博士論文要約（Summary）

平成 24 年入学
連合農学研究科　Bioscience and Biotechnology 専攻
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タイトル	**Engineering of yeast *Saccharomyces cerevisiae* strain appropriate for production of bioethanol from hot-compressed water-treated lignocelluloses** **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**

キーワード（ **yeast** ）（ **glycolaldehdye** ）（ **bioethanol** ）

「序論及び目的」Introduction

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 pre-treatment technology. Roughly about 60 inhibitory compounds have been identified as potent inhibitors in lignocelluloses hydrolysate. However, It has not been well characterized the inhibitory effect of HCWT-lig.hyd on yeast. We have for the first time discovered glycolaldehyde, which is formed through the retro-aldol condensation of liberated sugars in HCWT-lig. hyd (2-24 mM) as a key novel inhibitor in second generation bioethanol production (1). Hence, this study was implemented to unveil the toxic mechanisms of glycolaldehyde in both molecular and physiological levels of *S.cereviciae* and to identify the rational pathways for engineering glycolaldehyde tolerant yeast strain. The ultimate objective of this research is to develop efficient and robust *S. cerevisiae* for fermenting HCWT-lig. hyd to boost the second generation biofuel production.

「材料及び方法」Materials and methods

To accomplish the research objectives, wide variety of approaches were adopted. Briefly, comprehensive genomic approaches such as genome-wide analysis of mutant library of BY4743 and the microarray analysis were adopted to identify toxic mechanisms of glycolaldehyde and the genetic traits for engineering yeast strain for glycolaldehyde resistant yeast strain. The developed glycolaldehyde tolerant strains were further improved to detoxify glycolaldehyde and ethanol production through in-depth analysis of fermentation metabolic products perturbation in the presence of glycolaldehyde followed by metabolic engineering approaches. The significance of inhibitory activity of glycolaldehyde and its interactive inhibitory effect with other reported major fermentation inhibitors on yeast in the HCWT-lig. hyd were analyzed by transcriptomic, genomic, proteomic and megavariate data modeling. Through the analysis, rational molecular pathways for engineering yeast strains to combinational inhibitory effect of HCWT-lig. hyd were extracted.

「結果」 Results

We adopted novel approaches to develop *S.cereviciae* toward the inhibitory effect of HCWT-lig. hyd. First, we reported that glycolaldehyde significantly inhibits yeast growth (IC_{50}=10 mM) and fermentation (1). Furthermore, we adopted PLS modeling of growth to dissect the fermentation inhibitory effect of major inhibitors (glycolaldehyde, 5-HMF, furfural, methylglyoxal, and acetic acid) present in lignocelluloses hydrolysate, and confirmed glycolaldehyde is the most toxic compound in HCWT-lig. hyd (2). Furthermore, these results suggested glycolaldehyde is a key target to develop an inhibitor-tolerant yeast strain. We adopted a genome-wide analysis of glycolaldehyde toxicity towards yeast cells for insight into the molecular mechanism of inhibition of yeast ethanol fermentation by glycolaldehyde. As a result, 170 genes were identified as genes required for glycolaldehyde tolerance (1). The genome-wide analysis suggested that the dehydrogenases functions confer glycolaldehyde tolerance, and we speculated the enzymes that reduce the glycolaldehyde to ethylene glycol is effective to mitigate the damage.

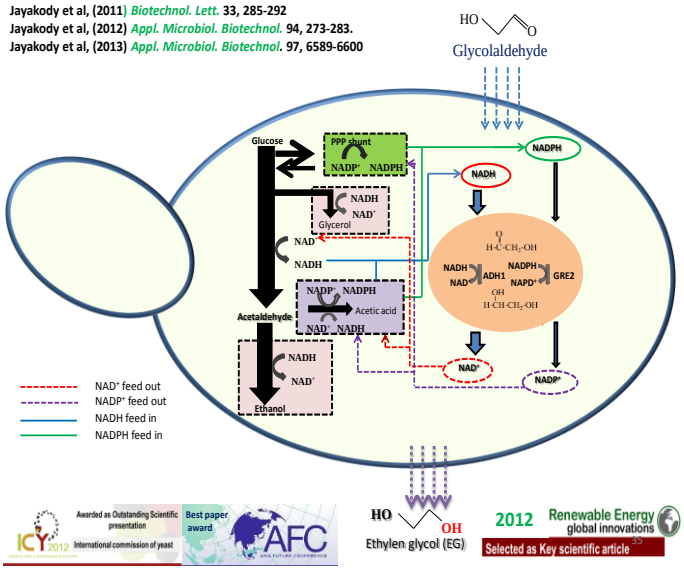

Fig. 1 Schematic elaboration of the coordination of redox cofactors in *S. cerevisiae* with the glycolysis pathway during the conversion of glycolaldehyde into ethylene glycol

Therefore, at the second stage of research, the reduction of plus charge of the carbonyl carbon of glycolaldehyde molecule by NADH-dependent Adh1 implemented as the principle strategy to develop a resistant strain (2). The developed *ADH1*-expressing strain (Japan open patent 2011-18441) exhibits an improved fermentation profile in a glycolaldehyde-containing medium as well as in an actual HCWT-lig. hyd. After in-depth analysis of metabolic fermentation products and redox cofactors in the developed glycolaldehyde-reducing strain, we identified NADH perturbation and concurrent redox imbalance is the major cause for the partial recovery of *ADH1*-expressing strain in high concentration glycolaldehyde-containing medium (5 mM ≥). Therefore, through novel metabolic engineering approach, we further improved the glycolaldehyde tolerance of *ADH1*-expressing strain by augmenting NADPH-dependent Gre2

glycolaldehyde-reducing pathway (3). The strain expressing both *ADH1* and *GRE2* showed significantly higher conversion activity of glycolaldehyde using NADH and NADPH as cofactors than those of the parent strains (Fig. 1). Moreover, the developed strain shows better redox balance and increases ethanol production by 7% in glycolaldehyde-containing medium relative to the control strain in the media without glycolaldehyde at the metabolic cost of reducing glycerol production. Taken together for the first time this research was developed hyper-glycolaldehyde tolerant and efficient ethanol producing yeast strain.

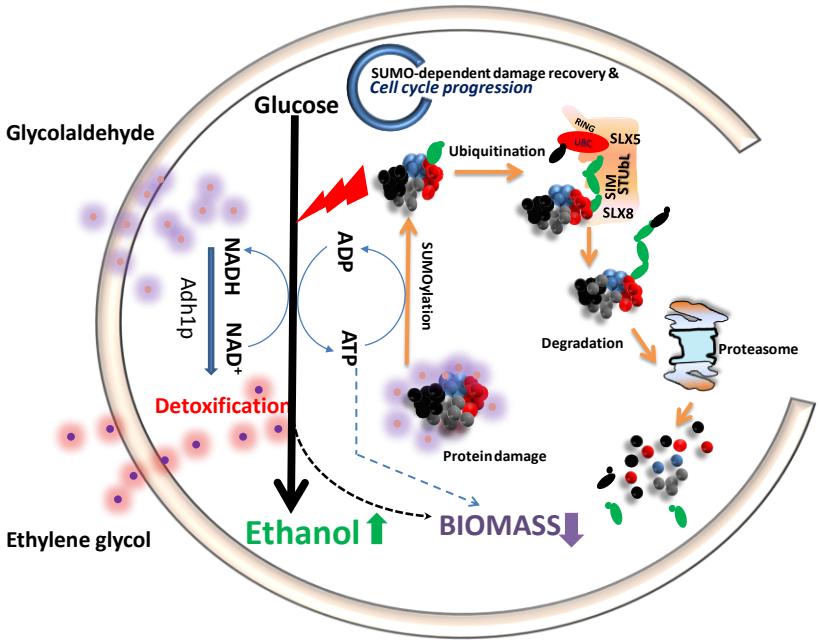

Fig. 2 Novel model of developing tolerant strain for combinational inhibitory effect of fermentation inhibitors

So far researchers in this field try to develop yeast strains for second generation biofuel production by targeting single inhibitors. However, none of these developed strains have shown better fermentation characteristics in actual hydrolysate owing to complex chemical stress of biomass hydrolysate. Indeed, through the factorial experiment, we identified major inhibitors present in HCWT-lig. hyd exert interactive inhibitory effect with glycolaldehyde. Moreover, glycolaldehyde or complex inhibitors mainly cause proteins damage, yeast cell cycle arrest at G2/M and leading cells to have long lag-phase in complex inhibitors medium. Therefore, third phase of our study we invented novel approach to develop tolerant yeast strains to complex inhibitors stresses of actual cellulose hydrolysate through the comprehensive genomic analysis followed by megavariate data modeling. We identified 68 putative genes, which are specifically involved in combinational inhibitory effect of inhibitors. Next, we identified key target molecular mechanisms to develop tolerant yeast strains by PCA analysis of identified genes. Indeed, we discovered SUMOylation as a novel target to develop a tolerant yeast strains. To the best of our knowledge, this work has demonstrated first successful utilization of SUMOylation pathway for conferring inhibitor tolerance to yeast cells (Japanese open patent 2013-246294).

Augmenting SUMO-dependent ubiquitin pathway genes such as *SMT3*, *SLX5*, *SLX8* and *UBC9* to *ADH1*-expressing strain totally rescued the cells from G2/M cell cycle arrest, improved energy status of cells, enhanced the glucose uptake, and significantly increased the growth rate of yeast (shortens the lag-phase of growth) in complex inhibitors medium. In addition, we identified SUMOylation regulates enzymes in the glycolysis pathway and might involve in energy and cofactor generation, and increases the ethanol yield of developed strain (BY4743*pADH1pSMT3-SLX5-SLX8-UBC9*) at the expense of biomass (Fig. 2).

「結論及び考察」**Conclusion**

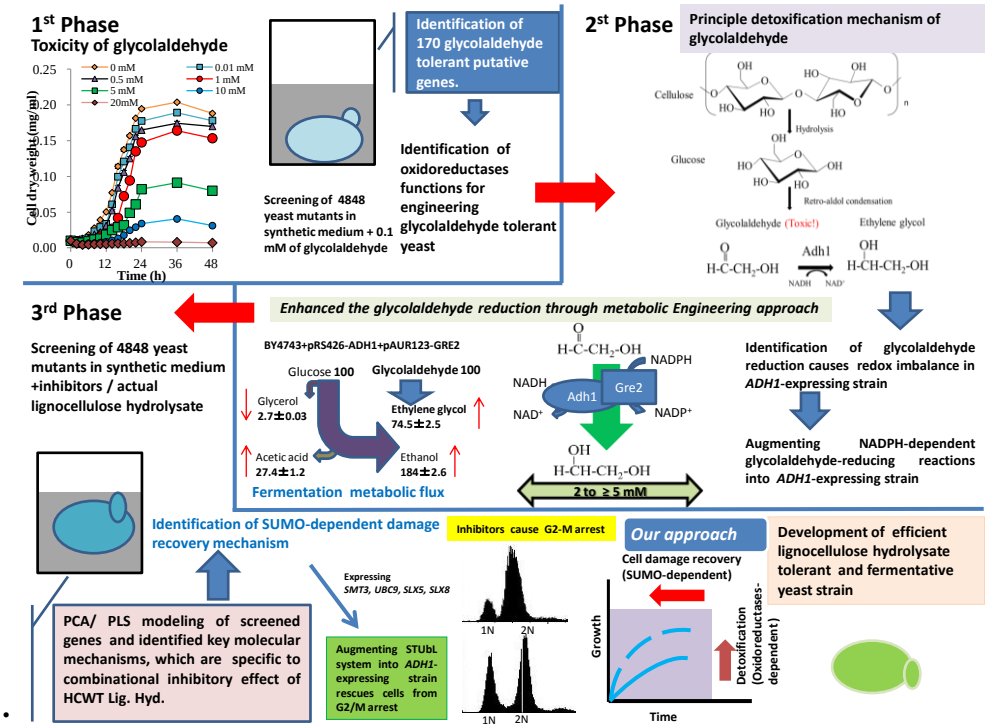

Fig. 3 Illustration of summary of research progress achieved in the dissertation study

As shown in Fig. 3, 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 NAD(P)H-dependent oxidoreductases. In addition, we identified SUMO-dependent ubiquitin pathway as a potent mechanism for shortening the inhibitors-mediated 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.

References

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序論、本論、結論などの各段落ごとに短くまとめる。

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