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REGULATION OF *SACCHAROMYCES CEREVISIAE* MALTOSE FERMENTATION BY COLD TEMPERATURE AND *CSF1*

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ABSTRACT

We studied the influence of cold temperature (10ºC) on the fermentation of maltose by a *S. cerevisiae* wild-type strain, and a *csf1*Δ mutant impaired in glucose and leucine uptake at low temperatures. Cold temperature affected the fermentation kinetics by decreasing the growth rate and the final cell yield, with almost no ethanol been produced from maltose by the wild-type cells at 10ºC. The *csf1*Δ strain did not grew on maltose when cultured at 10ºC, indicating that the *CSF1* gene is also required for maltose consumption at low temperatures. However, this mutant also showed increased inhibition of glucose and maltose fermentation under salt stress, indicating that *CSF1* is probably involved in the regulation of other physiological processes, including ion homeostasis.

Key words: refrigerated dough, maltose fermentation, baker's yeast, salt stress.

INTRODUCTION

Refrigerated doughs are of increasing importance in the bakery sector. These doughs permit the separation of the processes of dough production and baking, allowing large-scale production and distribution of doughs independent of the subsequent baking process (3). Various refrigerated dough products are currently available on the market, but since they are mostly leavened by chemical agents, they tend to have an inferior organoleptic quality compared with yeast-leavened dough products. Currently available commercial baker's yeasts are not applicable for such applications as they are too active under refrigerated conditions. Although glycolytic activity decreases with decreased temperature, baker's yeast still ferments even at extremely low temperatures when stored for days. The initiation of fermentation by backer's yeast is associated with a rapid loss of stress resistance, including cold resistance (1,15). Furthermore, the consumption of sugars in dough during storage decreases the browning of the crust during baking, an excess production of metabolites it is likely to deteriorate the flavor, and expansion of dough under refrigeration is undesirable because more storage space is needed. Although special dough preparation methods and/or additives have been developed to overcome these problems (6), these special techniques have restricted the spread of refrigerated dough usage. Therefore, it would be highly desirable to develop specific tailored backer's yeast strains with a strongly reduced fermenting activity under refrigeration, but maintaining normal leavening power at proofing temperatures (4,9).

Recently Kyogoku and Ouchi (5) described the isolation of *c*old *s*ensitive *f*ermentation (*csf*) mutants of baker's yeast which displayed substantially reduced fermentative activity at cold temperatures (below 15ºC), but with normal fermentation activity when the temperature is raised to 25ºC or above. The molecular analysis of one of such mutants (*csf1*) revealed that the *CSF1* gene corresponds to the ORF encoded by YLR087c located on chromosome XII (13). The predicted protein has a calculated molecular mass of 338 kDa containing four transmembrane motifs, and strains deleted on this gene (*csf1*Δ) do not grow or ferment only at low temperatures. This phenotype of the *csf1*Δ strain was a consequence of low glucose and leucine uptake at the restrictive temperature (10ºC), while at 30ºC the rates of transport were normal

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(13). Cold temperature effects on yeast fermentation performance have mainly been studied using glucose as carbon source (5,11-13). Although this has intrinsic fundamental value, the main sugar present in unsugared dough is maltose (8) and the response of *S. cerevisiae* to cold temperatures during fermentation of maltose has not been characterized in detail. In this work, we have analyzed the maltose fermentation performance of wild-type and *csf1*Δ strains under different temperatures and stress conditions.

MATERIALS AND METHODS

The *S. cerevisiae* wild-type strain CEN.PK2-1C (*MAT*a *ura3-52 his3Δ1 leu2-3,112 trp1-289 MAL2 8*^{c} *SUC2*) and the *csf1*Δ deleted mutant strain CEN.H113-6D (*MAT*a *ura3-52 his3Δ1 leu2-3,112 trp1-289* YLR087c::*URA3 MAL2 8*^{c} *SUC2*) were obtained from EUROSCARF (Institute for Microbiology, University of Frankfurt, Germany). Cells were grown aerobically in batch culture (160 rpm) at 10 or 30ºC on YEP medium (pH 5.0) containing 2% peptone, 1% yeast extract, and 2% of glucose or maltose. Solid medium plates contained 2% agar. When indicated the YEP medium was supplemented with 1 M NaCl, 1.3 M KCl, 1 mM tetramethylammonium (TMA), 0.2 M $CaCl_2$, or 0.1 mg hygromycin B (Hyg B) mL^{-1}. These last two compounds were added to the already autoclaved medium. Plates at pH 3.5 were prepared by adjusting a twofold-concentrated medium containing 50 mM succinic acid to the desired pH with Tris, autoclaving, and mixing with concentrated agar before pouring. Growth was measured at 570 nm on a UV-vis spectrophotometer after appropriate dilution of the medium. Samples were taken regularly, the cells harvested by centrifugation (2,600 g, 3 min), and the supernatant used to determine the consumption of sugars and ethanol production. Glucose and ethanol were determined using commercial enzymatic kits (Gold Analisa Diagnóstica Ltda. and Sigma, respectively). Maltose was assayed as described elsewhere (2). The experiments were repeated at least three times with consistent results. Representative results are shown.

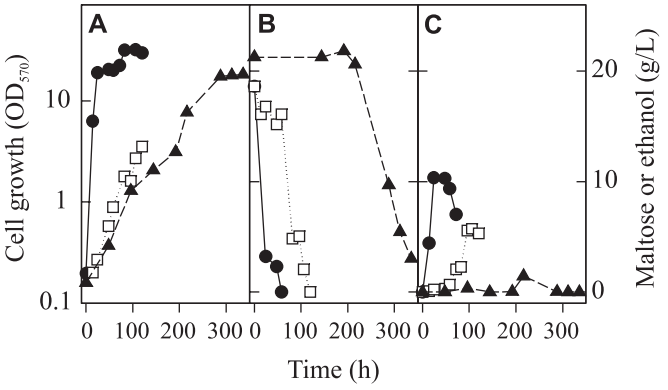

Figure 1. Growth and maltose fermentation by the wild-type CEN.PK2-1C strain. Cell density (A), sugar consumption (B) and ethanol production (C) were determined during growth at 10ºC (△), or at 30ºC in the absence (●) or presence of 1 M NaCl (□).

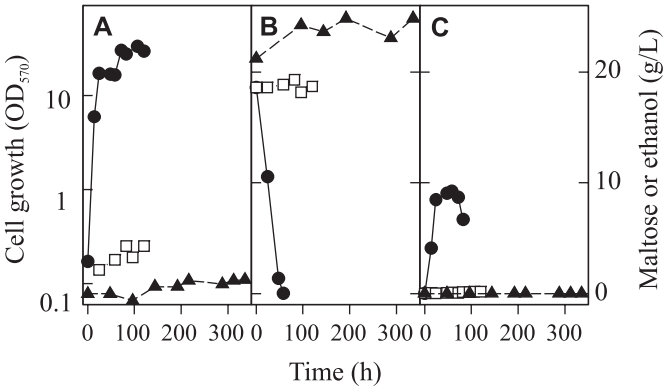

Figure 2. Growth and maltose fermentation by the *csf1*Δ mutant strain CEN.H113-6D. Cell density (A), sugar consumption (B) and ethanol production (C) were determined during growth at 10ºC (△), or at 30ºC in the absence (●) or presence of 1 M NaCl (□).

RESULTS AND DISCUSSION

The growth and maltose fermentation profile of the wild-type strain incubated at 10 and 30ºC (Fig. 1) shows that cold temperatures affect not only the fermentation kinetics (rates and length of fermentation), but also yeast metabolism. Although at 30ºC glucose or maltose were efficiently fermented reaching ~10 g ethanol L^{-1}, at 10ºC the growth rates decreased and almost no ethanol was produced from maltose (~1.5g ethanol L^{1}), while under this temperature glucose fermentation yielded ~4.5g ethanol L^{-1}. This probably is a consequence of a higher energy demand for maintenance under cold temperature, affecting maltose fermentation due to the further energy requirement for active maltose uptake by yeasts (16). Our results are also in agreement with a strong temperature dependence recently observed for maltose transport by yeast (10). The *csf1*Δ strain did not ferment or grow on maltose (Fig. 2) when cultured at 10ºC, but at 30ºC both glucose and maltose were fermented at rates similar to the ones obtained with the wild-type strain. Thus, our results clearly indicate that the *CSF1* gene is also required for maltose utilization at low temperatures.

We next analyzed the effect of high salt stress on the fermentation performance of both strains, since this stress is known to inhibit maltose fermentation by yeast cells while glucose fermentation is unaffected (14). The wild-type strain was able to produce ~5.5g ethanol L^{-1} from maltose in the presence of 1 M NaCl at 30ºC (Fig. 1), but maltose fermentation by the *csf1*Δ strain was completely inhibited under this condition (Fig. 2). Our results also showed that the salt stress affected glucose fermentation by the *csf1*Δ strain to the same degree as maltose fermentation is

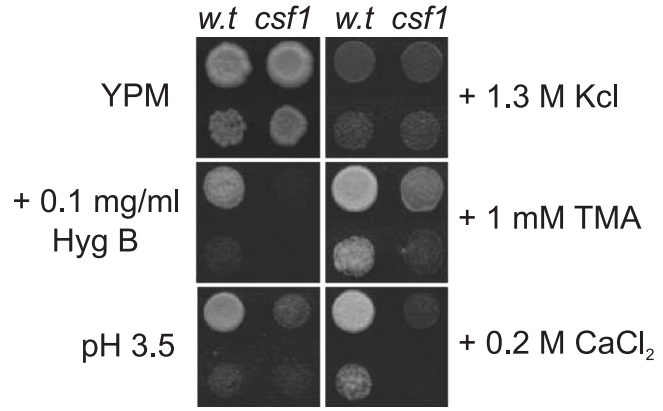

Figure 3. Sensitivity of the *csf1*Δ mutant to toxic cations. Serial dilutions (1/10 and 1/100 in water) of the wild-type CEN.PK2-1C strain (*w.t.*) and the *csf1*Δ mutant CEN.H113-6D strain (*csf1*) were spotted on YEP medium containing 2% maltose (YPM), or this medium containing the indicated concentrations of toxic cations or acidic buffer, and growth was monitored after 2 days at 30ºC.

inhibited by this stress (data not shown). Indeed, the *csf1*Δ strain showed an increased sensitivity (Fig. 3) to several toxic cations (calcium, hygromycin B, tetramethylammonium) and acidic pH, but not to high potassium concentrations. This phenotype is consistent with hyperpolarization of the plasma membrane, a phenomenon observed in strains lacking the Trk1-Trk2 potassium transporters, or lacking the kinases that activate these transporters (7). Although the mechanism by which *CSF1* allows normal nutrient uptake at low temperatures is still unknown, our results indicate that the *csf1*Δ mutant strain displays a complex pleiotropic phenotype which includes deficiencies in ion homeostasis and salt tolerance. Further research efforts will be directed towards the identification of the molecular mechanism(s) involved in the inhibition of sugar fermentation triggered by cold temperature or salt stress in the *csf1*Δ mutant.

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RESUMO

Regulação da fermentação de maltose em *Saccharomyces cerevisiae* por baixas temperaturas e *CSF1*

Foi estudado o efeito da baixa temperatura (10ºC) na fermentação de maltose por uma cepa de *S. cerevisiae* selvagem, e uma cepa *csf1*Δ mutante incapaz de transportar glicose e leucina a baixas temperaturas. A baixa temperatura afeta a cinética da fermentação por diminuir a velocidade de crescimento e rendimento celular final, com quase nenhum etanol produzido a partir de maltose pelas células selvagems a 10ºC. A cepa *csf1*Δ foi incapaz de crescer em maltose a 10ºC, indicando que o gene *CSF1* é também necessário para a utilização de maltose a baixas temperaturas. Entretanto, o mutante também mostrou inibição acentuada da fermentação de glicose e maltose por estresse salino, indicando que *CSF1* também estaria envolvido na regulação de outros processos fisiológicos, incluindo a homeostase iónica.

Palavras-chave: massa refrigerada, fermentação de maltose, levedura de panificação, estresse salino.

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