3Dp12 Functional redundancy of protein phosphatases Ptp2 and Msg5 prevents hyper-activation of the calcium-mediated signaling in *Saccharomyces cerevisiae*

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Reversible phosphorylation under the control of protein kinases (PKases) and protein phosphatases (PPases) is one of the important post-translational modifications for regulating many essential cellular processes. Previously, we reported that simultaneous disruption of two PPase genes, *PTP2* and *MSG5*, causes calcium sensitivity indicating a functional redundancy existing between the two PPases in response to high extracellular calcium. In this study, we aim to elucidate the roles of Ptp2 and Msg5 in the growth of *S. cerevisiae* under calcium exposure. Conversely, additional disruption of SLT2 cascade-related PKase genes BCK1, MKK1 or SLT2 in the *ptp2Δmsg5Δ* double disruptant background conferred calcium tolerance. Furthermore, genetic analyses revealed that calcineurin inactivation by the disruption of its regulatory subunit gene, *CNB1*, or treatment with a calcineurin inhibitor, FK-506, can also suppress the calcium sensitive phenotype of the *ptp2Δmsg5Δ* double disruptant. Thus, in a calcium-exposed environment, inactivation of either the SLT2 or calcineurin pathway suppresses the calcium sensitivity of the *ptp2Δmsg5Δ* double disruptant. In conclusion, we deduce that Ptp2 and Msg5 have key regulatory functions that prevent the over-activation of the calcium-induced signaling cascade under the parallel control of the SLT2 and calcineurin pathways.

Functional redundancy of protein phosphatases Ptp2 and Msg5 prevents hyper-activation of the calcium-mediated signaling in *Saccharomyces cerevisiae*

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Key words *Saccharomyces cerevisiae*, PTP2, MSG5, Calcium sensitivity

3Dp13 Increased transcription of *RPL40A* gene is important for the improvement of RNA production in *Saccharomyces cerevisiae*

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Yeast RNA is an important source of 5′- ribonucleotides to be used in food and pharmaceutical industry. Efficient transcription of rDNA is very important to construct yeast strain with high RNA content. *RRN10* gene, a component of upstream activation factor of Pol I pre-initiation complex, is essential to promote high-level transcription of rDNA in yeast. In previous study, we have isolated a dominant suppressor, SupE, which showed the ability to restore the severe growth defect and reduced RNA content caused by disruption of *RRN10* gene. Mutation in SupE strain is multiple and designated as *SUPE*. Genomic library of SupE strain was introduced into the *Δrrn10* strain to screen for the transformants which showed faster growth than the *Δrrn10* strain. Subcloning analysis indicated that the plasmid insert contain *RPL40A* gene involved in assembly of the ribosomal subunits was responsible for the suppression although we could not find any base change on it compared to that of parental *Δrrn10* strain. Additional copy of *RPL40A* gene partially suppresses the defects caused by *Δrrn10* disruption. Further analysis on copy number effect confirmed that increased transcription of *RPL40A* gene increased the growth rate and RNA content of the *Δrrn10* disruptant strain and when multiple copies of *RPL40A* gene is combined with *SUPE* mutation, resultant SupE strain showed higher RNA content than wild-type strain.

Increased transcription of *RPL40A* gene is important for the improvement of RNA production in *Saccharomyces cerevisiae*

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Key words Suppressor mutation, RPL40A gene, Yeast

3Dp14 Enhanced bioethanol production from sugarcane molasses using thermotolerant *Saccharomyces cerevisiae* strain TJ14-U54

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Although in comparison with starchy substances, sugar feedstock such as molasses is more favorable for ethanol production owing to its minimal energy requirement and greenhouse gases emission, economic issues still stand as the main concern to the usage of sugar crops by-products as substrate for bioethanol production. Use of thermotolerant *Saccharomyces cerevisiae* exhibiting tolerance to 41°C with high ethanol yield can be considered as a solution for feasible bioethanol production. This study focuses on improvement of the ethanol production from sugarcane molasses using multiple-stress-tolerant strain TJ14. UV mutagenesis was used to improve economical traits and consequently mutants are screened for enhanced high-temperature and ethanol resistance. The mutant TJ14-U54 which exhibits confluent growth at 42°C even in the presence of 8% ethanol was used to evaluate molasses fermentation performance and displayed more than 10% higher ethanol productivity (65g/l) than that of TJ14 from 20% molasses as a sole carbon source. Based on our results, the use of strain TJ14-U54 for ethanol production from molasses holds potential for scale-up studies.

Enhanced bioethanol production from sugarcane molasses using thermotolerant *Saccharomyces cerevisiae* strain TJ14-U54

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Key words high ethanol production, thermotolerance, molasses

3Dp15 Functional analysis of *HpFAD3* gene encoding Δ15-fatty acid desaturase in *Hansenula polymorpha*

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Hansenula polymorpha Δ12-fatty acid desaturase (*HpFAD2*) and Δ15-fatty acid desaturase (*HpFAD3*) genes were cloned in our previous study. To examine function and regulatory element for desaturation system in *H. polymorpha*, we constructed in this study the disruption of *HpFAD3*. *Hpfad3Δ* does not synthesize α-linolenic acid (αC18:3, Δ9, Δ12, Δ15), indicating that *HpFAD3* is the only one gene encoding Δ15-fatty acid desaturase in *H. polymorpha*. We have noted that C18:2 as a substrate to produce αC18:3 is not accumulated in *Hpfad3Δ*. In addition, we also found that C18:1 is accumulated in *Hpfad3Δ* and transcription of *HpFAD2* is reduced by C18:1 fatty acid supplementation. These observations suggest that C18:1 accumulated in *Hpfad3Δ* may repress *HpFAD2* transcription, resulting in the decrease in C18:2. In this connection, we found fatty acid regulated (FAR) like element, 5'-CCGGTTGGC-3' and 5'-GGGGACAGC-3' similar to that of *S. cerevisiae* Δ9-fatty acid desaturase (*ScOLE1*) in the upstream region of *HpFAD2* and *HpFAD3* genes, respectively. *ScOLE1* is controlled by ScRsp5 which acts as E3 ubiquitin ligase, ScSpt23 and ScMga2 as homologous transcriptional coactivators. We searched *H. polymorpha* genomic sequence to find out homologues of *ScRSP5*, *ScSPT23* and *ScMGA2*. The result revealed that *HpRSP5* shares 67% identity with *ScRSP5* while *HpSPT23* does 28% and 27% identity with *ScSPT23* and *ScMGA2*, respectively.

Functional analysis of *HpFAD3* gene encoding Δ15-fatty acid desaturase in *Hansenula polymorpha*

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Key words fatty acid desaturase, Hansenula polymorpha