การแสดงออกของยีน *MSN4* และยีนภายใต้การควบคุมในยีสต์ *Saccharomyces cerevisiae* สายพันธุ์ทนร้อน

Expression of *MSN4* and Genes under Its Regulation in Thermotolerant *Saccharomyces Cerevisiae*

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บทคัดย่อ

ในยีสต์ *Saccharomyces cerevisiae* โปรตีน Msn2 และโปรตีนคู่เหมือน Msn4 เป็นโปรตีนที่ควบคุมการถอดรหัสของยีนที่ตอบสนองต่อสภาวะเครียดทั่วไปรวมถึงความร้อน เพื่อช่วยในการปกป้องเซลล์จากการถูกทำลาย โปรตีน Msn2/Msn4 จับกับบริเวณเฉพาะที่มีชื่อว่า STREs และกระตุ้นในเกิดการถอดรหัสของยีนเป้าหมายนั้นๆ การศึกษานี้มุ่งเน้นบทบาทของโปรตีน Msn4 ในยีสต์ทนร้อนสายพันธุ์ธรรมชาติ โดยค้นหายีนที่ควบคุมโดยโปรตีน Msn4 ซึ่งถูกเหนี่ยวนำภายใต้การเจริญเติบโตที่อุณหภูมิสูง จากข้อมูลเบื้องต้นที่ศึกษาด้วย DNA microarray ของ *S. cerevisiae* สายพันธุ์ห้องปฏิบัติเพื่อค้นหายีนที่ควบคุมโดยโปรตีน Msn4p ที่อุณหภูมิโดยใช้โปรแกรม YEASTRACT บ่งชี้ว่ามียีนจำนวน 303 ยีน ที่มีการแสดงออกเพิ่มขึ้นมากกว่า 2 เท่า และจากจำนวนนี้มี 56 ยีนที่อยู่ภายใต้การควบคุมของโปรตีน Msn4 และมี STRE เมื่อทดสอบการเจริญเติบโตของยีสต์สายพันธุ์กลายที่ยีนดังกล่าวพบว่า สายพันธุ์กลายที่ 17 ยีนแสดงฟีโนไทป์ไม่ทนร้อน และจากการวิเคราะห์ด้วย real-time PCR ในสายพันธุ์ทนร้อนเปรียบเทียบกับสายพันธุ์ห้องปฏิบัติการที่ระยะต่างๆ ของการเจริญเติบโต ไม่พบการเปลี่ยนแปลงการแสดงออกของยีน *MSN4* ในขณะที่ยีนที่ใช้เป็นตัวแทนในการศึกษา *UBI4* มีการแสดงออกที่เพิ่มขึ้นอย่างมีนัยสำคัญที่อุณหภูมิสูง นอกจากนี้การแสดงออกของยีนอ้างอิง *NTH4* ซึ่งควบคุมโดยโปรตีน Msn4 และมี STREs มีลักษณะเพิ่มขึ้นเช่นเดียวกัน ผลการศึกษานี้เสนอแนะว่าการแสดงออกที่เพิ่มขึ้นของยีน *UBI4* ในสายพันธุ์ทนร้อนสัมพันธ์กับฟีโนไทป์ทนร้อน

ABSTRACT

Msn2p and its homolog Msn4p in yeast *Saccharomyces cerevisiae* are transcription factors which regulate the genes in response to general stresses including heat to protect cell damage. They bind specifically to stress-response elements (STREs) and activate the transcription of its target genes. In this work, we have focused on the role of Msn4p in heat stress of natural thermotolerant yeast strain. Based on DNA microarray data of *S. cerevisiae*, we searched for genes that are regulated by Msn4p under high temperature growth by computational analysis using YEASTRACT. Among 303 up-regulated genes (≥2-folds), 56 genes contained STREs at their upstream promoter. Investigation of yeast disruptants in those 56 genes revealed that 17 disruptants showed high temperature sensitive growth phenotype. By real-time PCR analysis, the change in expression level along growth phase at high temperature of *MSN4* was not observed whereas expression of *UBI4* significantly increased in both natural thermotolerant and laboratory strains. In addition, the expression level of *NTH1*, known as Msn4p-regulated gene, also elevated. These observations suggest that higher expression of *UBI4* at early-log phase could support the heat resistance phenotype in both of laboratory and thermotolerant strains.

คำสำคัญ: ยีน *MSN4*, สภาวะเครียด, ยีสต์ทนร้อน, การแสดงออกของยีน, เรียล-ไทม์ พีซีอาร์
Keywords: *MSN4*, heat stress, thermotolerant yeast, gene expression, real-time PCR

INTRODUCTION

During ethanol fermentation process, yeast cells are exposed to several types of stresses such as increase in temperature, osmotic pressure and ethanol concentration. Therefore, yeast cells have to reprogram their gene expression appropriately in order to maintain their metabolic activity to survive in stress conditions. High temperature fermentation by thermotolerant yeast can reduce cooling cost and the risk of contamination by performing the fermentation under high temperature condition, and can be applied for SSF (simultaneous saccharification fermentation).

In *S. cerevisiae,* heat response is governed by the transcription factors Hsf1p, Msn2p and Msn4p (Trott and Morano, 2003). Msn2/Msn4p are related to each other and activated in stress conditions, which results in translocation from the cytoplasm to the nucleus. Msn2/Msn4p binds to the stress-response elements (STREs), 5'-CCCCT-3', which are located in the promoter region of their target genes (Martinez-Pastor *et al.,* 1996). *MSN4* expression is itself Msn2/Msn4p dependent and induced by heat and other stresses, while *MSN2* expression is constitutive (Gasch *et al*., 2000, Causton *et al.,* 2001).

C3723-8B, natural thermotolerant strain (Choowong *et al*., 2012) was used as a model to investigate the special mechanisms that allow it to grow at high temperature. We have found the genes *CDC19* and *RSP5* encoding pyruvate kinase and ubiquitin ligase, respectively, which support the Htg^{+} phenotype of C3723 thermotolerant strain (Suthee *et al*, 2012 and Hosein *et al*, 2011). To search for genes that are responsible for growth at high temperature, we employed DNA microarray approach. This research aims to elucidate the expression of *MSN4* and genes under its regulation (*UBI4* and *NTH1*) in thermotolerant C3723-8B and thermosensitive BY4743 strains in different growth phase through the comparison of their expression between 30°C and higher temperatures of 41 ºC (C3723-8B) and 40°C (BY4743).

MATERIALS AND METHODS

A. Growth Condition

S. cerevisiae strains were cultured in nutrient rich broth (Yeast Extract Peptone Dextrose, YPD) at 30 °C and 41 °C or 40 °C with shaking at 180 rpm for 18 – 24 h. Samples were taken for OD_{660} measurement and collected for total RNA extraction at appropriate growth phases.

B. DNA Microarray Data Analysis

DNA microarray data was analyzed using SGD, GOTermMAPP and YEASTRACT (Dário *et al*, 2011) for the biological process and for identifying transcriptional regulators of up-regulated genes.

C. Screening for Heat Responsive Genes under Regulation of Msn4p

S. cerevisiae BY4742 were cultured in YPD broth for 24 h and 2.5 µl of 10-fold serial dilution were dropped on YPD agar, and incubated for 3 days at 30, 37, 39 and 40 °C.

D. Gene Expression Analysis by Quantitative Real-time PCR

Total RNA was extracted by hot acidic phenol method. A 1 µg of total RNA was purified and converted to cDNA by using QuantiTect® Reverse Transcription Kit (QIAGEN®, Germany). The expression level of genes was examined by using SYBR®*Premix Ex Taq*™ (TliRNaseH Plus) (Takara, Japan) in 7300 Real-Time PCR System (Applied Biosystem, USA). A standard curve was determined with cDNA from mid-log phase of *S. cerevisiae* at 30°C. *ACT1* was used as an endogenous control and data was calculated by relative standard curve method.

RESULTS AND DISCUSSION

A. DNA microarray analysis of S. cerevisiae BY4742 at 40°C and identification of genes involved in heat response

The DNA microarray data of *S. cerevisiae* BY4742 grown at 40 °C revealed that out of 5,868 genes, expression level of 303 genes were up-regulated by more than 2 folds under high temperature as compared with that at 30°C. Among 303 up-regulated genes, the computational analysis using YEASTRACT showed that 71 genes are under Msn4p regulation. By investigation of those 71 genes, only 56 genes contain STREs at their upstream promoter. This result suggested that Msn4p was directly or indirectly involved in their transcriptional regulation supported by the previous studied which showed that Msn2/4p could regulate the gene expression of *GRE1* even it does not contain any STREs. As in this case the researcher observed the PDS sequences at its upstream promoter which similar to STREs binding site so it could be recognized as STREs (Garray-Arroyo and Covarrubias, 1999). Screening of thermosensitive phenotype using *S. cerevisiae* BY4742 in single 56 genes disruption revealed that 17 disruptants exhibited thermosensitive phenotype at least equal to *S. cerevisiae* BY4742. Among those, *UBI4* was subjected to gene expression analysis since it was reported to be involved in stress response and it is the representative of protein turn-over process. *UBI4* encodes polyubiquitin which function as molecular specific chaperone in response to heat stress (Parsell and Lindquist, 1993) *NTH1* was used as positive control since it is known as Msn4p-regulated gene.

B. Gene expression in S. cerevisiae C3723-8B and BY4743

Gene expression of *MSN4* and two genes under its regulation, *UBI4* and *NTH1*, were examined at early-, mid- and late-log phases. The results revealed that the expression of *MSN4* at high temperature in natural isolated thermotolerant C3723-8B (41 ºC) and diploid laboratory BY4743 (40 ºC) strains displayed no significant change ($\leq$2-folds) when compared to its expression at 30°C (data not shown) (Fig. 1a). This suggested that the activation of *MSN4* was not regulated at transcriptional level as in the case of its homologue gene, *MSN2* which activated by hyperphosphorylation resulting in translocation from cytoplasm into nucleus (Garreau *et al*, 2000). For *NTH1*, expression was not significantly different between high temperature and 30°C ($\leq$2-folds). However, the expression in C3723-8B under high temperature growth was insignificantly higher than that in BY4743 strain (Fig. 1b). This gene encodes neutral trehalase which is required for trehalose degradation (Kopp *et al*, 1993). Interestingly, the expression level of *UBI4* in both C3723-8B and BY4743 significantly increased ($\geq$2-folds) when cells were exposed to high temperature (Fig. 1c) and it seems to be higher in C3723-8B strain than BY4743. *UBI4* is known as a stress response gene which is involved in ubiquitination and it is known as stress-inducible gene (Finley *et al*, 1987). The previous studies investigated that Ubi4p affected to free ubiquitin when yeast cells were exposed to heat stress. This protein is required for protein degradation and turnover (Berry *et al*, 2011).

C. Phenotype of ubi4 disruptant in response to heat stress

Quantitative real-time PCR analysis showed significant change in *UBI4* expression upon exposure to high temperature stress. Therefore, we analyzed growth behavior of *ubi4* disruptant (Fig. 2). *ubi4* disruptant showed high temperature sensitive growth phenotype as compared with parental strain BY4742. This suggests that Ubi4 plays important roles in high temperature resistance in BY4742 genetic background. In contrast to the observation in previous report, Δ*ubi4* disruptant showed no different growth in comparison to wild type strain when treated with heat shock at 40 ºC (Berry *et al*, 2011).

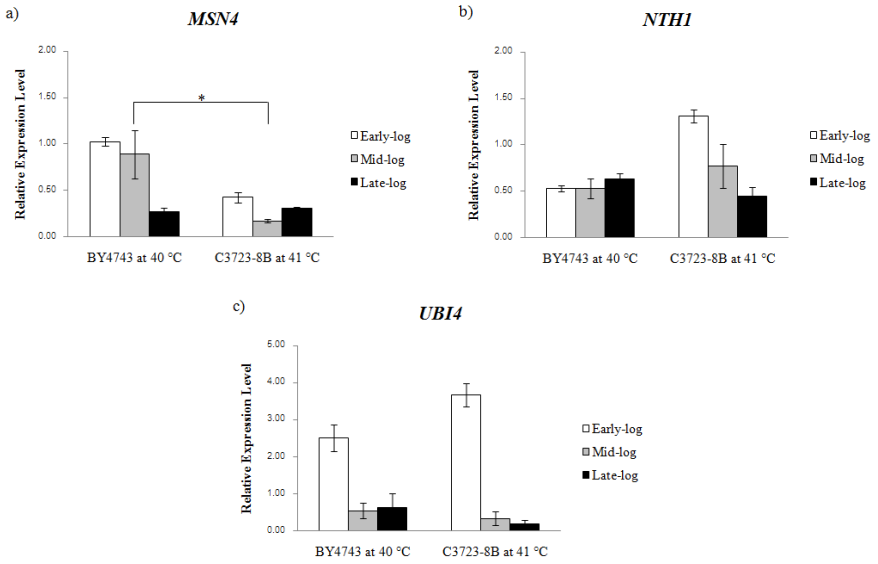

Figure 1 Expression of genes under Msn4p regulation in response to high temperature in *S. cerevisiae* C3723-8B and BY4743 strains. Cells were grown in YPD broth for 2 h (early-log phase), 5 h (mid-log phase) and 8 h (late-log phase) at indicated temperature. Transcription level of *MSN4* (a), *NTH1* (b) and *UBI4* (c) were normalized by *ACT1* transcription levels. Each bars show average value with standard deviation of three replication for two independent experiments.

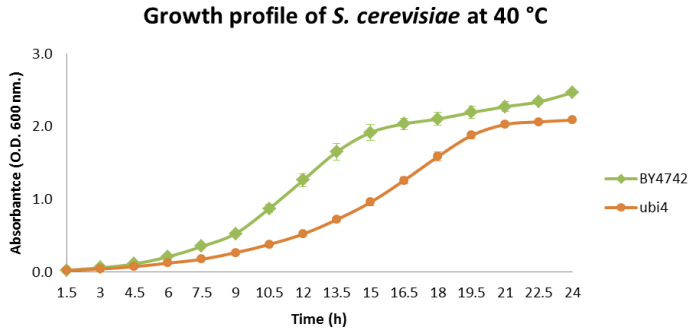

Figure 2 Growth of *S. cerevisiae* BY4742 Δ*ubi4* disruptant and its parent strain. Growth was monitored in nutrient-rich broth at 40 °C.

CONCLUSION

Our results showed that the expression of *MSN4* did not significantly change in thermotolerant strain. The slightly increased expression of *NTH1* and higher expression of *UBI4* in thermotolerant strain might correlate with its heat resistance phenotype in *S. cerevisiae.* The biological function related to heat resistance of *UBI4* should be further elucidated.

ACKNOWLEDGEMENTS

This study was supported in part by the joint program in the field of biotechnology under the Japan Society for the Promotion of Science (JSPS) and the National Research Council of Thailand (NRCT); and Thailand Research Fund, Master Research Grants (TRF - MAG).

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