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Effect of the kinetics of temperature variation on *Saccharomyces cerevisiae* viability and permeability

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Abstract

The variation rate of the temperature increase was found to have a great effect on the viability of *Saccharomyces cerevisiae* subjected to heat perturbations between 25° C and 50° C. A low intensity of the increase rate of temperature could maintain an important viability of the cells (about 34% of the initial population) with regard to the corresponding viability (about 1%) observed after a sudden step change for the same final temperature level of 50° C. A cell volume reduction more important (22% of the initial volume) has been observed in cells submitted to a heat shock than for the cells which have been submitted to a slow kinetic of temperature increase (9%). Such an observation allowed to propose a relation between the membrane permeability and the kinetics of temperature variation.

Keywords: Temperature; Cell viability; (Yeast)

1. Introduction

Numerous workers have shown the influence of the temperature level of the medium on the viability [1,2] and on the growth of microorganisms [3–5]. Several works have shown an increase in thermotolerance of *S. cerevisiae* when a preconditioning at intermediate temperatures was realized previously the heat shock [6–10]. Some workers have also studied the influence of the time of temperature stress application on the reduction of the viability of *S. cerevisiae* growing exponentially [6,11]. Such works and previous works (more than a century ago) have allowed the precise definition of heat-time treatments intended to reduce the contamination level of food or medical products. So, theoretical curves of temperature-viability dependence are very well known and this dependence has been related to the temperature effect on enzyme activity [12] which is quite well described by the Arrhenius law.

Nevertheless, a cell could not be considered as simple as an enzyme bag and the modification of the membrane permeability [13,14] and the variation of the solute internal composition through glucose and trehalose accumulation [2,10,15] or through heat shock proteins synthesis [8] could interfere with such a temperature influence. However, heat shock proteins may not be needed for stress-tolerance acquisition but for a rapid recovery from the stress-affected situation [16,17]. The trehalose accumulated after a heat shock might prevent the injury of cytosol by the heat shock or by associated dehydration [2,18] and/or the injury of the cell membrane [19]. Some workers [9,20] using mutants of trehalose synthesis have shown that the trehalose was a thermoprotector in yeasts but was not essential for the survival; mutants of *S. cerevisiae* lacking the trehalose carrier but being able to synthesize trehalose did not survive to a dehydration at 30° C [21]. They concluded that the trehalose is required on both sides of the yeast cell membrane to protect the cell during dehydration. On the other hand, these mutants were found to be thermoresistant due to an internal accumulation of trehalose; so in this case, trehalose protects proteins against denaturation [9].

Moreover, the temperature variation will passively induce other cellular variations, as membrane, mitochondria and nucleolus damage [1,13,22], physical state of intracellular water [23,24] and cell water potential which could induce passive water flow [25]. In reaction to external heat perturbation the cell could have possibilities of passive and active reactions or adaptations in order to prevent the dramatic effect of heat on cell activity.

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From these considerations this work was intended to study the influence of the kinetics of application of the heat perturbation. These kinetics could be influential on the passive and active reactions of the cells of which the time constants could be different than those for the heat transfer phenomena. Literature about these kinetic influences is very rare. Nevertheless, Dowben and Weidenmüller [3] have shown that the mesophilic bacteria *B. subtilis* can grow at elevated temperatures (72° C) through a slow increase in temperature, and Durvy and Magnand [26] have noticed an effect of the rate of increase and decrease of the external temperature on the luminescence intensity of an *Achromobacter sp.*

2. Materials and methods

2.1. Microorganism and cultivation conditions

Saccharomyces cerevisiae CBS 1171 was maintained on gelose slants, the composition of which was Malt Wickerham medium with 15 g/l of Pastagar A. The Malt Wickerman medium was composed of 10 g of glucose, 3 g of pancreatic peptone, 3 g of yeast extract and 1.5 g of NaH_2PO_4 in 1 l of solution of water and sorbitol which had an osmotic pressure of 1.38 MPa (100.59 g sorbitol/1 l water). Sorbitol has a thermoprotective effect on proteins during dehydration [27]. This osmotic pressure level corresponded to optimal growth conditions [28]. The total cell population was followed using a Malassez counting cell. Initial inoculum was about 10^5 cells for 1 ml. Cultures were led in 250 ml erlenmeyer for 48 h corresponding, in all cases, to the beginning of the stationary phase [29].

2.2. Experimental design for step change and linear increases of the temperature of the medium

For the viability experiments

Temperature step changes (shocks): at time t_0, 1 ml of yeast cultivation at the initial temperature is quickly added to 99 ml of a binary solution (water-sorbitol at 1.38 MPa) at the final temperature of 50° C. The obtained solution was maintained for one hour at the final temperature with a 300 rpm magnetic mixing in a water bath. Such an experiment will approach the ideal step change variation, nevertheless the mixing time will always exist and the realization of a perfect step change is physically impossible [30].

Temperature linear changes (slopes): 1 ml of cultivation sample was added in a conical flask containing 99 ml of a binary solution (sorbitol-water at 1.38 MPa) at the same temperature of 25° C. The conical flask was placed in a water bath previously calibrated in volume and temperature controlled in order to generate a specific slope of temperature. The rates of temperature increase in this paper were: 1.04° C min^{-1}, 0.625° C min^{-1}, 0.417° C min^{-1} and 0.208° C min^{-1}. As for step changes, the final solution was maintained during 1 h at the final temperature (50° C) with 300 rpm mixing.

In both cases for steps and slopes, after the exposure to 50° C for one hour, the temperature of the solutions was decreased progressively for 45 min to 25° C in order to limit the influence of this decreasing temperature stress. This controlled refrigeration corresponds to a time of 3.5 min in the critical range between 50° C and 45° C.

For the volume variation measurements

A visualization chamber of 113 mm^3 related to a microscope and an image analysis system was used. A 200 μl volume of cell suspension was fixed in the center of the chamber. This system was precisely described in a previous work [30]. A solution, temperature controlled, was then injected in the chamber through a peristaltic pump (Micropump corporation, USA) with a flow of 30 ml min^{-1}. For the slope realization the linear increase in temperature was realized as proposed for the design allowing the viability study. In all experiments latex particles (Coulter electronics, UK) were used to assure that cells were focused during the experiments in order to allow the correct measurement of the area variation of the cells submitted to a shock or to a slope of temperature. For all experiments temperature evolution was measured with a Ni-Cu thermocouple.

2.3. Measurement of cell viability after heat treatment

The viability measurement was performed through the CFU method by plating the cells in the Malt Wickerham medium with 15 g l^{-1} of pastagar A after the appropriate dilutions. The dilution was done after progressive return for 45 min to the room temperature (25° C). All dilutions were performed in water-sorbitol solutions (osmotic pressure: 1.38 MPa) without nutriments. For each dilution step 100 μl of solution was inoculated in three Petri dishes. Petri dishes were then incubated for 3 and 5 days at 25° C as described in a previous work [29].

2.4. Measurement of cell volume variation during heat treatment

The previous chamber is used in conjunction with an inverted microscope (Leitz-Labovert, Germany). An image analysis system (series 151, Imaging Technology, USA) allows images to be recorded via a camera CCD (model 6710, COHU, Japan) during the heat stress, and then permits their analysis using Visilog software (Noesis, France), enabling area measurement to be made [31]. Image recording began after a time of 5 s corresponding to the delay of the injection system.

In the selected visualization zone, 10 to 20 cells were individually analysed for one treatment (with three replications for each treatment) to find their area. *Saccharomyces*

cerevisiae CBS 1171 are spherical cells, so the volume is calculated from the cell area determination [30].

3. Results and discussion

3.1. Influence of the kinetics of heat perturbation on cell viability

For all experiments, the range 25° C–50° C, which appeared to be discriminating on the cell viability [2,9,22], was only studied in binary media (sorbitol-water). Results proposed in Fig. 1 show the dramatic difference in viability involved by the different kinetics of the heat perturbation. The mortality could be related to the rate of increase of temperature in the medium. After application of a shock, the viability was found to be about 1% (of the initial population) and in case of the different linear increases the viabilities were found to be 15%, 25%, 34% and 5% for respectively 1.04° C min^{-1}, 0.625° C min^{-1}, 0.417° C min^{-1} and 0.208° C min^{-1}. These results were found by counting the colonies after 3 days of incubation at 25° C. The results for 5 days of incubation show approximately the same differences between the different kinetics of temperature increases (4%, 25%, 35%, 42% and 12% for respectively shock, 1.04° C min^{-1}, 0.625° C min^{-1}, 0.417° C min^{-1} and 0.208° C min^{-1}).

The viability was found to increase as the kinetics of temperature variation were decreased until the maximal value of viability obtained for the 0.417° C min^{-1} slope (see Fig. 1). For the slowest linear temperature increase of 0.208° C min^{-1} the final viability was very low (5% of the initial population) which was close to the viability found after the shock application (1%). Such cell comportment could be explained through the opposition of two actions versus the cell viability: firstly, a positive action, which

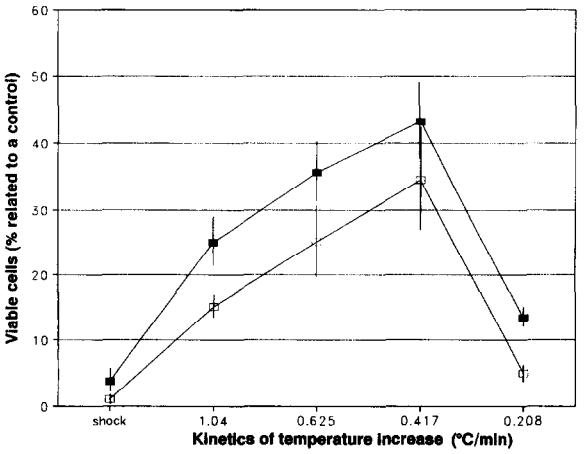

Fig. 1. Survival of *S. cerevisiae* related to the kinetics of temperature increase. (□) Colonies after 3 days and (■) after 5 days of incubation at 25° C. Confidence limit at 90% (vertical bars).

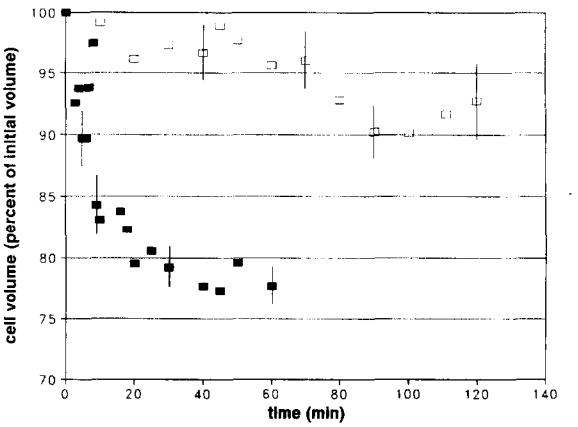

Fig. 2. Evolution of cell volume versus time in response to different kinetics of temperature increase from 25° C to 50° C: (■) step and (□) 0.417° C min^{-1}. The largest 95% confidence interval for both curves have been included (vertical bars).

was the slowness of the temperature variation, and secondly a negative action, which was the residence time of cells in the critical temperature range for *S. cerevisiae*, i.e., above 45° C [7]. Indeed more the slope was weak and more the residence time above the critical temperature was great (63.5 min, 68.3 min, 71.5 min, 75.5 min and 87.5 min for respectively shock, 1.04° C min^{-1}, 0.625° C min^{-1}, 0.417° C min^{-1} and 0.208° C min^{-1}). Moreover, these viability results could also be analysed in term of classical heat-treatment parameters, as it appears that in spite of an equal residence time at the same experimental final temperature for all treatments (exactly 1 h at 50° C), the corresponding cell viability was quite dependent on the kinetics of the temperature increase.

Such results allow to conclude that the kinetics of the application of the heat perturbation are of major importance on the yeast viability. Hottiger et al. [2] have not pointed out trehalose synthesis by *S. cerevisiae* when the cells were transferred to medium without glucose prior the heat shock. All previous experiments were made in binary medium (water-sorbitol), so trehalose synthesis could not be related to the influence of the kinetics perturbations. The use of non nutritive media in these experiments allow to explain the great observed viability differences by only passive mechanisms. In order to understand the kinetics of temperature increase on cell mortality, two mechanisms, which could be involved have been investigated in this work.

First, the existence of an eventual gradient of osmotic pressure between intra and extracellular medium generated by the temperature perturbation and due to the difference of time constants between water transfer (very fast) and heat transfer (slower). So, placing cells instantaneously (at 25° C) in a medium at 50° C will induce an immediate water exit due to an osmotic gradient and so the cell volume decrease. Previous works have shown that such a

sudden water exit from the cell could induce the cell mortality [25]. According to this assumption, as soon as the cell temperature increases due to heat transfer, the cell volume will increase until the recovery of the initial volume.

Second, the physicochemical modification of the plasma membrane due to changes in lipid order as fluidity and permeability could also damage the cells and induce mass transfer across the membrane [13,14].

Both mechanisms, water transfer and membrane permeability modification must induce cell volume modifications. In order to verify such assumptions and through the use of a specific device, the modification of the cell volume has been followed during the application of the heat perturbation.

3.2. Influence of the kinetics of the heat perturbation on cell volume variation

In order to verify if an increase in temperature will involve water and solute transfer, the cell volume evolution was followed during two previous temperature treatments: shock and 0.417° C min^{-1} linear increase. Results concerning the volume variation are proposed in Fig. 2. In case of heat shock, an important decrease of the cell volume was found to occur until the volume reached 78% of the initial volume. Initially, the volume decrease occurred from 2 to 3 min after the heat treatment application and the final volume was reached 17 min after. No recovery of the initial cell volume was observed during the following hour. In the case of the heat slope, the volume decrease was found to be slower and less important. The final volume was about 91% of the initial one and was reached after 90 min.

These results allow some remarks to be made:

Firstly, no recovery of the initial volume was observed after the first decrease in volume following the heat perturbation. So, the assumption of an osmotic shock induced by the temperature gradient between the cell and the medium did not seem to be possible. Moreover, the calculation of theoretical maximal osmotic gradient generated by a temperature gradient of 25° C (between 25° C and 50° C) has confirmed this conclusion by applying the classical relationship between osmotic pressure (Π) and temperature (T) level [29]. Calculations showed that the 25° C temperature increase of the system was equivalent to a sudden osmotic pressure increase of 0.115 MPa which was very inferior to the normal turgor pressure of *S. cerevisiae* [32–34] corresponding to the cell envelope (between 0.3 and 1.3 MPa). So, a temperature stress from 25° C to 50° C cannot be sufficient to increase the osmotic pressure of the medium in order to produce a volume decrease.

The second assumption, which related the volume evolution of the cell at high temperature to a variation of the permeability of the plasma membrane was in agreement with previous works. Van Zoelen et al. [35,36] have shown the greatest non-electrolyte permeability of liposomes at elevated temperatures (30° C). Piper [37] has reviewed numerous results showing the increase of the permeability of *Saccharomyces cerevisiae* with temperature increase. Such increase in permeability has been related to a sudden release of the intracellular glycerol in the alga *Asteromonas gracilis* [14] and to a leakage of yeasts contents and sorbose [13] above the maximum temperature of growth. This leakage was due to a rupture of yeast cytoplasmic membrane. Such a sudden release was necessarily accompanied by a water exit from the cell in order to balance the osmotic equilibrium and so could correspond to an important and sudden volume decrease as observed in our results (22% for the heat shock). With this assumption the final volume which corresponds to a thermodynamic equilibrium must stay constant as observed in our experiments. In the case of the temperature slope application, the cell would have the time to regulate its plasma membrane fluidity and the permeability would be then less affected. The release of solutes (e.g., glycerol) could be then controlled involving a volume reduction less important as observed in our experiments for the temperature slope of 0.417° C min^{-1} (9%). So, the high viability of cells submitted to a progressive heat treatment could be related to the weak variation of corresponding cell volume which is the measured parameter related to the cell permeability modification.

Such results demonstrate the important effect of the kinetics of application of the heat perturbations on *S. cerevisiae* viability. If these results could be extrapolated to others microorganisms this conclusion would have technological applications in biological areas. Indeed applying a sudden heat shock to contaminated products at low temperature (about 50° C) would reduce of about 2 log the proportion of microorganisms without, in the most of the cases, physicochemical perturbations of the products. In the same way, special care should be focused on food pasteurization treatments, and on heat disinfection treatments of industrial materials: slow kinetics of temperature increase could maintain a high contamination level for a same time-temperature treatment. Nevertheless, the high time constant value of heat transfer would be the limiting factor for the industrial realization of a temperature step change on food or biological products.

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