Industrial production of dried yeast: plasma membrane as a survival indicator of air drying process

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INDUSTRIAL PRODUCTION OF DRIED YEAST: PLASMA MEMBRANE AS A SURVIVAL INDICATOR OF AIR DRYING PROCESS

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Abstract: Preservation of microorganisms by desiccation is a major industrial interest. However, study of cell survival mechanisms that occur during desiccation is complex. In this work, the impact of the magnitude and the kinetics of dehydration on yeast survival were evaluated in either hyperosmotic liquid medium or a gaseous environment. A same lethal magnitude of dehydration and a same lethal kinetic effect were found. As previously shown for osmotic stress, this work demonstrate that yeast survival after drying was also related to plasma membrane disorganization, suggesting a similar passive rearrangement of the membrane components. Hyperosmotic treatment in liquid medium represents an opportune environment to optimize industrial drying processes.

Keywords: Yeast, Air-drying, Osmotic dehydration, Microscopy, Plasma membrane

INTRODUCTION

Cell stabilization is a complex process that requires the removal of water without altering viability and functionality. The plasma membrane (PM) located at the interface between the cell and the external medium is often described as a major site of injury during variations in hydration. The PM is a vital component whose structure and organization can be drastically challenged by dehydration. Therefore, understanding PM behavior during dehydration is important for biological research and for the improvement of preservation methods of biological samples (drying, freezing). The study of PM mechanisms that occur during air-drying requires a continuous, technically complex approach, with the transition of samples from liquid to solid state. Many data connecting the evolution of the PM to the progress of the dehydration process have been collected in liquid media by studying cells in increasingly concentrated solutions (Simonin *et al.*, 2007). Recently, PM mechanisms behind cell survival were observed during progressive dehydration in liquid medium (Dupont *et al.*, 2010). Observation during such treatment revealed the existence of lateral PM reorganization. PM structural rearrangement is characterized by the dissipation of membrane surface excess, forming "big pleats" which remain integrated in the PM. These pleats allow PM re-expansion during rehydration without permeabilization.

With air-drying, phenomena are initially relatively similar since the early stages correspond to the maintenance of cells in a concentrated liquid environment. However, the subsequent events are more complex due to matrix solidification. The aim of this work was to study mechanical cell damage due to hydric disturbance by comparing the survival of yeast to dehydration either in liquid medium or through air-drying. We compared the kinetic and magnitude effects on *S. cerevisiae* survival. In order to measure the involvement of PM damage, we focused on the behavior of specific proteins fused with GFP, which constitute a permanent fluorescent PM marker during dehydration.

MATERIALS AND METHODS

The *Saccharomyces cerevisiae* strain BY4742 (Euroscarf, Frankfurt, Germany) in early stationary phase was used in this study.

Osmotic treatments

Three osmotic treatments were used: moderate (a_w=0.80), which is slightly higher than that allowing osmoregulation, and severe (a_w=0.45 and a_w=0.30). Hyperosmotic shock was induced by quickly introducing 1 mL of a binary water–glycerol solution to the pellets. For progressive perturbations, the external osmotic pressure was increased linearly by slowly adding pure glycerol. The cells were maintained under hyperosmotic conditions for one

hour before rehydration. For rapid rehydration, the solution (a_w=0.99) was added abruptly to the cell pellet. Progressive rehydration was performed using successive dilutions in solutions with increasing osmotic pressure to reach a_w=0.99.

Air-drying treatments

The air-drying chamber consists of an airtight box controlled in relative humidity with saturated salt solutions (Greenspan, 1977). CH_3COOK, K_2CO_3 or NaCl were used to obtain water activities of 0.30, 0.45 and 0.80. For the fast treatment, a thin layer of yeast was deposited on a glass microscope slide and placed in the drying chambers for one hour. For the progressive treatment, yeasts were extruded forming small filaments, as in industry. Slow rehydration was performed by placing samples at RH=100%.

Measurement of yeast viability

Yeast viability was estimated in triplicate using the CFU method. CFU were recorded as CFU/mL or CFU/mL/g of hydrated paste.

Confocal microscopy

Yeast strains with Sur7-GFP were observed using a Nikon Eclipse TE 2000 U microscope with multispectral confocal head D Eclipse C1. Excitation was performed at 488 nm, and the emission signal was measured between 500 and 545 nm. Images were acquired with a ×100 (NA: 1.4) Plan Apo oil-immersion objective (Nikon), and collected using EZ-C1 software 3.50 (Nikon).

RESULTS

Impact of dehydration and rehydration kinetics

Cell viability was estimated by the CFU method after osmotic and air-drying to a_w=0.30, maintenance for 60 minutes and rehydration to a_w=0.99.

Table 1: Influence of dehydration and rehydration kinetics after osmotic and air-drying treatments on yeast survival (%)

	Slope-Slope	Slope-Shock	Shock-Slope	Shock-Shock
Osmotic dehydration	78.7 ± 3.6	42.6 ± 3.6	19.2 ± 1.1	0.3 ± 0.2
Air-drying desiccation	42.3 ± 13	27.5 ± 9.1	21.6 ± 8.6	2.8 ± 0.7

Firstly, for osmotic dehydration and air-drying desiccation, the lowest survival rates were observed when the dehydration and rehydration steps were performed rapidly while the highest survival rates were observed with both techniques after progressive dehydration followed by progressive rehydration (78.7% and 42.3%, respectively). The two other treatments that combine a rapid and a progressive step showed intermediate cell survival rates. So, for osmotic dehydration and air-drying desiccation, survival of the yeast strain depended on the kinetics. With both techniques, greater yeast preservation was obtained with slow water flow.

Impact of magnitude of dehydration on yeast survival rates

Yeast cells were treated with a shock-shock cycle for 60 min and then rehydrated.

Table 2: Yeast viability (%) after dehydration in liquid medium or in air-drying chambers

	a_w=0.80	a_w=0.45	a_w=0.30
Osmotic dehydration	83.5 ± 2.5	14.6 ± 9.1	0.35 ± 0.2
Air-drying desiccation	65.3 ± 6.3	30.0 ± 8.4	2.8 ± 0.8

Higher viability ratios were observed at a_w of 0.80 with 83.5% and 65.3% in liquid and gaseous environments respectively. Dehydration in liquid medium or in drying chambers led to similar survival rates. Moreover, for both techniques, cell viability clearly decreased with increasing osmotic pressure.

Impact of air-drying on PM organization

PM organizations after air-drying dehydration and rehydration have been studied by confocal microscopy.

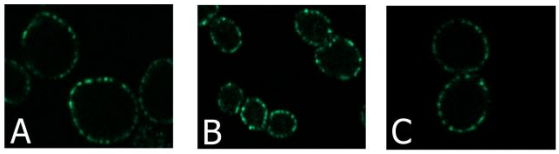

Fig. 1. Impact of dehydration and rehydration on Sur7-GFP microdomain distribution in the PM. Images show control cells (A), cells after progressive air-drying dehydration (B) and rehydration (C)

After progressive dehydration in a gaseous environment, several distribution profiles were observed. Nevertheless, most cells showed more diffuse areas, larger and fewer than the control as already observed in liquid medium (Dupont *et al.*, 2010). After rehydration, numerous patches were distributed in the PM. The reorganization of the PM in yeast cells dehydrated in liquid medium or by air-drying is similar to the profiles of the control cells. For both methods of dehydration, survival rates were strongly related to perturbations kinetic and more particularly to the dehydration step.

DISCUSSION

This study was conducted to understand yeast survival mechanisms to hydric perturbations. Yeast

viability depended on the variation rate of water activity with both methods of dehydration. The comparison of yeast viability after shock-slope and slope-shock cycles showed the importance of controlling dehydration and rehydration kinetics to enhance yeast survival. As in liquid medium, the choice of adequate drying kinetics improved cell preservation (Dupont *et al.*, 2010). Cell behavior in liquid or gaseous environments was comparable, with a decrease in viability with decreasing a_w. At a_w of 0.80, cell growth and metabolism stopped but the vital structures were not completely altered whereas dehydration attaining water activities of 0.45 or 0.30 resulted in significant cell death in both liquid and gaseous environments. This may be due to the lipid phase transition (Simonin *et al.*, 2007). To understand desiccation survival mechanisms the lateral organization of the PM through GFP localization was observed for both dehydration methods. After progressive disturbance in liquid and gaseous environments, a redistribution of GFP was observed. These observations are related to significant yeast survival and may be linked to membrane rearrangement during the lipid phase transition. The mechanisms responsible for cell resistance are perhaps not exactly identical but in both cases they result in yeast survival.

CONCLUSION

Therefore, whether in case of cell death or cell survival, results suggest similar mechanisms during drying to those observed in hyperosmotic liquid medium, with similar PM behavior. During air-drying, the PM is a key structure in cell survival. This work emphasizes the major role of kinetics to preserve yeast cells during air-drying. Thereby, osmotic disturbance with glycerol represents an opportune environment to optimize industrial processes. Moreover, PM could be considered as an "*in situ*" indicator of yeast integrity during dehydration process.

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