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Isolation of an Osmotolerant ale Strain of *Saccharomyces cerevisiae*

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Abstract

Saccharomyces cerevisiae (ale *strain) grown in batch culture to stationary phase was tested for its tolerance to heat (50 °C for 5 min), hydrogen peroxide (0.3 M) and salt (growth in 1.5 M NaCl/YDP medium). Yeast cells which have been exposed previously to heat shock are more than controls. Their fermentative activity as judged by glucose consumption and their viability, as judged by cell number and density have higher levels when compared with cells not previously exposed to heat shock. Experimental condition facilitated the isolation of* S. cerevisiae *(ale strain) which was tolerant to heat and other agents such as hydrogen peroxide and sodium chloride.*

Keywords: *Osmotolerant, heat shock, fermentative activity, glucose consumption.*

Introduction

Stress co-tolerance is the phenomenon of tolerance to one type of stress, tending to be associated with tolerance to another (Lewis *et al.* 1997). In the yeast, *Saccharomyces cerevisiae*, relationships have been recorded between tolerance to heat and osmotic stress, freezing and dehydration stress, freezing and alcohol stress (Trollmo *et al.* 1988).

Tolerance to hydrogen peroxide stands out as being important in two ways. First, there are data that all yeast strains demonstrate exceptionally high levels of tolerance towards hydrogen peroxide (Lewis *et al.* 1997). Second, tolerance to all stress, except ethanol, shows a significant, positive correlation with tolerance to hydrogen peroxide. Most *ale* strains of *S. cerevisiae* show a decline in fermentative activity, thus salt concentrations in the 2-2.5% range cause a considerable inhibition in yeast activity. This common osmotic phenomenon was observed with yeast in sugar or high-salt brewing by Trollmo *et al.* (1988). In consequence, it was of interest to study the hydrogen peroxide and the salt tolerance of the individual ale strain of *S. cerevisiae* C1028 (UK) before and after heat shock.

Materials and Methods

The yeast used in this study was the individual *ale* strain C1028 (UK) of *S. cerevisiae*. Active colonies of the yeast were grown on yeast peptone dextrose (YPD) agar slopes at 37°C for 24 hr and then maintained at 4°C. Experimental fermentation was carried out by inoculating yeast cells in YPD medium, in which agar was omitted and H_2O_2 was added to a final concentration of 0.3 M. In another series of experiments fermentation was carried out in the presence of 1.5 M NaCl.

To investigate cell co-tolerance to H_2O_2 or high salt a 1 ml sample of cells, at a density of 1 x 10^7 CFU/ml was heated at 50°C for 5 min. Post-stress viability was assessed by diluting the cells in YPD medium and plating them onto YPD agar plates. A colony of cells, which survived the shock, was inoculated into 10 ml of YPD medium and incubated overnight at 37°C with shaking at 1880 rpm prior to transfer to 250 ml of fresh YPD medium, containing 0.3 M H_2O_2 or 1.5 M NaCl. Fermentation was carried out at 37°C for 168 h. Every 24 h aliquots were taken and the cell density, number of yeast cells, as well as the glucose concentration were measured. Controls containing 0.3 M H_2O_2 and 1.5 M

NaCl in YPD agar were inoculated with unstressed yeast cells.

The cell density was measure using a model Spectronic Genesys absorption spectrophotometer. The cell number was counted using an emulsion microscope (Nikon, Japan). The residual glucose concentration in the yeast culture supernatants was measured by refractometer (Tamco, Japan).

Results and Discussion

The fermentation ability of the industrial *ale* strain of *S. cerevisiae* C1028 was investigated in the presence of 0.3 M hydrogen peroxide or 1.5 M sodium chloride, as judged by glucose consumption. The experiments were performed after heat shock at 50°C for 5 min. Fig. 1 shows that glucose consumption was higher when the yeast cells were previously exposed to heat shock with a consequent growth in YPD medium containing 0.3 M H_2O_2 or 1.5 sodium chloride compared with yeast cells which had not undergone heat chock. Thus, preliminary heat shock seems to develop tolerance in yeast cells, making them more tolerant to other kinds of osmotic or chemical stress.

The cell density, as well as the cell number, was affected, depending on whether or not the yeast cells had undergone heat shock (Figs. 2 and 3). Cell density and the cell number were higher when the cells were exposed first to heat shock and then to H_2O_2 or NaCl, compared with the controls, providing cells which have not been heat shocked were grown in a medium containing high concentrations of hydrogen peroxide or sodium chloride.

These results indicate that the tolerance of yeast cells to heat may be correlated with tolerance to hydrogen peroxide and high salt concentrations. A role of oxidative processes in stress-induced injury has been previously suggested for such stresses, including freezing (Swartz 1971), acetic acid (Martin *et al.* 1976), dehydration (Beudeker *et al.* 1990), and ethanol (Costa *et al.* 1993). The mechanistic connection between oxidative processes and stress has had a number of explanations, including the suggestion that stress may induce increased levels of free radicals within the cell (Collinson and Dawes 1992). There is much evidence which suggests a link between oxidative processes and cellular damage associated with a variety of types of stress. The association between H_2O_2 and other stress tolerance found in this work adds substantially to this evidence.

References

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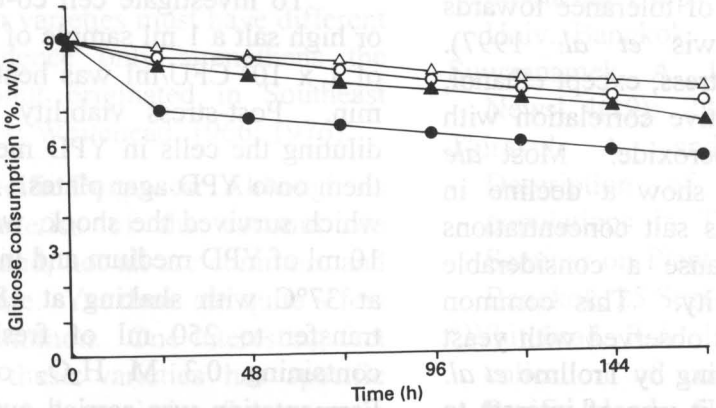

Figure 1 Glucose consumption by *S. cerevisiae*. ○, Control fermentation by yeast cells not exposed to heat shock in the presence of 0.3 M H_2O_2 in YPD medium. ●, Fermentation by yeast cells after heat shock in the presence of 0.3 M H_2O_2 in YPD medium. △, Control fermentation by yeast cells not exposed to heat shock in the presence of 1.5 M NaCl in YPD medium. ▲, Fermentation by yeast cells after heat shock in the presence of 1.5 M NaCl in YPD medium.

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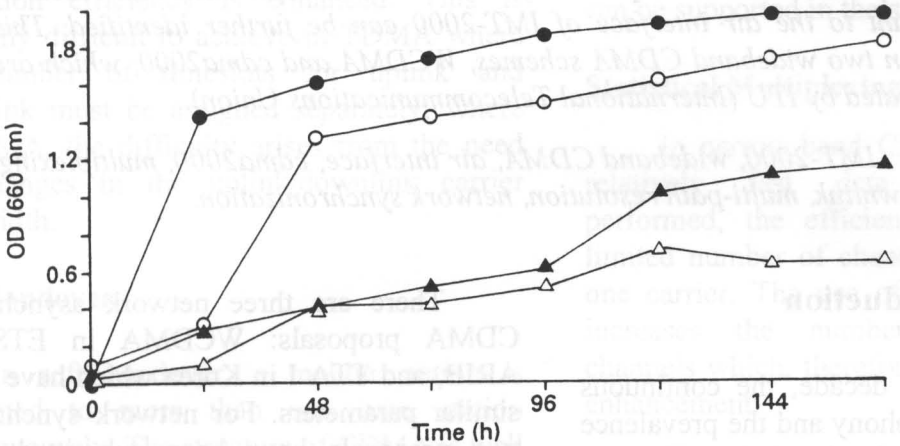

Figure 2 Yeast growth in time range of 168 h as measured by the optical density at 660 nm wavelength. ○, Growth of control cells not exposed to heat shock in the presence of 0.3 M H_2O_2 in YPD medium. ●, Cell growth of yeast cells after heat shock in the presence of 0.3 M H_2O_2 in YPD medium. △, Growth of control cells not exposed to heat shock in the presence of 1.5 M NaCl in YPD medium. ▲, Growth of yeast cells, after heat shock in the presence of 1.5 M NaCl in YPD medium.

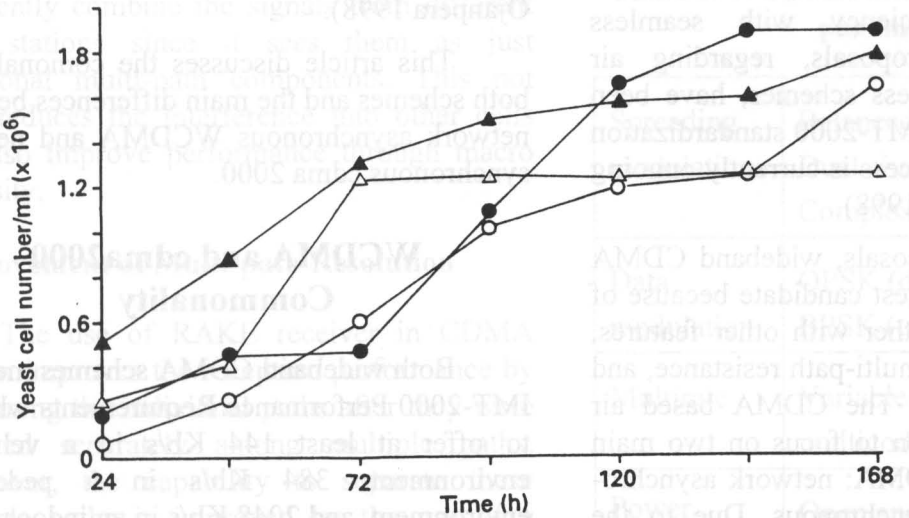

Figure 3 Viable counts of yeast cells in the time range of 168 h. ○, Number of cells, not exposed to heat shock in the presence of 0.3 M H_2O_2 in YPD medium. ●, Number of cells, after heat shock in the presence of 0.3 M H_2O_2 in YPD medium. △, Number of cells, not exposed to heat shock in the presence of 1.5 M NaCl in YPD medium. ▲, Number of cells, after heat shock in the presence of 1.5 M NaCl in YPD medium.