

Respiration and Viability of Thermally Injured *Saccharomyces cerevisiae*†

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Resting cells of *Saccharomyces cerevisiae* Y25 were heated at 56°C for 0 to 2 min. Respiratory activity of the cells reflected the severity of the heat stress. The endogenous respiration was approximately 50 μ l of O₂/mg per h for cells heated for 2 min at 56°C as compared with 2 μ l of O₂/mg per h for nonheated cells. There was a distinct decrease in respiration after 1 to 3 h, and after 20 h the respiration rate of heated cells was less than that of nonheated cells. Along with increased rates of endogenous respiration, respiratory quotients of cells were altered after heat stress. Addition of 2,4-dinitrophenol stimulated O₂ uptake in nonheated cells but decreased O₂ uptake of heated cells. Due to the high rate of endogenous respiration, addition of glucose resulted in no substantial change in the rate of respiration of heated cells. However, addition of glucose prolonged the presence of the high rates of respiration observed in heated cells.

Recently, several investigators have reported the influence of thermal stress on fungi. One of the manifestations of thermal injury in yeasts and molds is a change in the metabolic activity of the cells. Altered respiratory activity associated with thermal injury was reported in a number of investigations on fungi (1, 2, 5, 13, 14). Also, decreased fermentative activity is found in fungi exposed to supramaximal temperatures (7, 14).

However, there have been few reports of reversible thermal injuries associated with respiratory activities of fungi. E. D. Meyer (Ph.D. thesis, University of Arizona, Tucson, 1975) investigated the effects of thermal stress on *Candida* P25, a psychrophilic yeast. Thermal injury affecting endogenous respiration is irreversible when cells are heated in the presence of glucose, but is reversible when cells are heated in the absence of glucose. The presence of glucose during storage does not affect recovery. In contrast, Baldy et al. (1) reported that glucose inhibits recovery from thermal injury during storage of heat-stressed conidia of *Penicillium expansum*.

Previously we reported that cells of *Saccharomyces cerevisiae* Y25 are injured during heating at 56°C. Repair of injured cells takes place in water at 22°C, but not at 4°C or in the presence of 2,4-dinitrophenol (DNP). The present investigation reports on the respiratory ac-

tivity of *S. cerevisiae* Y25 cells after heating and during recovery from thermal injury.

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MATERIALS AND METHODS

Organism. *S. cerevisiae* Y25, a diploid yeast from the culture collection of the Michigan State University Food Microbiology Laboratory, was used in all experiments. Procedures for culture maintenance, cultivation, and preparation of cell suspensions were as described by Graumlich and Stevenson (8).

Thermal stress. Cells of *S. cerevisiae* were subjected to thermal stress by utilizing the flask method described by the National Canners Association (11). An 11-ml amount of cell suspension was added to 99 ml of water preheated to 56°C in a 250-ml, screw-capped Erlenmeyer flask. Temperature was maintained in a water bath heated by a Bronwill 20 constant temperature circulator (Bronwill Scientific Co., Rochester, N.Y.). The contents of the flask were mixed with a magnetic stirring bar to help provide a uniform temperature and to maintain suspension of the yeast.

Samples of the cell suspensions were withdrawn after heating for 0, 1, or 2 min and placed in test tubes. The tubes were cooled by immersion in cold tap water.

Enumeration procedures. Samples of heated cell suspensions were plated immediately or, for delayed plating, were held for 20 h at room temperature (22°C) in test tubes on a Model TC-6 rotating drum (New Brunswick Scientific Co., New Brunswick, N.J.) operating at 6 rpm to provide aeration and to maintain the yeast in suspension. The samples were diluted in water, and duplicate pour plates were prepared of the

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appropriate dilutions. Samples were plated on plate count agar (PCA; Difco) and potato dextrose agar (PDA; Difco). Plate counts were determined after incubation for 5 to 6 days at 25°C.

Manometric measurements. Respiration of heat-stressed and nonstressed cells suspended in water at 30°C was studied by conventional manometric techniques (15), using a Gilson differential respirometer, model GR-14 (Gilson Medical Electronics, Inc., Middleton, Wis.). In some experiments, heat-stressed and nonstressed cells were washed by filtration on a 0.40- μ m membrane filter (Nucleopore Corp., Pleasanton, Calif.) and suspended in water. Alternately, heat-stressed and nonstressed cells were added directly to respirometer flasks after appropriate dilution. Each flask contained 2.0 ml of yeast suspension (2.0 to 4.5 mg/ml, dry weight). The center well contained 0.2 ml of 20% KOH or water and a folded strip (2 by 2 cm) of Whatman no. 1 filter paper. In some experiments, 2.2 or 11 μ mol of glucose in 0.2 ml or 0.2 ml of 10^{-3} M 2,4-dinitrophenol (DNP) were added from the side arm. In addition, sometimes DNP was added immediately after heating. The gas phase was air. A dry-weight turbidity curve was utilized to determine the dry weight of the yeast. Production of CO₂ was determined by the direct method (15).

RESULTS

Respiration of heat-stressed cells. Differences in endogenous O₂ uptake between heat-stressed and unheated cells of *S. cerevisiae* Y25 were noted. Oxygen uptake of cells heat stressed at 56°C for 1 or 2 min was considerably higher than that of unheated cells (Fig. 1). Cells heated for 1 min at 56°C had a high initial uptake of O₂ which declined shortly after measurements were started. Cells heat stressed for 2 min at 56°C also had a high initial rate of O₂ uptake which continued through the h 1 of measurement. The prolonged high rate of O₂ uptake was associated with thermal injury, as indicated by reduced recovery of heat-stressed cells on PDA (Table

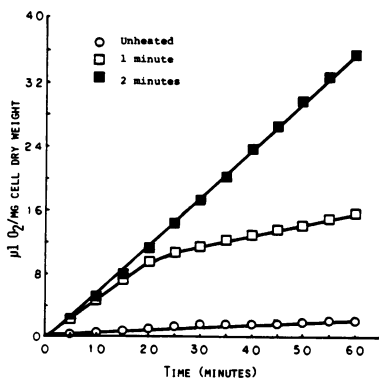


FIG. 1. Endogenous respiration at 30°C of heat-stressed *S. cerevisiae* in water. Cells were heated for 0, 1, or 2 min at 56°C.

1). Plate counts on PCA and PDA of unheated cells and cells heated for 1 min were similar. Plate counts of cells heated for 2 min were initially lower on PDA in comparison with controls. Plate counts on PCA and PDA of cells heat stressed for 2 min and stored for 20 h in water were similar.

Endogenous O₂ uptake rates of unheated and heated cells varied during the first 4 h after heating (Table 2). Unheated cells had a low Q_{O_2} (μ l of O₂/mg of dried yeast per h) of approximately 2.0 during the 4h period. Cells heat stressed for 1 min had an average initial Q_{O_2} of 40.6, which declined to 7.5 during h 2. Cells heated for 2 min had an initial Q_{O_2} of 39.7, which declined to 33.5 during h 2 and then gradually declined to 6.0 during h 4. Interestingly, after 20 h of storage, endogenous rates of O₂ uptake of heated cells were actually lower than those of unheated cells; the Q_{O_2} of unheated cells was 2.0, and those of cells heated for 1 and 2 min were 0.8 and 0.9, respectively.

Respiratory quotients (RQ) of heat-stressed cells were also different from those of unheated cells (Table 3). The respiratory quotient is defined as the ratio of CO₂ produced per O₂ consumed. Unheated cells had RQ values at or near 1.00 during the first 3 h of measurement. Cells heat stressed for 1 min had an initial RQ of 0.70, associated with the high initial rates of O₂ uptake, which then increased to 0.91 to 0.93 by h 3 or 4. In contrast, cells heat stressed for 2 min had an initial RQ of 1.04 which dropped to 0.72 during the h 2 and increased to 0.95 and 0.93 during h 3 or 4.

TABLE 1. Effect of storage at 22°C on plate counts of heat-stressed *S. cerevisiae*

Heating time, (min)	Plate counts after (h of storage):			
	0		20	
	PCA	PDA	PCA	PDA
0	1.4×10^8	1.4×10^8	1.3×10^8	1.3×10^8
1	1.5×10^8	1.3×10^8	1.4×10^8	1.3×10^8
2	1.3×10^8	7.1×10^7	1.2×10^8	1.1×10^8

TABLE 2. Rate of endogenous oxygen-uptake at 30°C for unheated and heat-stressed *S. cerevisiae*

Heat stress (min at 56°C)	Q_{O_2} at (h after heating): ^a			
	0	1-2	2-3	3-4
0	2.0	1.9	1.8	1.8
1	40.6	7.5	6.4	5.4
2	39.7	33.5	16.7	6.0

^a Q_{O_2} as defined in the text.

TABLE 3. RQ values at 30°C for endogenous respiration of unheated and heat-stressed *S. cerevisiae*

Heat stress (min at 56°C)	RQ at (h after heating):			
	0	1-2	2-3	3-4
0	1.00	0.98	1.00	0.80
1	0.70	0.78	0.91	0.93
2	1.04	0.72	0.95	0.93

Rates of oxygen uptake in unheated cells and cells heat stressed for 1 min increased when DNP was added (Fig. 2). However, oxygen uptake in cells heat stressed for 2 min declined when DNP was added. The results were similar when DNP was added immediately after heating (data not shown).

Addition of glucose (2.2 or 11 μ mol) stimulated oxygen uptake in unheated cells and cells heat stressed for 1 min; however, little stimulation in the rate of oxygen uptake was observed in cells which were heat stressed for 2 min (Fig. 3). In addition, after 4 h the total oxygen uptake resulting from addition of glucose was more than 2 times greater for cells heat stressed for 2 min than it was for unheated cells or cells heat stressed for 1 min.

DISCUSSION

Respiration of heat-stressed cells. Rates of endogenous respiration appeared to reflect thermal injury. Prolonged high rates of endogenous respiration were related to evidence of differential recovery of heat-stressed cells on PCA and PDA. After 20 h of storage when plate counts on PCA and PDA of heat-stressed cells were similar, rates of oxygen uptake were diminished to levels approximating or lower than those of unheated cells.

Brandt (3) reports a high endogenous oxygen uptake in cells of *S. cerevisiae* heat stressed at 50°C, including evidence of trehalose disappearance from cell reserves concomitant with high rates of oxygen uptake. Although attempts were not made to measure trehalose in this investigation, the initial RQ of cells heat stressed for 2 min appeared to reflect carbohydrate utilization. In a recent investigation, Baldy et al. (1) report that endogenous oxygen uptake of unheated and heated *Penicillium expansum* spores are similar; however, viability of heated spores is less than 1.0% of unheated spores. In contrast, viability of heated cells utilized in this investigation were only slightly reduced in comparison with unheated cells, even though endogenous rates of O₂ uptake were drastically different. In studies of *Candida* P25, a psychrophilic yeast, Meyer

(Ph.D. thesis) reported declines in endogenous oxygen uptake during exposure of heat-stressed cells to supramaximal temperatures. The reduced Q_{O₂} correlates with decreased viability. Viability and damage to respiratory activity are repairable after cells are heat stressed in water, but are irreparable when cells are heat stressed in the presence of glucose. In addition, decreases in viability are greater when cells are heat stressed in the presence of glucose.

Changes in the RQ values of heat-stressed *S. cerevisiae* Y25 may have reflected changes in endogenous substrate utilization or assimilation of substrates for repair of injury. Although the

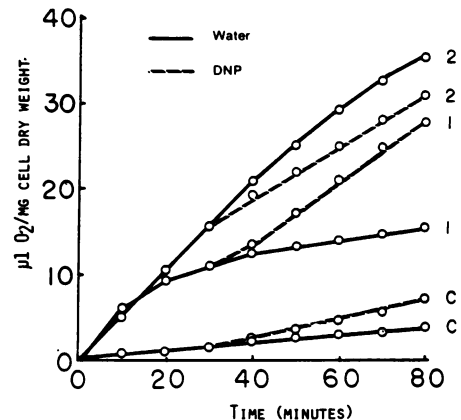


FIG. 2. Effect of addition of 0.10 mM DNP on endogenous respiration at 30°C of heat-stressed *S. cerevisiae* in water. Cells were heated for 0, 1, or 2 min at 56°C, and DNP was added after 30 min.

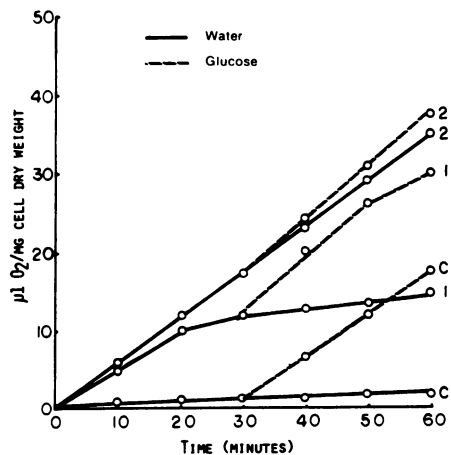


FIG. 3. Effect of addition of 1.0 mM glucose on respiration at 30°C of heat-stressed *S. cerevisiae* in water. Cells were heated for 0, 1, or 2 min at 56°C, and glucose was added after 30 min.

theoretical RQ is 1.0 for carbohydrates, 0.9 for amino acids and proteins, and between 0.7 and 0.8 for lipids (6), "oxidative assimilation" of substrates may result in less than theoretical oxygen uptake (4). Furthermore, Wilson and McLeod (17) show that the endogenous respiration of *S. cerevisiae* is a complex process which concurrently utilizes glycogen, trehalose, proteins and fatty acids.

Spiegelman and Nozawa (12) and Wilson et al. (18) report a decrease in respiratory quotients when endogenous respiration of *S. cerevisiae* is measured over a period of a few hours. This investigation found a similar trend, since the RQ of unheated cells declined to 0.80 after 3 h. In contrast, heat-stressed cells were observed to undergo interesting fluctuations in RQ. Cells heat stressed for 1 min demonstrated no detectable injury with respect to differences in plate counts on PCA and PDA; however, the RQ was 0.70 initially and increased to 0.93 after 3 h of storage. Furthermore, cells heat stressed for 2 min, which sustained thermal injury as demonstrated by differences in plate counts on PCA and PDA, were observed to have an RQ of 1.04 immediately after heating. The RQ dropped to 0.72 during the h 2 of storage, and then increased to between 0.93-0.95.

In cells heat stressed for 2 min at 56 C respiration was temporarily uncontrolled. This may have been due to activation of mitochondrial adenosine triphosphatase or uncoupling of oxidative phosphorylation or both. Since RQ values of heat-stressed cells returned to values near 0.95 after dropping to values near 0.70 after heat stress, the injured yeasts presumably altered their metabolism during the repair process. The initial RQ of 1.04 plus the high Q_{O_2} of cells heat stressed for 2 min may have represented uncontrolled respiration which resulted from thermal injury. Ward (16) postulates uncoupling of respiration as an explanation for anomalous respiratory activity in the presence of DNP and reduced incorporation of substrate of *Sclerotinia borealis* exposed to maximal temperatures for growth. Obviously, without further evidence such as studies of substrate utilization and P/O ratios, the postulate above must remain as speculation.

Further evidence of thermal injury was provided by the rates of oxygen uptake observed in the presence of DNP. Addition of DNP to unheated cells or cells heat stressed for 1 min resulted in increased oxygen uptake typical of uncoupler activity. On the other hand, oxygen uptake in cells heat stressed for 2 min was depressed by the addition of DNP. One possible explanation is that intracellular concentrations

of DNP were increased in heat-injured cells due to enhanced entrance of DNP through damaged membranes. Alternatively, low concentrations of DNP may have uncoupled oxidative phosphorylation of thermally injured mitochondria in a manner similar to that observed at higher concentrations of DNP with mitochondria of non-stressed cells.

Lee (10) determined that stimulation or inhibition of respiration of *S. cerevisiae* by DNP is dependent on DNP concentration, pH, and metabolic state of the cells. Inhibition of respiration is observed at high concentrations of DNP, 5×10^{-4} M or higher, and at a pH of 5.0 or lower. Stimulation of respiration is observed at lower concentrations of DNP or at higher pH. The inhibition is also dependent on the metabolic state of the cells.

Oxygen uptake in response to the addition of glucose was related to thermal stress in this investigation. Although oxygen uptake due to glucose addition was not appreciably stimulated initially in cells heat stressed for 2 min, the high rate of oxygen-uptake in those cells may have precluded or masked its effect. However, glucose increased the total oxygen uptake of cells heat stressed for 2 min in comparison with unheated cells and cells heat stressed for 1 min. The increased oxygen consumption may have reflected utilization of added glucose to meet increased energy demands of heat-stressed cells. Alternatively, Hagler and Lewis (9) reported that addition of glucose results in damage to yeast cytoplasmic membranes after thermal stress. Thus, addition of glucose may have increased oxygen consumption due to further injury. Baldy et al. (1) also reported that glucose inhibits recovery from thermal injury in *P. expansum* conidia.

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LITERATURE CITED

- Baldy, R. W., N. F. Sommer, and P. M. Buckley. 1970. Recovery of viability and radiation resistance by heat-injured conidia of *Penicillium expansum* Lk. ex Thom. *J. Bacteriol.* 102:514-520.
- Baxter, R.M., and N. E. Gibbons. 1962. Observations on the physiology of psychrophilism in a yeast. *Can. J. Microbiol.* 8:511-517.
- Brandt, K. M. 1941. Über die Reservekohlenhydrate der Presshefe. Das Verhalten der Trehalose beim Wachstum und bei der Warmeschädigung der Hefe. *Biochem. Z.* 309:190-201.
- Dawes, E. A., and E. W. Ribbons. 1962. The endogenous metabolism of microorganisms. *Annu. Rev. Microbiol.* 16:241-264.
- Evison, L. M., and A. H. Rose. 1965. A comparative study on the biochemical bases of the maximum tem-

- peratures for growth of three psychrophilic microorganisms. *J. Gen. Microbiol.* **40**:349-364.
6. Geise, A. C. 1962. Cell physiology. W. B. Saunders Co., Philadelphia.
 7. Grant, D. W., N. A. Sinclair, and C. N. Nash. 1968. Temperature-sensitive glucose fermentation in the obligately psychrophilic yeast *Candida gelida*. *Can. J. Microbiol.* **14**:1005-1010.
 8. Graumlich, T. R., and K. E. Stevenson. 1978. Recovery of thermally injured *Saccharomyces cerevisiae*: effects of media and storage conditions. *J. Food Sci.* **43**:1865-1870.
 9. Hagler, A. N., and M. J. Lewis. 1974. Effect of glucose on thermal injury of yeast that may define the maximum temperature of growth. *J. Gen. Microbiol.* **80**:101-109.
 10. Lee, D. C. 1970. Inhibition of respiration in baker's yeast (*Saccharomyces cerevisiae*) by 2,4-dinitrophenol, p. 199-206. In D. A. Ahearn (ed.), Recent trends in yeast research. School of Arts and Sciences, Georgia State Univ., Atlanta.
 11. National Canners Association. 1968. Laboratory manual for food canners and processors, vol. 1. Microbiology and processing. Avi Pub. Co., Westport, Conn.
 12. Spiegelman, A., and M. Nozawa. 1945. On the inability of intact yeast cells to ferment their carbohydrate reserves. *Arch. Biochem.* **6**:303-322.
 13. Shibasaki, I., and T. Tsuchido. 1973. Enhancing effect of chemicals on the thermal injury of microorganisms. *Acta Aliment.* **2**:327-349.
 14. Sinclair, N. A., and J. L. Stokes. 1965. Obligately psychrophilic yeasts from the polar regions. *Can. J. Microbiol.* **11**:259-269.
 15. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1972. Manometric and Biochemical Techniques. Burgess Pub. Co., Minneapolis, Minn.
 16. Ward, E. W. B. 1968. The low maximum temperature for growth of the psychrophile *Sclerotinia borealis*: evidence for the uncoupling of growth from respiration. *Can. J. Bot.* **46**:524-525.
 17. Wilson, K., and B. J. McLeod. 1976. The influence of conditions of growth on the endogenous metabolism of *Saccharomyces cerevisiae*: effect on protein, carbohydrate, sterol and fatty acid content and on viability. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **42**:397-410.
 18. Wilson, K., B. J. McLeod and R. Cooper. 1977. The influence of conditions of growth on the endogenous metabolism of *Saccharomyces cerevisiae*: effect on respiratory activity. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **43**:233-244.