The Journal of Microbiology (2012) Vol. 50, No. 3, pp. 544–546
DOI 10.1007/s12275-012-2043-3
Copyright ⓒ 2012, The Microbiological Society of Korea

NOTE

# Ectopic Expression of Sweet Potato *MuS1* Increases Acquired Stress Tolerance and Fermentation Yield in *Saccharomyces cerevisiae*

**Il-Sup Kim[1], Sun-Young Shin[1], Sun-Hyung Kim[2], and Ho-Sung Yoon[1*]**

[1]*Department of Biology, Kyungpook National University, Daegu 702-701, Republic of Korea*
[2]*Department of Environmental Horticulture, University of Seoul, Seoul 130-743, Republic of Korea*

(Received January 27, 2012 / Accepted April 12, 2012)

**The *MuS1* gene is highly homologous to many stress-related proteins in plants. Here, we characterized whether a new candidate gene, *MuS1*, is related to multiple stress tolerance in yeast as it is in plants. Transgenic yeast strain expressing *MuS1* were more resistant to hydrogen peroxide, menadione, high salinity, metals (i.e., cadmium, copper, iron, and zinc), ethanol, and lactic acid than wild-type strain transformed with a vector alone. In addition, the alcohol yield of the transgenic yeast strain was higher than that of the wild-type strain during the batch fermentation process. These results show that *MuS1*-expressing transgenic yeast strain exhibits enhanced alcohol yield as well as tolerance to abiotic stresses, especially metal stress.**

***Keywords***: *MuS1* gene, stress response, fermentation, *Saccharomyces cerevisiae*

Plants are continuously challenged by various environmental stresses such as high salinity, drought, extreme temperature, ozone, and flooding throughout their life cycle. To overcome unfavorable conditions, plants express a large number of genes associated with stress adaptation or tolerance via a fast and efficient gene expression program. Consequently, plants naturally undergo morphological and physiological changes (Ashraf, 2009). Numerous stress-induced genes have been identified in various plant species, and the functions of their products have been elucidated (Lushchak, 2011). Among these genes, multiple stress responsible gene I (*MuS1*), is induced in response to dehydration in sweet potato roots (*Ipomoea batatas* L. cv. Yulmi) (Seo *et al.*, 2010). To date, the potential of *MuS1* as a valuable genetic source has not been investigated. Here, we used a yeast model system to determine whether or not stress tolerance is mediated by *MuS1*. The objective of this study was to identify the potential of *MuS1*-expressing transgenic yeast for stress-tolerant yeast strain improvement and bioethanol production.

The *MuS1*-inserted TA cloning vector was provided by Dr. Sun-Hyung Kim. After confirming the sequence correction, *MuS1* was digested with *Bam*HI and *Eco*RI, and ligated into the corresponding sites of yeast expression vector p426GPD (Fig. 1A). The construct harboring *MuS1* was transformed into *Saccharomyces cerevisiae* BY4741 (*MATa*, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*) using the PEG/LiCl method (Gietz and Woods, 2001). Transformants were selected by a yeast synthetic drop-out medium without uracil. Ectopic expression of *MuS1* was confirmed by semiRT-PCR. Total RNA was isolated from mid-log phase yeast cells ($A_{600}$=2.0) using an RNA purification kit (Promega, USA). RT-PCR was carried out with sense 5′-TCGGTCCCGTTTACGATAAG-3′ and antisense primers 5′-ATGGAAGATACGACGCAACC-3′ using a One-Step RT-PCR PreMix kit (Intron, Korea) according to the manufacturer's instructions. The PCR conditions were as follows: 1 cycle for cDNA synthesis at 45°C for 30 min and 94°C for 5 min; 25 cycles at 94°C for 30 sec, 56°C for 30 sec, and 72°C for 1 min; and a final extension at 72°C for 7 min. *PDA1* was used as a housekeeping control (Wenzel *et al.*, 1995). As shown in Fig. 1B, a single band was detected in the transgenic yeast strain (TG) harboring *MuS1*, whereas no signal was detected in the transformed yeast strain (WT) with vector alone. Because ectopic *MuS1*-overexpression enhances tolerance to cadmium stress in transgenic plants (Kim *et al.*, 2011), these results led us to postulate that *MuS1* may also be able to enhance acquisition tolerance when microorganisms are imposed to stress. To examine whether *MuS1* expression improves stress responses against exogenous stimuli, a spotting assay was performed. Mid-log phase cells were exposed to 30 mM $H_2O_2$, 0.6 mM menadione (MD), 10 mM $CdCl_2$, 10 mM $CuCl_2$, 0.1 M $FeCl_2$, 0.2 M $ZnSO_4$, 5 M NaCl, 12% or 16% ethanol, or 1 M lactic acid for 1 h at 30°C with shaking (160 rpm), and then serially diluted ($10^0$ to $10^{-4}$). Diluted solution (5 μl) was spotted onto YPD agar medium and incubated for 2–3 days at 30°C. Consistent with the results obtained from plants, the ectopic expression of *MuS1* enhanced the acquired tolerance of TG strain to cadmium stress compared to the WT strain (Fig. 1F). In addition, TG strain was more resistant than WT strain in the presence of $H_2O_2$, MD, $CuCl_2$, $FeCl_2$, $ZnSO_4$, NaCl, ethanol, and lactic acid (Figs. 1C–1M). However, *MuS1*-expressing TG strain was sensitive to *tert*-butylhydroperoxide (*t*-BOOH), acetic acid, and high temperatures (data

*For correspondence. E-mail: hyoon@knu.ac.kr; Tel.: +82-53-950-5348; Fax: +82-53-955-5522

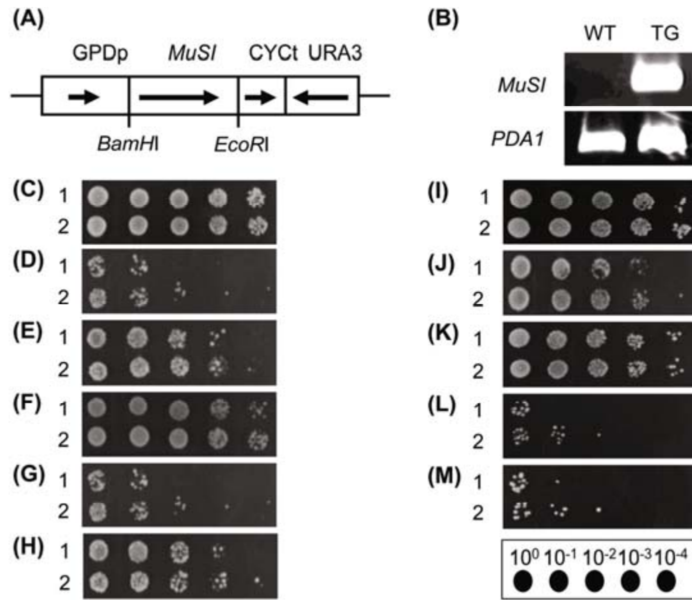


**Fig. 1. Stress tolerance assay in *MuS1* transgenic yeast.** (A) Schematic diagram of the *MuS1*-expression construct. GPDp, GPD promoter; CYCt, CYC terminator; URA3, gene encoding uracil as a selectable marker. Arrows indicate the expression direction. (B) *MuS1* expression analysis at the transcriptional level using semiRT-PCR. The products of *PDA1* were used as a housekeeping standard. WT, wild-type strain with vector alone; TG, *MuS1*-expressing yeast strain. Mid-log phase yeast cells ($A_{600}$=2.0) were exposed to exogenous stimuli without (C) or with exogenous stimuli containing 30 mM $H_2O_2$ (D), 0.6 mM menadione (E), 10 mM $CdCl_2$ (F), 10 mM $CuCl_2$ (G), 0.1 M $FeCl_2$ (H), 0.2 M $ZnSO_4$ (I), 5 M NaCl (J), 12% (K) or 16% (L) ethanol, or 1 M lactic acid (M) for 1 h at 30°C with shaking and then serially diluted ($10^0$ to $10^{-4}$). Diluted solution (5 μl) was spotted onto YPD agar medium and incubated for 2–3 days at 30°C. 1, wild-type strain with vector alone; 2, *MuS1*-expressing yeast strain.

not shown). It is reported that *MuS1* expression is highly induced by various stressors, including dehydration, high salinity, heavy metals oxidation, and plant hormones in sweet potato plants (Seo *et al*., 2010). *MuS1* expression in transgenic tobacco plants increases stress tolerance to cadmium (Kim *et al*., 2011), salt, high temperature, and osmotic stress (Seo *et al*., 2010), and the potential for phytoremediation of soils contaminated with cadmium (Song *et al*., 2003). In *Escherichia coli*, *MuS1* overexpression also increases stress tolerance to cadmium (Kim *et al*., 2011). However, the tolerance mechanism related to *MuS1* expression is largely unknown in many species. Recently, the cadmium resistance mechanism was partially explained in tobacco plants; this mechanism involves the formation of complexes containing cadmium thiol compounds and translocated metallothioneins and phytochelatins, and the subsequent accumulation of these complexes in the vacuoles of transgenic tobacco plants (Kim *et al*., 2011). Taken together, these results suggest that *MuS1* functions as a positive regulator of various stress responses and may be useful for improving stress-tolerant transgenic yeast strains.

Because *MuS1*-expressing TG strain was more resistant than WT strain to high ethanol concentrations and exogenous stimuli, laboratory-scale batch fermentation involving TG and WT yeast strains was performed at 30°C for 72 h in YG medium containing 1% yeast extract and 20% glucose. The alcohol yield, residual glucose content, and cell survival at 30°C (the general temperature most frequently used for industrial fermentation) were significantly different between TG and WT strains. During batch fermentation, the alcohol

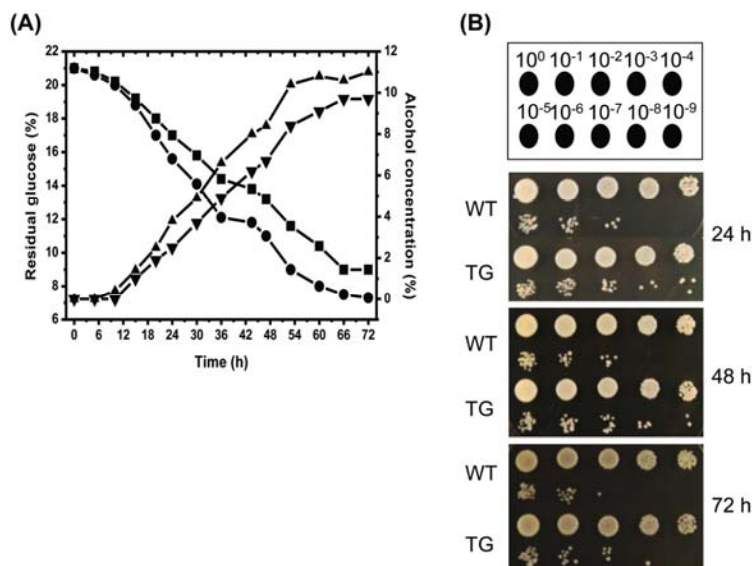


**Fig. 2. Alcohol yield and cell survival during batch fermentation.** (A) Batch fermentation was initiated by inoculating 4% of the cultures (initial $A_{600}$=0.2) in YG medium. Alcohol concentration was determined from the percentage (v/v) of alcohol in the distillate after fermentation according to an alcohol hydrometer. Residual total glucose concentration was measured using a hand-held refractometer after centrifugation to spin down cells. Upward triangle, alcohol yield in TG strain; downward triangle, alcohol yield in WT strain; circle, residual glucose in TG strain; square, residual glucose in WT strain. (B) Cell survival was determined by performing a spotting assay from cells harvested after 24, 48, and 72 h of fermentation. Cells were diluted serially from $10^0$ to $10^{-9}$ and then spotted onto YPD agar plates. WT, wild-type strain with vector alone; TG, *MuS1*-expressing yeast strain.

yield in TG strain was 10%-higher than that in WT strain. Residual glucose was inversely proportional to alcohol yield (Fig. 2A). The growth kinetics of TG strain increased sharply when they were grown in YG medium (Fig. 2B); however, the growth kinetics of both types of strain did not differ significantly when they were grown in YPD medium (Fig. 1C). These results indicate that *MuS1* expression affects fermentation yield, which is a well-chosen factor for the corresponding calculated fermentation yield during fermentation in TG strain. However, the acquisition mechanism of *MuS1* during the fermentation process remains unknown.

The results of the present study show that *MuS1*-expression, at least with respect to its functionality in eukaryotic systems, may have a protective role in fighting environmental stress, which could lead to improved redox homeostasis. The results also highlight the potential for using *MuS1*-expressing transgenic yeast in biofuel production from sugar biomass. Thus, further studies should focus on the stress resistance mechanisms in *MuS1* transgenic yeasts as well as the suitability of *MuS1* for the bioremediation of heavy metals such as those involved in *MuS1* transgenic plants.

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No. PJ0080602011), Rural Development Administration, Republic of Korea.

## References

**Ashraf, M.** 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.* **27**, 84–93.

**Gietz, R.D. and Woods, R.A.** 2001. Genetic transformation of yeast. *Biotechniques* **30**, 816–820.

**Kim, Y.N., Kim, J.S., Seo, S.G., Lee, Y., Baek, S.W., Kim, I.S., Yoon, H.S., Kim, K.R., Kim, S.H., and Kim, K.H.** 2011. Cadmium resistance in tobacco plants expressing the *MuSI* gene. *Plant Biotechnol. Rep.* **5**, 323–329.

**Lushchak, V.I.** 2011. Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **153**, 175–190.

**Seo, S.G., Kim, J.S., Yang, Y.S., Jun, B.K., Kang, S.W., Lee, G.P., Kim, W., Kim, J.B., Lee, H.U., and Kim, S.H.** 2010. Cloning and characterization of the new multiple stress responsible gene I (*MuSI*) from sweet potato. *Genes Genom.* **32**, 544–552.

**Song, W.Y., Sohn, E.J., Martinoia, E., Lee, Y.J., Yang, Y.Y., Jasinski, M., Forestier, C., Hwang, I., and Lee, Y.** 2003. Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nat. Biotechnol.* **21**, 914–919.

**Wenzel, T.J., Teunissen, A.W., and de Steensma, H.Y.** 1995. *PDA1* mRNA: a standard for quantitation of mRNA in *Saccharomyces cerevisiae* superior to *ACT1* mRNA. *Nucleic Acids Res.* **23,** 883–884.