How does the yeast *Saccharomyces cerevisiae* cope with stresses?

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ABSTRACT Stress response is currently one of the leading subjects of interest, and has been widely investigated for several decades. Yeasts – in particular, *S. cerevisiae* – have been successfully used as a model to gain a better understanding of the molecular mechanism behind this fundamental and critical process. Some transcriptional regulators play important roles in mediating stress response by controlling expression of some drug resistance genes, oxidative stress response genes and genes involved in alternative carbon source utilization. They belong to the Gal4 family of zinc cluster proteins which comprise a major group of sequence-specific transcriptional regulators in *S. cerevisiae*. In addition, members of the Basic leucine zipper (bZIP) transcription factors Yap1 and Yap2 as well as Msn2 and Msn4 regulators play important functions in response to such stressful oxidative events by up-regulating the expression of some antioxidant genes. Here, we further investigate the roles of these zinc cluster proteins as well as new less known members in stress tolerance and cellular response of *S. cerevisiae* in responses to different stresses.

1. INTRODUCTION

Basic leucine zipper (bZIP) transcription factors Yap1 and Yap2 as well as Msn2 and Msn4 regulators are known to play important functions in response to such stressful oxidative events by up-regulating the expression of some antioxidant genes. Recently, we have characterized new roles for zinc cluster transcriptional regulators called Rds2, Tog1 and Znf1 and implicated them in alternative carbon source utilization. They are shown to mediate expression of genes involved in gluconeogenesis, and related pathways, required for non-fermentative mode of growth. In attempt to gain insight into this complex regulatory process, yeast strains lacking genes encoding these regulators are also tested under different environmental conditions to uncover novel roles in stress responses, including high temperature, in the presence of salt, under acid or alkaline pHs, weak acid stress. In deed the results show that they play important role. *Saccharomyces cerevisiae* has been used as a model for extensive studies of zinc cluster proteins function in biological processes, they function as transcriptional regulators of genes involved metabolism of alternative

carbons under glucose limitation. For examples, *Sip4*, *Cat8 or Rds2*, members of zinc cluster transcription regulators, have been revealed to regulate the expression of gluconeogenic genes in response to glucose exhaustion (Vincent and Carlson, 1998; Haurie et al., 2001; Soontorngun et al., 2007; Tangsombatvichit et al., 2015). Yeasts including *S. cerevisiae* can switch to use alternative carbohydrates or non-fermentable carbon sources, such as lactate, acetate, ethanol, and glycerol in the place of glucose. This phenomenon is known as "carbon catabolite repression" or "glucose repression". They are hypothesized to be involved in the regulaton of genes in response to changing environmental conditions such as nutrient limitation, heat shock, high osmolarity, acidic or alkaline pH, and weak acid stress. Strains lacking putative zinc cluster genes are examined via spot test for their role in stress response survival to these stresses. In summary, we have identified potentially new transcription regulators of stress responses in *S. cerevisiae.*

2. EXPERIMENT

2.1 Experimental Apparatus

Phenotypic screening of the zinc cluster deletion strains in *S. cerevisiae*

Wild-type FY73 and the isogenic zinc cluster deletion strains were grown in YP-dextrose (YPD) medium overnight at 30°C. Yeast cells were harvested and resuspended in distilled water prior to being diluted to an optical density at 600 nm (OD_{600}) of 0.1. Identical volumes of serially diluted cell cultures at an OD_{600} of 0.1, 0.02, 0.004 and 0.0008 were spotted onto the indicated agar plates, containing different carbon sources, including 2% glucose, 3% glycerol, 3% ethanol, 3% potassium acetate. To test for alkaline pH, strains were grown on glucose or ethanol plates containing 0.4 M HEPES (free acid) adjusted to pH 8.0 with NaOH. For the temperature sensitivity, the wild-type FY73 and the zinc cluster strains were spotted on YP-glucose, YP-ethanol, YP-acetate and YP-glycerol plates and incubated under various high temperature of 30 ^{0}C, 37^{0}C and 39^{0}C. Growth was monitored after incubation at 30°C for a period of 3-5 days. Assays were performed in triplicate and representative images are shown.

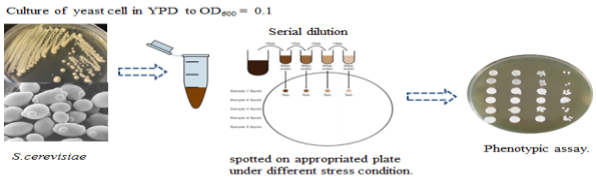

Fig. 1 Phenotypic assays of zinc cluster deletion strains under different stress conditions

ANALYSIS

3.1 Phenotypic screening of the zinc cluster deletion strains in *S. cerevisiae* under different stress conditions

The results showed that the zinc cluster deletion strains show impaired growth on YP-plates, containing increasing salt concentration, ethanol, glycerol and acetate as a sole carbon source, at elevated temperature and at different pH levels. Therefore, these zinc cluster proteins are involved in non-fermentative carbon metabolism and stress responses in yeast

S. cerevisiae.

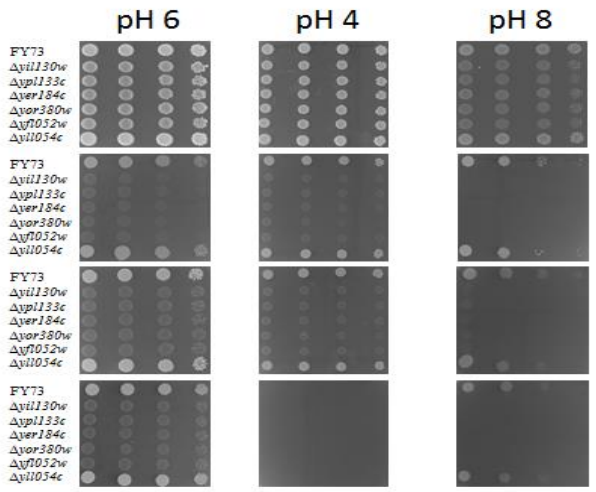

Figure 2. Phenotypic growth of the wild-type FY73 strain and the zinc cluster deletion strains using different carbon sources including 2% glucose, 3% ethanol, 3% glycerol and 3% potassium acetate under various stressful conditions for examination of pH sensitivity under various under acid or alkaline pHs concentrations of 6, 4, 8.

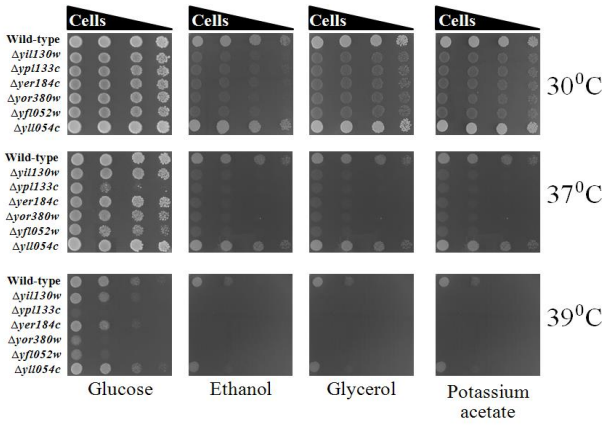

Figure 3. Phenotypic growth of the wild-type FY73 strain and the zinc cluster deletion strains using different carbon sources under various stressful conditions for examination of heat sensitivity under various high temperature of 30 ^{0}C, 37^{0}C and 39^{0}C.

CONCLUSION We have identified some potential regulators of stress response in *S. cerevisiae*. Further experiments involving gene expression studies are in progress to identify the function and target of these putative transcriptional regulators. Their characterization in the stress response will assist in genetic engineering of S. cerevisiae for stress tolerance which may be useful for some industries such as for biofuel production.

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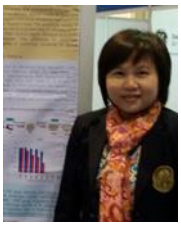

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Her research interests include Yeast Genetics and Molecular Biology.