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EXPERIMENTAL ARTICLES

The Absence of a Direct Relationship between the Ability of Yeasts to Grow at Elevated Temperatures and Their Survival after Lethal Heat Shock

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Abstract—The study of the growth of the yeasts *Rhodotorula rubra*, *Saccharomyces cerevisiae*, and *Debaryomyces vanriji* at elevated temperatures and their survival after transient lethal heat shock showed that the ability of these yeasts to grow at supraoptimal temperatures (i.e., their thermoresistance) and their ability to tolerate lethal heat shocks (i.e., their thermotolerance) are determined by different mechanisms. It is suggested that the thermotolerance of the yeasts is mainly determined by the division rate of cells before their exposure to heat shock.

Key words: maximum growth temperature, growth rate, thermoresistance, thermotolerance, yeasts.

Any microorganism is characterized by its own specific temperature range within which it can grow. According to their inherent growth temperatures, all yeasts can be classified into psychrophilic, mesophilic, thermotolerant, and thermophilic [1]. At supraoptimal temperatures, the growth of yeasts slows down. At temperatures exceeding the maximum growth temperature, yeasts cannot grow. As a rule, the decline in the growth rate of yeasts at elevated temperatures is accompanied by an increase in cell mortality. In contrast, van Uden found that the cessation of cell growth in the yeasts *Candida curiosa*, *Lipomyces kononenkoae*, and *Cryptococcus neoformanus* exposed to temperatures exceeding the maximum growth temperature by 3–4°C was not accompanied by cell death and explained this phenomenon by the fact that these three yeasts had a dissociative temperature profile, while the other yeasts studied had an associative temperature profile [2].

A cell exposed to heat shock synthesizes the so-called heat shock proteins (HSPs). The mild heat pretreatment of *Saccharomyces cerevisiae* cells enhances their tolerance to subsequent lethal heat shock [3, 4]. This phenomenon, known as acquired thermotolerance or thermal hardening [3, 4], is mainly due to the induced synthesis of the 104-kDa HSP and the accumulation of trehalose in the cells exposed to heat shock. The deletion of the HSP104 gene in *S. cerevisiae* cells did not affect their ability to grow at the maximum growth temperature (37.5°C) [3]. Similarly, the deletion of the trehalose-6-phosphate synthetase gene *TPS1* in *Hansenula polymorpha* cells did not affect their ability to grow at elevated temperatures but diminished their acquired thermotolerance [5]. In contrast, the deletion of the *S. cerevisiae HSC82* and *HSP82* genes coding for highly homologous proteins of the HSP90 family made this yeast unable to grow at elevated temperatures but did not affect the survival of yeast cells exposed to lethal heat shock [6]. These experimental data suggest that the ability of cells to grow at supraoptimal temperatures (i.e., their thermoresistance) and their ability to tolerate transient lethal heat shocks (i.e., their thermotolerance) are determined by different mechanisms.

This work was undertaken to verify this suggestion. To this end, we compared the growth rates of three yeasts (*Rhodotorula rubra*, *Saccharomyces cerevisiae*, and *Debaryomyces vanriji*) at different temperatures and their survival rates after exposure to lethal heat shock.

MATERIALS AND METHODS

Experiments were carried out with the strains *Debaryomyces vanriji* (formerly *D. vanriji*) GK46-2 and *Rhodotorula rubra* Dz40-1 isolated form the hot springs of Buryatia and the strain *Saccharomyces cerevisiae* Ψ-74-D694 kindly provided by S. Lindquist from the University of Chicago, United States. The strains were grown either in a YEPD medium containing (g/l) yeast extract, 5; peptone, 10; and glucose, 20 or in a minimal medium containing (g/l) KH_2PO_4, 0.9; K_2HPO_4, 0.1; $MgSO_4$, 1; $(NH_4)_2SO_4$, 1; and glucose, 20. The minimal medium was either supplemented with 0.2 mg/ml thiamine or not. Solid media were prepared

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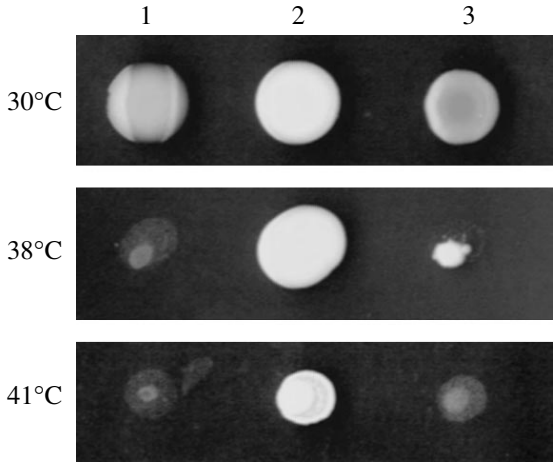

Fig. 1. The growth of (1) *R. rubra*, (2) *D. vanriji*, and (3) *S. cerevisiae* on YEPD agar at 30, 38, and 41°C. Cells for inoculation were grown in a liquid YEPD medium at 30°C. The agar plates were incubated for 48 h.

by adding 15 g/l agar to the aforementioned media. During experiments, the strains were maintained on a YEPD medium at 30°C.

Cells for inoculation were grown on a shaker at 30°C either in a YEPD or minimal medium for 14–24 h and then transferred, in a certain amount, to fresh medium. The growth of yeasts in a YEPD medium was monitored nephelometrically by measuring culture turbidity at 600 nm in a Specol 20 spectrophotometer. The generation time (g) of cells was calculated by the formula

$$g = \frac{t - t_0}{3.32(\log N - \log N_0)},$$

where N_0 and N are the numbers of cells at times t_0 and t, respectively [7].

The effect of cultivation temperature on yeast growth was studied by plating logarithmic-phase cells onto YEPD agar and incubating the plates at 30, 38, and 41°C for 48 h. The effect of thiamine on the ability of *D. vanriji* to grow at elevated temperatures was studied by plating the exponential-phase cells of this yeast onto minimal agar either containing thiamine or not. The plates were incubated at 30 and 41°C for 72 h and then examined for the ability of yeasts to grow.

The effect of lethal heat shock on cell survival was studied by incubating test tubes with 1 ml of yeast suspensions at 45 and 50°C for 2 to 60 min. The effect of thiamine on the thermotolerance of *D. vanriji* cells was studied by growing yeast cells at 30°C in a liquid minimal medium with or without thiamine and then exposing them to heat shock at 45°C. Alternatively, thiamine was added to the incubation medium of yeast cells 15 min prior to their exposure to heat shock. After exposing to heat shock, the suspension of yeast cells was cooled, appropriately diluted, and plated onto YEPD agar. The number of grown colonies on the plates was determined after 24–48 h of their incubation at 30°C. The survival rate was calculated as a percent of colonies grown from heat-exposed cells relative to the number of colonies grown from unexposed yeast cells.

RESULTS AND DISCUSSION

The growth temperatures of the yeasts known to date range from –2 to 45°C [1]. All of the three yeasts under study showed good growth on YEPD agar at 30°C (Fig. 1, the upper photograph). At 38°C, *D. vanriji* grew well, *S. cerevisiae* grew poorly, and *R. rubra* showed no growth at all (Fig. 1, the middle photograph). At 41°C, only the yeast *D. vanriji* showed growth (Fig. 1, the lower photograph). Thus, among the three yeasts, *R. rubra* had the lowest maximum growth temperature.

The study of the effect of lethal heat shock on the yeast cells grown at 30°C gave unexpected results: after 60-min exposure to 45°C, 70% of *R. rubra* cells remained viable (Fig. 2, curve *1*), whereas only 3.4% of *S. cerevisiae* cells and 1.4% of *D. vanriji* cells survived this treatment (Fig. 2, curves *3* and *2*, respectively). The comparison of the survival rates of *S. cerevisiae* and *R. rubra* cells grown at 30°C and then exposed to 50°C showed that the latter yeast was much more tolerant to this temperature (Fig. 3). Thus, *R. rubra*, which has the lowest maximum growth temperature among the three yeasts studied, turned out to be the most thermotolerant under the conditions used in these experiments (cells of all three yeasts were grown at the same growth temperature equal to 30°C). In other words, there is no direct relationship between the survival rate of cells exposed to lethal heat shock and their maximum growth temperature.

The situation may change if yeasts are grown at their maximum growth temperature before exposure to heat shock. For instance, unlike the *D. vanriji* cells grown at 30°C, the same cells grown at 41°C are fairly resistant to heat shock at 46°C [8]. Similarly, the *S. cerevisiae* cells grown at 37°C are much more resistant to heat shock than the same cells grown at 30°C [9]. This phenomenon is known as “temperature adjustment” [4].

It is known that the mild heat pretreatment of *S. cerevisiae* brings about a delay in the G_1 phase of the cell cycle of this yeast and enhances its tolerance to lethal heat shock [10]. This effect is under the negative control of the cAMP-dependent protein kinase A. Raising artificially the level of cAMP in cells results in the continuation of their growth at elevated temperatures and eventual cell death at a high rate [10, 11]. In contrast, mutants with the altered gene of adenylate cyclase (the enzyme that synthesizes cAMP) poorly grow at normal temperature but remain fairly viable when exposed to heat shock [12, 13].

The rate of HSP synthesis likely depends on the proliferating activity of cells, as is evident from the obser-

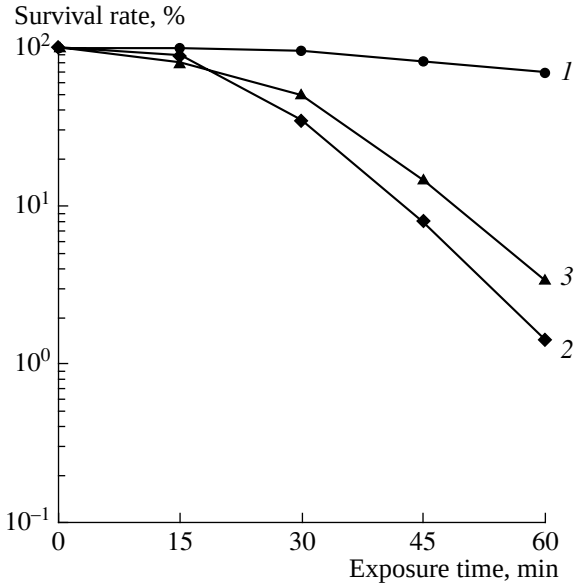

Fig. 2. The survival rates of (*1*) *R. rubra*, (*2*) *D. vanriji*, and (*3*) *S. cerevisiae* cells grown in a liquid YEPD medium at 30°C and exposed to heat shock at 45°C.

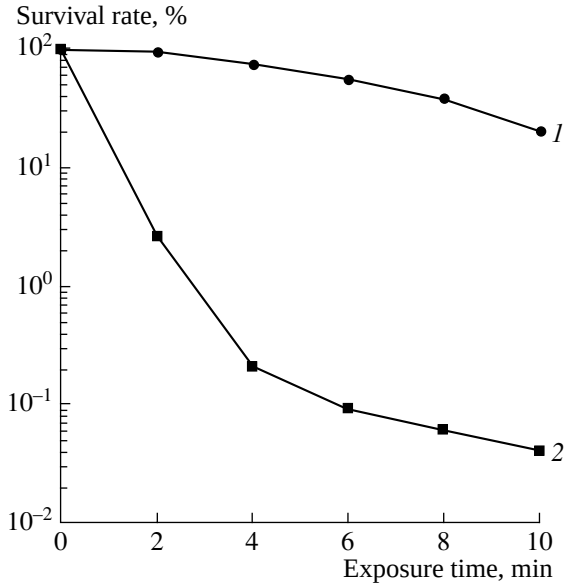

Fig. 3. The survival rates of (*1*) *R. rubra* and (*2*) *S. cerevisiae* cells grown in a liquid YEPD medium at 30°C and exposed to heat shock at 50°C.

vations that mutants with an altered adenylate cyclase bring about the constitutive synthesis of HSPs [12–14], whereas, when exposed to mild heat shock, the *bcy1* mutants with the cAMP-independent protein kinase A continue to divide, do not synthesize some HSPs, and do not acquire tolerance to subsequent lethal heat shock [14].

These data suggest that the ability of cells to withstand lethal heat shock depends on their growth rate prior to exposure to heat shock. Indeed, among the three yeasts studied, the generation time of *R. rubra* in liquid YEPD medium at 30°C in the logarithmic growth phase was maximum (2.2 h) (Fig. 4, curve *1*), whereas that of *D. vanriji* was minimum (1.5 h) (Fig. 4, curve *2*). The generation time of *S. cerevisiae* was intermediate (1.86 h) (Fig. 4, curve *3*). After 8 h of growth, the latter yeast showed a diauxic shift from glucose to ethanol, which also correlated with an abrupt increase in cell thermotolerance [11].

The inverse dependence of the thermotolerance of cells on their growth rate does not exclude the possibility of existence of other mechanisms allowing cells to tolerate heat shocks. For instance, the high thermotolerance of *R. rubra* can be related to the enhanced synthesis of carotenoid pigments, which scavenge reactive oxygen species formed in cells in response to heat shock [15] and thus reduce the toxic action of the oxidative stress induced by heat shock [16].

Our earlier investigations showed that the thermophilic bacterium *Bacillus* sp. isolated from a hot spring in association with the yeast *D. vanriji* secretes thiamine into the culture medium. This vitamin was found to stimulate yeast growth at normal temperature and to

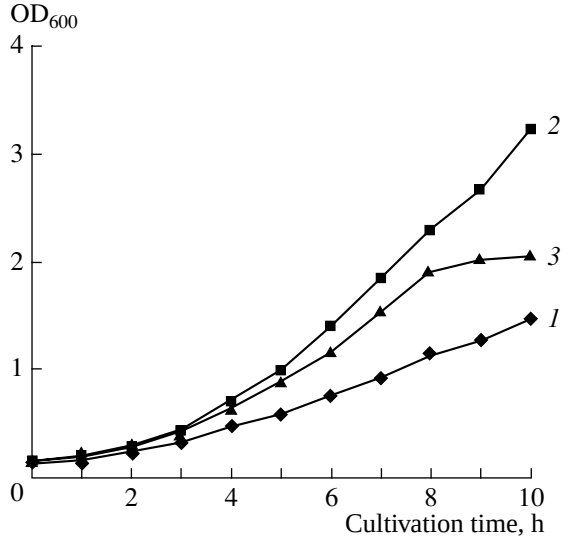

Fig. 4. The growth of (*1*) *R. rubra*, (*2*) *D. vanriji*, and (*3*) *S. cerevisiae* in a liquid YEPD medium at 30°C.

increase the maximum growth temperature [17]. In this work, we also found that thiamine added to the cultivation medium made *D. vanriji* capable of growing at 41°C (Fig. 5, photographs 3, 4). The control experiments (cultivation at 30°C) showed that the yeast *D. vanriji* is able to grow without thiamine, albeit at a low rate (Fig. 5, photographs 1, 2).

The study of the effect of thiamine on yeast tolerance to 45°C showed that the addition of this vitamin to the suspension of yeast cells grown in the thiamine-free minimal medium was ineffective (Fig. 6, curve *2*).

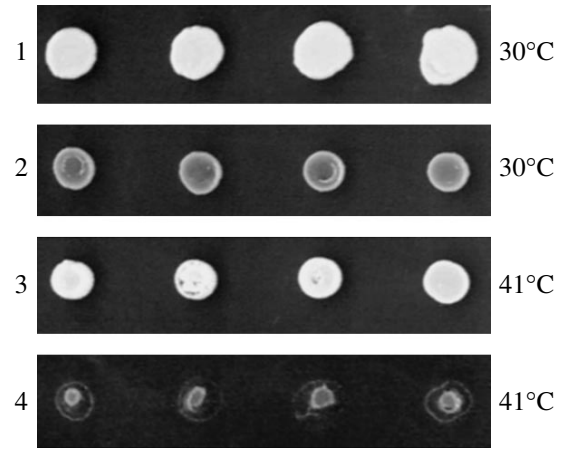

Fig. 5. The effect of thiamine on the growth of *D. vanriji* on an agar medium at 30 and 41°C. Yeast cells were grown at 30°C in a liquid minimal medium without thiamine, plated onto minimal agar with (1, 3) and without (2, 4) thiamine, and incubated for 72 h at the indicated temperatures.

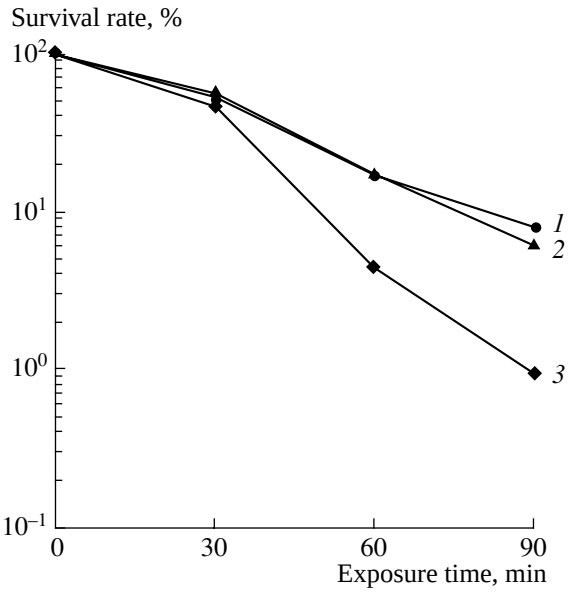

Fig. 6. The effect of thiamine on the survival rates of *D. vanriji* cells exposed to heat shock at 45°C. Yeast cells were grown at 30°C in a liquid minimal medium with (*3*) and without (*1*, *2*) thiamine and then exposed to the heat shock. In the experimental variant *2*, thiamine was added to the medium 15 min before the exposure of cells to the heat shock.

Moreover, the *D. vanriji* cells grown in the presence of thiamine turned out to be more sensitive to heat shock than those grown in the thiamine-free medium (Fig. 6, curves *1*, *3*). In view of the fact that thiamine stimulates the growth of *D. vanriji* in a minimal medium [17], these data confirm the suggestion that enhanced growth rates diminish the thermotolerance of yeast cells. Thus, thiamine favors the growth of *D. vanriji* at elevated temperatures but does not enhance the ability of this yeast to tolerate lethal heat shock.

It can be suggested that the survival rate of a yeast exposed to heat shock is determined by its previous growth rate (the survival rate being the greater, the lower the previous growth rate) rather than by the maximum growth temperature of this yeast. This suggestion is in agreement with the observations of Elliott and Futcher [18], who showed that slower-growing *S. cerevisiae* cells are characterized by a higher tolerance to lethal heat shock.

It is believed that the major cause of heat-induced damage to cells is largely determined by the aggregation and denaturation of cell proteins, especially newly synthesized proteins, which have no time to acquire native conformation [10]. This suggests that the survival of yeast cells exposed to lethal heat shock must depend on the rate of protein synthesis, which, in turn, depends on the rate of cell growth and division. This suggestion is likely to be valid not only for heat shock but also for other types of stresses. For instance, Park *et al.* [19] showed that, when exposed to a freeze–thaw cycle, *S. cerevisiae* cells became tolerant to a second such cycle, but lost the acquired tolerance if they were allowed to divide between the first and second freeze–thaw cycles. The same phenomenon may be responsible for so-called cross-tolerance, when one mild stress induces tolerance to another stress [4, 10].

Thus, the ability of yeasts to grow at extreme temperatures (i.e., their thermoresistance) and their ability to tolerate lethal heat shocks (i.e., their thermotolerance) are determined by different mechanisms. As an aside, N.F. Reimers treats the term *resistance* (of an organism) as its ability to withstand (or tolerate) external stresses and the term *tolerance* as the ability of an organism to withstand the deviation of ecological parameters from optimal values [20]. As is evident from these definitions, the terms thermoresistance and thermotolerance are used as synonyms in the scientific literature. In particular, both of these terms are used to evaluate the ability of organisms to grow at elevated temperatures and to survive lethal heat shock. The experimental data presented in this paper show that cell thermoresistance and thermotolerance are governed by different, although similar, mechanisms. In view of this, we propose using the term thermotolerance to indicate the ability of organisms to tolerate lethal heat shock and the term thermoresistance to indicate their ability to grow at supraoptimal temperatures.

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