indicates that trehalose and the other, unknown factor must confer higher stress resistance by entirely different mechanisms. If the other factor is heat shock proteins, then it is well conceivable that their function would be to correct damage (i.e., unfolding) to proteins while the role of trehalose would consist more in physical prevention of damage. The dependency of trehalose on the presence of the other factor for improving stress resistance can then be explained in different ways which are not mutually exclusive. Trehalose might be unable to prevent all protein damage completely, even when present in very high concentrations. If no factor or at least not enough of it is present to correct the damage, a harsh stress treatment would still be lethal. Alternatively, trehalose might be unable to prevent damage to certain proteins which are critical for survival. Obvious candidates in this respect are proteins localized in specific compartments in which no trehalose accumulates. As far as is known, trehalose is localized in the cytosol. Even with very high levels of trehalose accumulated in the cytosol, the protective effect on proteins localized in other compartments might be insufficient. Another factor possibly involved in the difference in stress resistance between fermenting and nonfermenting cells is membrane lipid composition (51). No data are available, however, on rapid changes in membrane lipid composition during the initiation of fermentation.

Because of the close correlation between trehalose levels and stress resistance in yeast cells, an obvious strategy to preserve stress resistance in frozen doughs was to delete the gene(s) encoding the enzyme(s) responsible for trehalose mobilization (14). Alternatively, overexpression of enzymes responsible for trehalose biosynthesis might also reduce the rate of trehalose mobilization and therefore the loss of stress resis-

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tance (33). Our results, however, show that retention of more than 10% trehalose (dry weight) does not abolish the loss of stress resistance. In addition, frozen doughs prepared with cells of a mutant lacking the neutral trehalose did not show any significant improvement in leavening power by comparison with that of frozen doughs prepared with the isogenic wild-type strain. Hence, it will be important first to identify the real factor(s) whose nutrient-induced disappearance is responsible for the loss of stress resistance. After constitutive expression of the gene(s) encoding this factor(s) in strains with or without trehalose mobilization, it will be possible to assess whether prevention of trehalose mobilization could result in additional improvement of stress resistance during the initiation of fermentation.

Why is there in general such a close correlation between trehalose levels and stress resistance in yeast cells if both properties can, at least under some conditions, be uncoupled? Since fermenting cells also arrest in the G_1 phase of the cell cycle and enter the G_0 state when deprived of other essential nutrients like nitrogen, phosphate, and sulfate, the disappearance of stress resistance probably requires a complete, fermentable growth medium. We have proposed the name "fermentable-growth-medium-induced pathway" for the signaling pathway responsible for a series of glucose-induced regulatory events which also depend on the presence of all other nutrients essential for growth (53). The expression of several heat shock proteins is known to be regulated by nutrients in such a way. Since the trehalose level is also regulated by the fermentable-growth-medium-induced pathway, regulation of stress resistance by the same pathway provides an explanation for the striking correlation previously observed between trehalose levels and stress resistance.

ACKNOWLEDGMENTS

This work was supported by grants from the Flemish Biotechnology Action Programme (VLAB/TOP-041), the Belgian National Fund for Scientific Research, the Belgian National Lottery, and the Research Fund of the Katholieke Universiteit te Leuven.

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