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Changes in protein composition of *Saccharomyces* brewing strains in response to heat shock and ethanol stress

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SUMMARY

Heat shock and ethanol stress of brewing yeast strains resulted in the induction of a set of proteins referred to as heat shock proteins (HSPs). At least six strongly induced HSPs were identified in a lager brewing strain and four HSPs in an ale brewing strain. Four of these HSPs with molecular masses of approximately 70, 38, 26 and 23 kDa were also identified in two laboratory strains of *Saccharomyces cerevisiae*. The appearance of HSPs correlated with increased survival of strains at elevated temperatures and high concentrations of ethanol. These results suggest that HSPs may play a role in the ethanol and thermotolerance of yeasts. The properties of these proteins and membrane fatty acids in relation to heat and ethanol shock are being investigated.

INTRODUCTION

All organisms examined to date, including yeasts, are known to respond to temperatures above their normal growth temperature by inducing the synthesis of a family of specific proteins referred to as heat shock proteins (HSPs) [10,12,15,18,29]. The synthesis of these proteins is also induced in response to a variety of other environmental stresses including ethanol [12,17,21,22]. A close correlation between the synthesis of HSPs and the acquisition of thermotolerance has been reported in many organisms including yeasts [12,14,15,20,28]. It has been suggested that this highly conserved group of proteins protect cells from stress-related injuries [7,15]. The induction of HSPs and thermotolerance in yeasts by heat shock has also been shown to induce cross-tolerance to ethanol [21,26,28]. Similarly, prior exposure of cells to ethanol, which induced HSPs, also conferred protection against subsequent lethal temperatures [21]. Thus, the induction of HSPs either by heat shock or ethanol appears to protect cells against environmental stresses.

Although there are several reports on the induction of HSPs in yeasts by heat shock [15,16,18,21,26], the role of these proteins in ethanol and thermotolerance of brewing yeasts is yet to be determined. In this study, changes in cell protein composition of lager and ale brewing yeast strains in response to heat shock and ethanol stress were examined. These changes were correlated with increased survival of yeast cells in high concentrations of ethanol and high temperatures.

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MATERIALS AND METHODS

Yeast strains and growth medium. The yeast strains employed in this study were, with their Labatt culture collection numbers, *Saccharomyces cerevisiae* brewing ale strain 3001, *Saccharomyces uvarum (carlsbergensis)* brewing lager strain 3021, *Saccharomyces diastaticus* strain 62 and *Saccharomyces cerevisiae* strain 67. The yeast cells were subcultured in YNB medium and incubated at 21 °C on a rotary shaker prior to use in this study. The YNB medium consisted of yeast nitrogen base with amino acids (Difco), 6.7 g; glucose, 20 g; sodium citrate, 5.2 g; citric acid, 7.0 g; all dissolved in 1 liter of distilled water.

Conditions of heat and ethanol shock. Cultures, grown to exponential phase at 21 °C, were subjected to heat shock at 37 °C for 30 min. Cultures were cooled to 21 °C prior to testing for their tolerance to high temperatures and ethanol. Yeasts' ethanol and thermotolerance were determined by their viability after 5 min heat shock at 45–50 °C or ethanol shock in 20% (v/v) ethanol for 60 min. Control cells did not receive heat shock prior to testing for their ethanol and thermotolerance.

Determination of viability. Cell viability was determined by the colony count method. Samples were diluted in

sterile physiologic saline [0.89% (w/v) NaCl] and plated on PYN agar plates supplemented with 2% (w/v) glucose. Cultures were incubated at 21 °C for 3 days and the number of colony forming units per ml of sample was determined.

Radiolabelling. Radiolabelling of yeast cells was carried out as described previously [21]. Yeast cells were grown to exponential phase of growth in YNB medium supplemented with 2% (w/v) glucose. [^{35}S]methionine (approximately 1097 Ci/mM, ICN Biomedicals, Inc.) was added to 4 ml of yeast cultures at 15 μCi/ml and these cultures were incubated at 21 °C, 37–50 °C or at 21 °C in the presence of 10–15% (v/v) ethanol for at least 10 min or more. Incorporation of [^{35}S]methionine into cells was halted by adding ice directly into cultures. Radiolabelled cultures were centrifuged at 10 000 × *g* for 5 min and the pellet was kept frozen at − 70 °C until required for analysis of cell proteins.

Analysis of radiolabelled proteins. Radiolabelled proteins were extracted as described previously [5], separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [8] and visualized by autoradiography.

RESULTS

Effect of heat preincubation on survival of yeasts from heat and ethanol stress

Exponentially growing yeast cells were shifted from 21 °C to 37 °C for 30 min. The cultures were allowed to cool to 21 °C and then subjected to heat shock at 45 °C or 50 °C. The effect of heat shock on yeast cell viability at these temperatures is shown in Table 1. The viability of *S. diastaticus* strain 62 and *S. cerevisiae* strain 67 was unaffected after heat shock at 45 °C with or without preincubation at 37 °C. These strains appeared tolerant to a

TABLE 1

Effect of pre-incubation of yeast strains at 37 °C for 30 min on survival at 45 °C or 50 °C for 5 min

Yeast strain	Labatt code	Preincubation temp. (°C)	Percent survival[a] at	
			45 °C	50 °C
S. diastaticus	62	21	92.5	16.8
S. diastaticus	62	37	98.6	82.9
S. cerevisiae	67	21	85.6	2.7
S. cerevisiae	67	37	97.5	75.6
S. uvarum (carlsbergensis)	3021	21	6.4	0
S. uvarum (carlsbergensis)	3021	37	85.5	3.5
S. cerevisiae	3001	21	14.7	0.97
S. cerevisiae	3001	37	89.4	16.1

[a] Data represent the average of 3 separate experiments.

TABLE 2

Effect of pre-incubation of yeast strains at 37 °C for 30 min on survival in 20% (v/v) ethanol for 60 min

Yeast strain	Labatt code	Preincubation temp. (°C)	Percent survival[a] in 20% (v/v) ethanol
S. diastaticus	62	21	90.5
S. diastaticus	62	37	96.8
S. cerevisiae	67	21	82.4
S. cerevisiae	67	37	94.6
S. uvarum (carlsbergensis)	3021	21	52.4
S. uvarum (carlsbergensis)	3021	37	98.5
S. cerevisiae	3001	21	49.2
S. cerevisiae	3001	37	78.5

[a] Data represent the average of 3 separate experiments.

5-min heat shock at this temperature. However, their viability declined to less than 20% after a 5-min heat shock at 50 °C, without preincubation at 37 °C. Preincubation at 37 °C resulted in a significant increase in the survival of these strains after heat shock at 50 °C for 5 min.

The two brewing strains, *S. uvarum (carlsbergensis)* strain 3021 and *S. cerevisiae* strain 3001, were less tolerant to a 5-min heat shock at 45 °C without preincubation at 37 °C. Their viability was less than 20% after heat shock at 45 °C and less than 1% at 50 °C. Preincubation at 37 °C for 30 min resulted in a significant increase in survival of these strains at these temperatures. Thus, preincubation at 37 °C prior to heat shock at higher temperatures protected yeast cells from thermal death. Similar results were obtained when yeast cells were preincubated at 37 °C for 30 min prior to incubation in 20% (v/v) ethanol (Table 2). Strains 62 and 67 appeared tolerant to a 60-min incubation in 20% (v/v) ethanol with or without preincubation at 37 °C. On the other hand, the survival of the two brewing strains in 20% (v/v) ethanol improved significantly as a result of preincubation at 37 °C. Thus, protection against heat shock and ethanol effects may involve similar cellular factors.

Induction of HSPs by heat shock

Yeast cells in exponential growth phase were radiolabelled prior to heat shock at 37 °C for 15–180 min as described in Materials and Methods. Fig. 1 shows autoradiograms of proteins from lager strain 3021 separated by SDS-PAGE before and after heat shock at 37 °C for 15–180 min. The molecular masses of HSPs identified were approximately 70, 50, 38, 35, 26 and 23 kDa. These HSPs and several other weakly induced HSPs are indicated by the arrows. These proteins were clearly visible after 30 min of heat shock at 37 °C. Incorporation of radiolabel into cellular proteins within 15 min of incubation either at 21 °C or 37 °C was very low. The optimum time for induction of HSPs was 30 min. No additional HSPs were identified after 30 min heat shock at 37 °C.

Similar results were obtained when *S. cerevisiae* ale brewing strain 3001 was subjected to heat shock at 37 °C for 15–180 min. At least four HSPs of molecular masses 70, 38, 26 and 23 kDa were identified in this strain. These HSPs and other weakly induced HSPs are indicated by arrows (Fig. 2). The optimum incubation time for induction of HSPs in this strain was also 30 min.

Comparison of various heat shock proteins identified in these two brewing strains with those of *S. diastaticus* strain 62 and *S. cerevisiae* strain 67 revealed four HSPs which are shared in common by these strains. The molecular masses of these proteins are approximately 70 kDa (arrow I), 38 kDa (arrow II), 26 kDa (arrow III) and 23 kDa (arrow IV) (Fig. 3). Other HSPs identified in these strains are indicated by arrows. A summary of the molecular weights of various heat shock proteins which

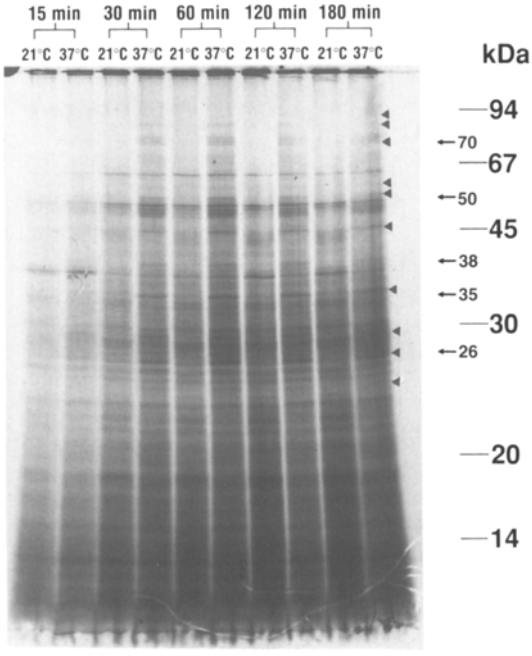

Fig. 1. Heat shock proteins of lager brewing strain *S. uvarum (carlsbergensis)* 3021 induced by heat shock at 37 °C for 15–180 min. Heat shock proteins are indicated by arrows.

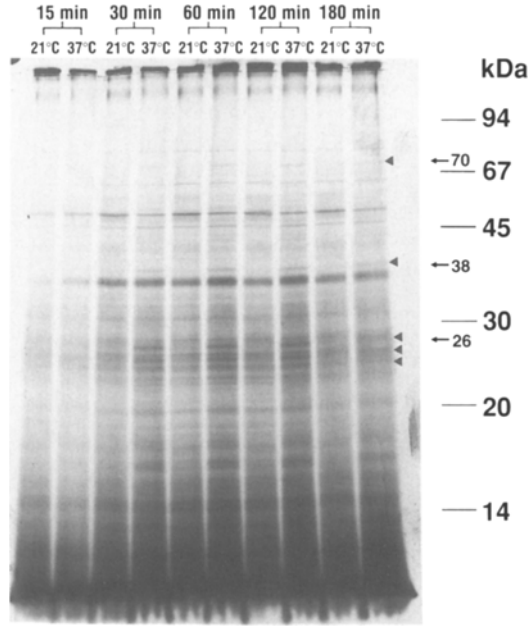

Fig. 2. Heat shock proteins of ale brewing strain *S. cerevisiae* 3001 induced by heat shock at 37 °C for 15–180 min. Heat shock proteins are indicated by arrows.

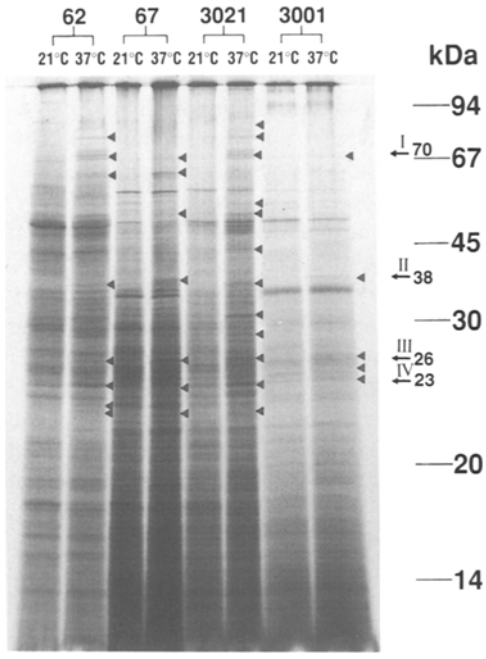

Fig. 3. Heat shock proteins of various yeast strains. Heat shock proteins were induced by heat shock at 37 °C for 30 min. These proteins are indicated by arrows.

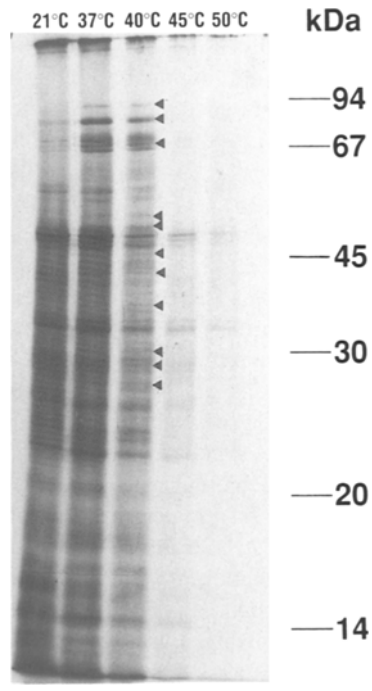

Fig. 4. Induction of heat shock proteins in *S. uvarum (carlsbergensis)* 3021 at 37 °C to 50 °C. Heat shock proteins are indicated by arrows.

are strongly induced by heat shock is shown in Table 3. Lager strain 3021, the most heat shock sensitive strain, produced the highest number of HSPs. The appearance of these proteins correlated with protection of yeast cells against heat shock at 45 or 50 °C. It is possible, therefore, that these proteins play a role in protecting the cells from the lethal effects of elevated temperatures.

Induction of HSPs by heat shock at various temperatures

Yeast cells were subjected to heat shock at 37, 40, 45 and 50 °C, in an attempt to determine if higher temperatures induce the synthesis of more HSPs. Fig. 4 shows the autoradiograms of proteins from lager strain 3021 heat shocked at these temperatures. Temperatures above 37 °C appeared to inhibit the incorporation of radiolabel into yeast proteins. The viability of this strain decreased with an increase in heat shock temperatures above 37 °C (Table 1). Thus the loss of cell viability may account for the reduction in the incorporation of radiolabel. Similar results were obtained with the ale brewing yeast strain 3001. Therefore, the optimum temperature for the induction of HSPs in these brewing yeast strains with minimum loss of cell viability was 37 °C.

TABLE 3

Heat shock proteins of yeast strains

Yeast strain	Labatt code	Approximate molecular weights of HSPs (kDa)
S. diastaticus	62	70, 38, 26, 23
S. cerevisiae	67	70, 50, 38, 26, 23
S. uvarum (carlsbergensis)	3021	70, 50, 45, 38, 35, 26, 23
S. cerevisiae	3001	70, 38, 26, 23

Induction of HSPs by ethanol shock

Exposure of exponentially growing yeast cells to 10% (v/v) ethanol at 21 °C for 60 min also induced the synthesis of HSPs. Fig. 5 shows autoradiograms of proteins of lager strains 3021 and ale strain 3001 before and after exposure to 10% (v/v) ethanol for 60 min. Heat shock proteins of approximately 70, 38, 26, 23, 18 and 14 kDa are indicated by arrows. The numbers of HSPs induced by ethanol shock were smaller than that of heat shock at 37 °C (Fig. 4). Ethanol shock induced 23, 18 and 14 kDa proteins in the lager strain but not in the ale strain. These proteins were observed as early as 30 min of incubation of cultures with 10% (v/v) ethanol. No additional HSPs

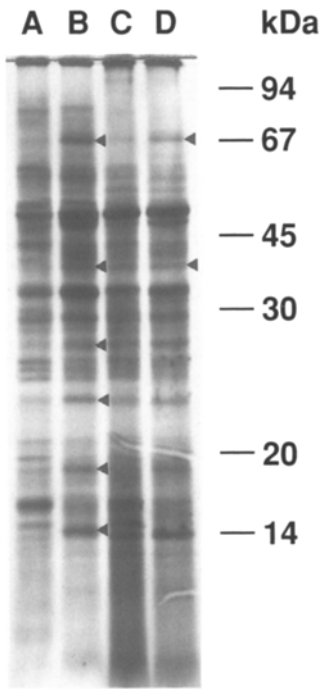

Fig. 5. Induction of heat shock proteins in *S. uvarum (carlsbergensis)* 3021 and *S. cerevisiae* 3001 by ethanol shock with 10% (v/v) ethanol for 60 min. Lanes: A, 3021 at 21 °C; B, 3021 at 21 °C + 10% ethanol; C, 3001 at 21 °C; and D, 3001 at 21 °C + 10% ethanol. Heat shock proteins are indicated by arrows.

were identified in cells exposed to 15% (v/v) ethanol for 30–60 min (data not shown).

DISCUSSION

In many organisms, induction of thermotolerance is directly correlated with induction of a specific set of HSPs [9,11,12,19,28]. These proteins are induced by a variety of stress conditions most notably heat shock [7,13,17,21,28] and ethanol stress [2,17,21]. This paper examines the effects of heat shock and ethanol stress on the cell protein composition and viability of two brewing yeast strains: *S. cerevisiae* ale brewing strain 3001 and *S. uvarum (carlsbergensis)* lager brewing strain 3021, and two laboratory strains: *S. diastaticus* strain 62 and *S. cerevisiae* strain 67. The viability of the brewing strains decreased with increases in heat shock temperatures above 37 °C, while the laboratory strains remained viable with increases in heat shock temperatures up to 45 °C. However, their viability declined rapidly at heat shock temperatures above 45 °C. These results confirmed previous reports in which these two laboratory strains were found to be more thermotolerant than the brewing strains [4]. Preincubation of yeast strains at 37 °C for 30 min resulted in the induction of HSPs and protection of cells against the lethal effects of elevated temperatures and high ethanol concentrations. *S. diastaticus* strain 62 and *S. cerevisiae* strain 67 were found to be more tolerant to 20% (v/v) ethanol than the two brewing strains.

Several HSPs were identified in the four strains examined. HSPs with molecular masses of 70, 38, 26 and 23 kDa were shared in common by these strains. These HSPs have also been reported in other *S. cerevisiae* strains [1,3,5,16,24]. The optimum temperature and incubation time for the induction of HSPs in yeast strains was 37 °C for 30 min. Ethanol also induced HSPs in these strains but the number was smaller and the level of induction was weaker than that of heat shock at 37 °C. This difference in HSPs induction may be related to the severity of the stress conditions. Heat shock above 37 °C produced a more dramatic decline in viability compared to ethanol shock with 20% (v/v) ethanol (Tables 1 and 2). Ethanol shock induced the synthesis of 23, 18 and 14 kDa proteins in the lager strain but not in the ale strain (Fig. 5). The ale strain has been shown in our laboratory to be more ethanol tolerant than the lager strain (unpublished data). The 70, 23 and 14 kDa proteins reported in our previous study (submitted for publication), were similar to those reported in this study. These proteins were present in cells as of 24 h of fermentation at which time more than 2% (v/v) ethanol was present in the growth medium. The role(s) of these proteins in ethanol tolerance remain to be determined although there are reports that the synthesis of these proteins are also related to the growth cycle of yeasts [7,23,25].

S. uvarum (carlsbergensis), the most thermosensitive strain, produced the highest number of HSPs. Perhaps some of these HSPs are synthesized constitutively in the thermotolerant strains and these proteins may account for their thermotolerance. Iida and Yahara [7] isolated a heat shock-resistant mutant of *S. cerevisiae* which constitutively synthesized six proteins which were not synthesized, or were synthesized at reduced rates, in the parent strain. This mutant was found to be approximately 1000-fold more resistant to lethal heat shock than the parent strain [7]. Thus, HSPs appear to play a role in the acquisition of thermotolerance in yeasts. Other studies, however, have shown that thermotolerance in yeasts may be acquired in both the presence or absence of protein synthesis [6,27]. It is therefore possible that other cellular factors in addition to HSPs may be involved in the acquisition of thermotolerance in yeasts. We are presently investigating the role of these HSPs and other cellular factors such as membrane fatty acids in the ethanol and thermotolerance of brewing yeasts.

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