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Influence of the temperature variation on the *Saccharomyces cerevisiae* cells viability

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Abstract

The objective of this work is to study the influence of the temperature variation and of the time of maintenance at low temperature in the range between 30°C and °C on the Saccharomyces cerevisiae kinetics. Cellular viability according to the thermal kinetics of disturbance after various times of maintenance to 0°C was given. The behaviour of the microorganisms is a function of the physiological stage, the kinetics of cooling and the duration of the time of maintenance at low temperature in growth media nutritive or stress media. Our results showed that the cells resulting from the exponential phase of growth are much more sensitive to the thermal stresses applied than those in stationary phase.

Keywords: *Saccharomyces cerevisiae*; stress; temperature; cells viability.

Introduction

Nowadays, more the standard of living improves, the more the need for high quality nutritional and organoleptic foodstuffs consumption increase. The use of living organisms in the transformation of food is extremely common. The use of the microorganisms implies the need for being able to preserve them for rather long periods without their metabolic activity not being affected. Consequently, the industrialists currently tend to turn for the processes of stabilization to the application of "soft techniques" in order to minimize the modifications of properties of the products. These techniques can be for example the temperature treatments low, the high hydrostatic pressures, the pulsated electric fields or the pulsated light [3].

Materials and methods

The culture stock of *Saccharomyces cerevisiae* W303-1A (MATa leu 2-3, 112 his 3-11, 15 trp 1-1 can 1-100 ade 2-1 ura 3-1) [5] was used in this study. It comes from Laboratory of the Cellular and Molecular Biology of the Catholic University of Leuwen (Netherlands). From the preserved strain at -80°C, a sowing was carried out on medium with agar Malt Wickerman modified (MW). The pre-culture was prepared by the inoculating in a flask of 250 ml, containing 100 ml of medium MW, from the gelose stock culture. This flask is shaken at 250 rotations per minute at 30°C during 24 h. The culture was then prepared in a flask containing the same medium and placed under the same conditions, during 6 h to obtain the culture in exponential phase or 24 h for the use of the culture in stationary phase.

Several media were used in this study: medium Malt Wickerham (MW), binary solution of Water-Glycerol (WG), Water-Glycerol with Glucose (WG+Glu), Water-Glycerol with pancreatic Peptone (WG+Pep) and Water-Glycerol with yeast extract (WG+YE). The culture was initially centrifuged with 4000 rotations per minute during 5 minutes and a temperature of 30°C for *Saccharomyces cerevisiae* W303-1A.

The supernatant then was removed and replaced by a binary solution of water glycerol or water-glycerol with glucose, water-glycerol with pancreatic peptone or water-glycerol with extract yeast. The cellular suspension and the solutions used for the experiments were then placed during 15 minutes in a bain-marie thermostated in order to reach the desired temperature.

Two kinetics of cooling (2 and 2000°C.min-1) have been developed like follows:

- For the slope, the slowest kinetics (2°C.min-1) was used a cryotube containing 1 ml of cellular suspension. This cryotube was then placed in a thermostated bain-marie with 30°C for *Saccharomyces cerevisiae* W303-1A, then a linear and progressive reduction in temperature of 30°C was obtained thanks to a programmer of temperature.
- The shock (2000°C.min-1) was carried out by the fast introduction of 0,1 ml of cellular suspension from 30°C into 9,9 ml of binary solution (WG, WG+Pep, WG+Gluc, WG+ YE) of 0°C. For all the experiments, a thermocouple of the type T was used to follow the kinetics of variation in temperature. After all the heat treatments, the samples were heated until ambient temperature in order to measure cellular viability.

The reheating was carried out by putting the samples in a thermostated bain-marie at 30°C (for *Saccharomyces cerevisiae* W303-1A). This speed of reheating corresponded of a speed of 20°C.min-1.

In this study, the measurement of cellular viability was determined by the Plate Count Method on medium with agar on Petri dishes. Each counting of the sample having undergone the thermal stress was reported to a control not having undergone any variation in the temperature. The dishes were incubated at the temperature corresponding to the growth conditions of the microorganisms. Each experiment was repeated at least twice. We used usual media and selective media in order to determine the effect of the cold stress on the physiological state of the cells. These mediums would make it possible to determine the number of wounded cells [4]. Indeed, the wounded cells did not survive on the selective medium.

The method employed consisted of a NaCl addition in the solid medium. The NaCl concentration applied was 0,75% in mass for *S. cerevisiae*. The growth dishes and those with NaCl were used to determine the number of cells on nonselective medium and selective medium. The loss of viability is defined by the difference of UFC/mL of microorganisms between the enumeration of a control and the experimental enumeration on selective and nonselective medium. The "wounded survivors" are indicated by the difference of UFC/mL between the control and the samples with micro-organisms stressed on selective medium and nonselective medium. The measurement of the number of the wounded cells makes it possible to highlight the non-lethal effect of the thermal stress.

$$\% \text{ viability} = \left(\frac{UFCcontrol}{UFCsample}\right) \times 100$$

Results and discussions

The objective of this work was to examine the influence of the kinetics of disturbance at low temperatures, without freezing, on the viability of the yeast *S. cerevisiae*. The aim it was to prove if the resistance at low temperature is a function of the kinetics of variation in temperature and the stress medium in which we kept the samples during the maintenance time at low temperatures. The level of survival of the micro-organisms depends in particular on four parameters: the speed of cooling, the type of microorganism, the phase of growth and duration of the maintenance time at low temperature. We have noticed that the level of survival microorganisms also depends on stress medium used.

The figure 1 represents the viability of *Saccharomyces cerevisiae* W303-1A after a stress in various media and maintenance times at 30°C during 1 day, 3 days and a week. Two very different behaviors of the cells from the exponential and stationary phase of growth were observed. During one week, the control in exponential phase records an important increase, whereas the cells resulting from the stationary phase of growth record a significant loss.

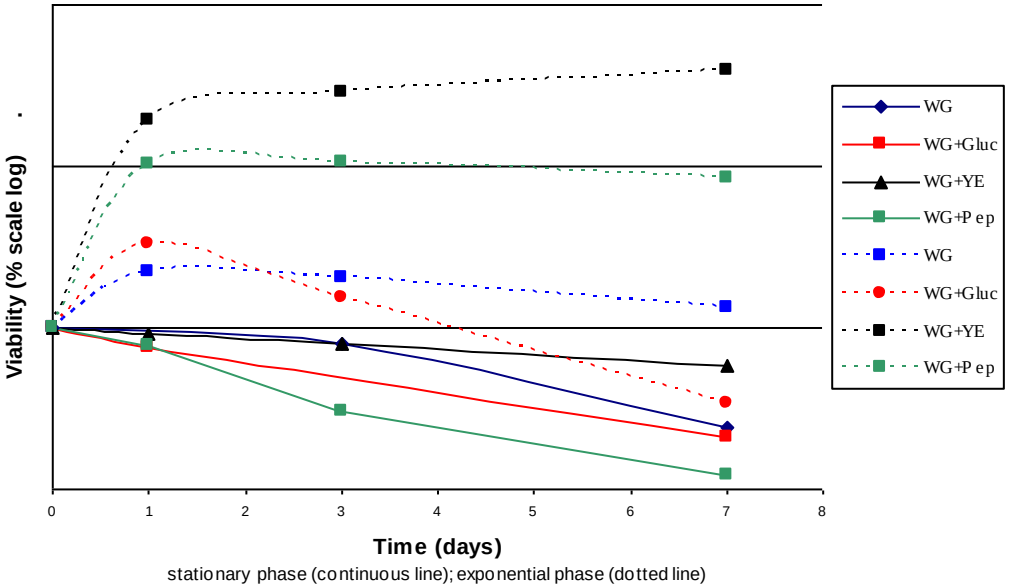

Figure 1. Influence of media content during a maintenance time at 30°C (control) on *S.cerevisiae* W303-1A growth

The viability of *Saccharomyces cerevisiae* W303-1A after a cold stress from 30°C to 0°C at a 2°C.min-1 speed, in various media and maintenance times during 1 day, 3 days and a week is shown in figure 2. We was noticed that after one week the level of cellular viability is comparable for the exponential phase and the stationary phase of growth.

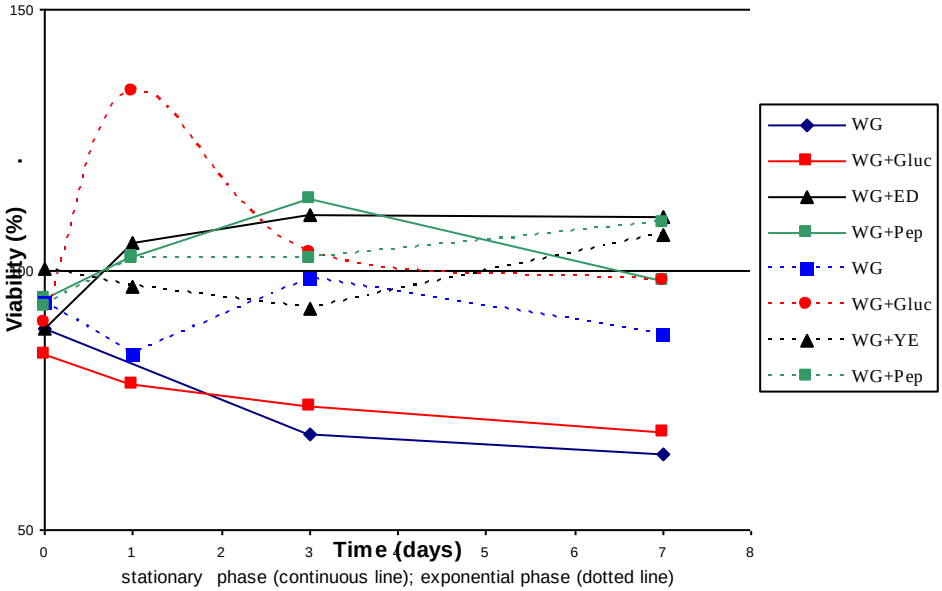

Figure 2. Influence of the maintenance time at 0°C, after a slope, on S.cerevisiae W303-1A growth; (2°C.min-1 cooling speed)

The viability of *Saccharomyces cerevisiae* W303-1A after a cold stress of 30°C to 0°C with a speed of 2000°C.min-1 in various media and maintenance time at 0°C during 1 day, 3 days and a week is shown in Figure 3. Three different behaviors from the cells resulting from the exponential and stationary phase of growth were observed: an increase in a number of alive cells if the samples in stationary phase are kept in Water-glycerol medium with peptone or Water-glycerol with yeast extract, a reduction in viability if the samples in exponential phase are kept in Water-glycerol medium or Water-glycerol with glucose and a stabilization for the samples in stationary phase kept in medium Water-glycerol and Water-glycerol with glucose. We notice same stabilization for the samples in exponential phase of growth kept in medium Water-glycerol with extract yeast and pancreatic Water-glycerol peptone.

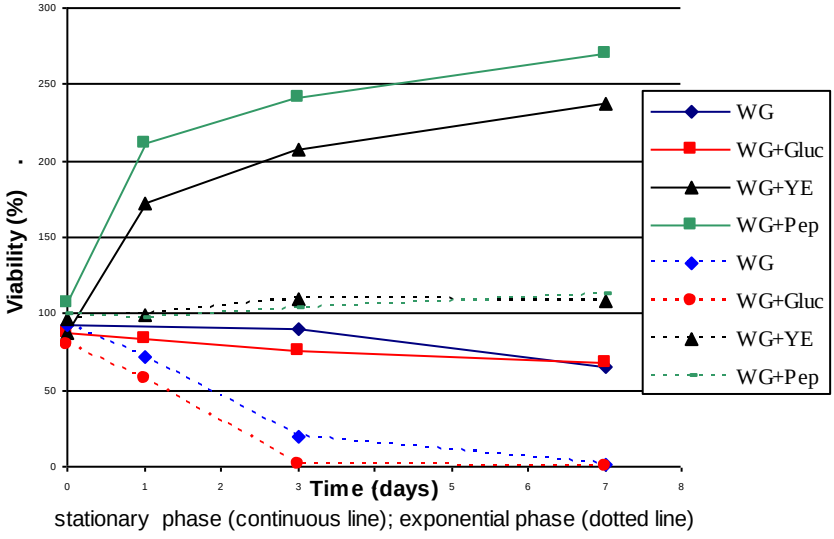

Figure 3. Influence of maintenance time at 0°C, after a shock, in various media on *Saccharomyces cerevisiae* W303-1A; (2000°C.min-1 cooling speed)

The kinetics of the temperature variation between 30 and 0°C influence the death of the microorganisms at the time of cold stress. Two assumptions seem reasonable for the explanation of cellular mortality after cold thermal stresses:

- a modification of the membrane characteristics such as the permeabilisation and fluidity;
- a denaturation of membrane and/or intracellular proteins. Our results showed that the viability of the cells depends in a drastic way to the speed of application of the thermal stresses. On one hand, viability is higher when the cells are cooled slowly that brutally.

Some authors [6] assumed that during the application of a slow cooling, the cells are likely to develop an adaptive response to the stress which makes them more resistant to temperatures normally lethal [5]. The synthesis of proteins of stress known as "cold shock protein" (Csp), trehalose and sterol (only for the case of yeasts) could play a very important part in the resistance of the cells after a thermal slope. The recent studies showed that sterols of the membrane of the yeasts which are not synthesized in the bacteria, are a factor making it possible yeasts to fight against stresses of the environment. It was observed that the viability of *S. cerevisiae* after a thermal stress and an ethanol stress increases with the proportion of the ergosterol in the membrane. The presence of sterols in the membrane of yeasts seems to be thus a factor of protection of the cells. It is obviously that the effect of the cold stress changes according to the type of cell, because the microorganisms are quite different on the level from their physiological and thus from their behavior to the cold stress.

There is a sharp difference in the resistance to the cold thermal stresses between the exponential phase and the stationary phase of growth. The cells in stationary phase are more resistant to the stress than the cells in exponential phase. In the exponential phase after a thermal shock the cells can remain on the same level of viability, for the samples kept in WG+Pep and WG+YE media, or can go down up to 0%, for the samples kept in medium WG and WG+Glu media. At the same time the cells resulting from the stationary phase of growth can remain on the same level of viability, for the samples kept in medium WG and WG+Glu media, or can go up to 87.1% up to 236.9% for the samples kept in WG+YE medium and of the 107.1% up to 269.5% for the samples kept in WG+Pep. It should be noted that the cells are known for a long time to have membrane compositions different according to their phase from growth. The membranes of the young cells are more fluid, less sterols (only for the case of yeasts). They are thus more fragile. The fast changes of the environmental parameters thus seem to disturb these membrane structures. We assume that the more fluid the membrane of the cell is, the more the cell is sensitive to the disturbance of the temperature.

Following cooling, the integrity of the cells is related to the time of maintenance at low temperature. Indeed, an important progressive loss of the viability of the surviving cells appears according to the maintenance time at low temperature, for example in the case of *S. cerevisiae* W303-1A in exponential phase of growth after a shock of 30°C to 0°C (2000°C.min-1) and 7 days maintenance on 0°C in medium WG and WG+Glu, or in stationary phase of growth after a thermal shock of 30°C to 0°C (2000°C.min-1) and 7 days maintenance on 0°C in medium WG andWG+Glu. At least two explanations are possible:

- the maintained cells at low temperature suffer membrane damage and lose a part of their material necessary to their survival
- the damage and the losses cannot be repaired as long as the temperature is maintained at a temperature allowing a metabolic activity.

Whatever the speed of cooling, a higher loss of viability appears with the increase of spent time at reduced temperature. Moreover, more the cells are cooled quickly, plus that

involves an important cellular mortality and this whatever the duration of time passes at low temperature. We can thus think that the first lesions are induced during the phase of cooling by the speed of disturbance of temperature, but also by the time of maintenance at low temperature.

These lesions results in either an immediate death, or a death in the course of time if the cell does not have of capacities to be regenerated. In the case of a shock, we can distinguish three types of cell whose two types are wounded by the thermal disturbance: non-wounded resistant cells, the wounded cells which die immediately and the wounded cells which die during variable times of conservation at low temperature.

In the case of a slope, two types of cells are distinguished: the wounded cells which die and resistant cells. The part of resistant cells was more important because the thermal stress in the form of slope was him even less drastic.

On the level of the culture medium, the stationary phase is regarded as a phase of stress, in particular nutritive and acid, which causes at the cells the installation of active protection systems enabling him to survive future stresses.

If we look at figure 3, after a shock, we can observe that the samples kept in WG+Pep and WG+YE media record an increase for the cells resulting from the stationary phase and a stabilization for the cells resulting from the exponential phase. On the other hand, the samples kept in WG and WG+Glu media record a loss of viability after one week.

We can think that the source of carbon, which in this case is glucose does not have a very important role in surviving it of the cells after a cold stress, on the other hand the source of the proteins, which in case is pancreatic peptone and the extract yeast, plays a very important part in surviving it and multiplication of the cells.

On the level of their metabolism, trehalose is known for its role of guard in particular on the levels of three macromolecules of the cell: proteins, the membrane and the ADN. As the cells in stationary phase have more sugar of reserve than those in exponential phase [2], the capacity of these cells to preserve their macromolecules at the time of cold stress will be more important. The protection of proteins, the membrane and the ADN was to play an important part in the resistance of the cells at the time of the cold stress [1].

Conclusions

In the literature, the temperatures with the top of 0°C are often associated an adaptive metabolic response of the cells, while the temperatures with the lower part of 0°C do not allow this type of answer and cell multiplication.

In the range of positive temperatures (30-0°C), our work showed that cellular resistance during cooling, without presence of freezing, strongly depends on the kinetics of reduction in temperature. This resistance is one function of the type of cells, the age of cells and the time of maintenance at low temperature in mediums of stress. The phenomena leading to the mortality of the microorganisms at the time of variations in temperature are generally related to membrane modifications or the denaturation of proteins.

We observed that a better safeguarding could be obtained when the process of cooling is done slowly.

The control of the kinetics of variation in temperature could be applied in biotechnological treatments so stabilizing food. A better safeguarding could be obtained when the process of cooling and reheating is done slowly.

Of the same way, the effect of the kinetics of application of such a treatment of safeguarding could be used in combination with the cumulative effect of a double stress, for

example temperature/pressure of water or hydrostatic temperature/pression, which will contribute to the development of "soft techniques".

Lastly, since the traditional use into agro-alimentary of the cold is the conservation and the stabilization of food, this study will allow a new use of the cold, by the weakening and/or the destruction of the microorganisms in order to stabilize food by associating four following factors: a low temperature, a speed of cooling rather fast and a time of maintenance at low temperature sufficiently long in the mediums of stress. This exploitation of the cold treatments makes it possible to think of the development of new soft technology of decontamination of food.

Acknowledgments

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