（学位第３号様式）

学 位 論 文 要 旨

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題　目	加圧熱水処理リグノセルロースからのバイオエタノールの生産に適した酵母 *Saccharomyces cerevisiae* の育種（**Engineering of yeast *Saccharomyces cerevisiae* strain appropriate for production of bioethanol from hot-compressed water-treated lignocelluloses)**

Inhibitory compounds present in hot-compressed water-treated lignocelluloses hydrolysate (HCWT-lig. hyd.) hamper the growth and ethanol fermentation of yeast *Saccharomyces cerevisiae*, and one of the key barrier for industrialization of this advanced biomass pretreatment technology. Incorporation of physiochemical understanding into the study of fermentation inhibitors, we, for the first time, discovered glycolaldehyde, which is formed through the retro-aldol condensation of liberated sugars in HCWT-lig. hyd. as a key novel inhibitor in HCWT-lig. hyd.. Hence, this dissertation study was implemented to unveil the toxic mechanisms of glycolaldehyde in both molecular and physiological levels of *S. cerevisiae* and to identify the rational pathways for engineering glycolaldehyde tolerant yeast strain.

We used omics- and megavariate-oriented approaches to develop *S. cereviciae* toward the inhibitory effect of HCWT-lig. hyd. First, we identified glycolaldehyde is the most toxic inhibitory compound in HCWT-lig. hyd . and key target to develop inhibitor tolerant yeast strain. The molecular mechanism of glycolaldehyde toxicity on yeast was elucidated by genome-wide analysis and the transcriptome analysis. The results suggested that dehydrogenases functions confer glycolaldehyde tolerance, and we speculated the enzyme that reduce the glycolaldehyde to ethylene glycol is an effective mechanism to mitigate the damage.

Consistence with uncovered toxic mechanism of glycolaldehyde on yeast, at the second stage of research, we developed the glycoladehyde tolerant yeast strain by expressing NADH-dependent *ADH1*. The strain capable to reduce glycolaldehyde efficiently into less toxic ethylene glycol. Moreover, we further improved the glycolaldehyde tolerance of *ADH1*-expressing strain by engineering redox cofactor for glycolaldehyde-reducing reaction. Indeed, expression of NADPH-dependent *GRE2* in addition to *ADH1* restored the redox balance, enhanced NADPH-dependent glycolaldehyde-reducing reaction, improved growth and ethanol production of yeast in glycolaldehyde-containing medium. Taken together we successfully demonstrated the development of inhibitor tolerant yeast by simultaneous expression of NADH- and NADPH-dependent glycoldehyde detoxification systems.

However, the developed strain has long lag phase of growth mainly owing to complex inhibitory effects of HCWT-lig.hyd. Therefore, third phase of our study, we invented novel approach to develop tolerant yeast strains to complex inhibitory stresses of HCWT-lig. hyd. First, we confirmed the existence of interactive inhibitory effect of major inhibitors present in HCWT-lig.hyd. Second, through systems biology approaches, we dissected the complex fermentation inhibitory stress responces of yeast in HCWT-lig.hyd. and identified sumolysation as a novel potential mechanism to overcome combinational inhibitor effect of HCWT-lig.hyd. Indeed, augmenting STUbL system into *ADH1*-expressing strain significantly recovers the total growth, reduces the lag phase of growth and enhanced the ethanol production in combinational inhibitors-containing medium.

In sum, this dissertation research for the first time has established and comprehensively characterized glycolaldehyde as a key fermentation inhibitor in HCWT-lig.hyd, Moreover, we invented novel efficient glycolaldehdye detoxification mechanisms through expression of NADH- and NADPH-dependent oxidoreductases, and identified SUMO-dependent pathway as a potent mechanism for reducing inhibitors mediate lag phase of yeast growth. The uncovered completely novel knowledge in this dissertation and the developed biocatalyst will certainly beneficial for boosting second generation biofuel production.