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Fundamental researches on increasing applicability of *Saccharomyces cerevisiae* as the host cells for production of useful metabolites

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Research aims

As for the glucose metabolism in yeast, it is well known that yeast shows crabtree effect exhibiting high ethanol formation under high glucose concentration due to the repression of the expression of the genes responsible for respiration. By using this phenomenon, the yeast *Saccharomyces cerevisiae* has been mainly used for production of alcoholic beverages and bioethanol. However, it has been sometimes difficult to use *S. cerevisiae* for producing useful metabolites other than ethanol under high glucose concentration due to the decreased metabolic flow to the metabolic reactions leading to such metabolites. Thus, the yeast strains that exhibit lower ethanol production are highly desirable for the production of useful metabolites other than ethanol. In this study, the metabolic pathway(s) whose disruption leads to the decreased ethanol production was identified by the use of two different methods, namely, screening of a single gene deletion strain library for mutants exhibiting low ethanol production and *in silico* simulation using a genome-scale metabolic model.

Methods

(1) Identification of the single gene deletion strains that exhibited low ethanol production

Each strain of a single gene deletion strains set (Yeast *MAT*alpha collection; Open Biosystems)[1] was cultivated in YP10D medium (2% bacto yeast extract, 1% bacto peptone and 10% glucose) using 96-well microtiter plates covered with plastic films at 30ºC for two days with shaking to prevent cell sedimentation. After cultivation, culture supernatant was obtained by centrifugation and was used for the measurement of ethanol concentration. For measuring ethanol concentration in the culture supernatant, E-kit ethanol (Scil Diagnostics GmbH) and microplate reader (Perkin Elmer) were used. The deletion strains that showed significantly low ethanol production were identified.

(2) Identification of metabolic pathway whose disruption exhibited decreased ethanol production by simulation using a genome-scale model

In this study, a modified genome-scale model of *S. cerevisiae* iND750[2] including 1,268 reactions and 646 metabolites was used. In this model, cellular compartment such as cytoplasm, mitochondria, peroxisome, nucleus, golgi body, vacuole and endoplasmic reticulam, and the transport reaction between inside and outside of cells were taken into account. The effect of disruption of each metabolic reaction on flux distribution was simulated by calculating maximum cellular growth (i.e. flux for biomass formation) using linear programming. In the simulation, upper and lower limits of the flux for each metabolic reaction were set to –1,000 and 1,000 (mmol/g dry cell/h), respectively. For simulation, programming software Matlab 2006b (The Mathworks Inc.) and LINDO API (LINDO Systems Inc.) were used.

Results

(1) Identification of the single gene deletion strains that exhibited low ethanol production

To identify the deletion strains of *S. cerevisiae* that exhibited low ethanol production, 4,826 single gene deletion strains were cultured in YP10D medium and amounts of ethanol produced by these strains were measured. Cultivation was performed twice. The mean of ethanol productions in all the deletion strains was 33 g/l, and the deletion strains whose ethanol productions were less than 10 g/l were identified as the strains whose ethanol productions were decreased by single-gene deletion. As a result, 75 deletion strains were determined as the strain whose ethanol production was significantly low. However, among these strains, seven strains exhibited a significant growth defect. Thus, these seven strains were omitted from further analysis. It is notable that the deletion strain of *ADH1*, a gene encoding the major alcohol dehydrogenase in *S. cerevisiae,* was found to be included in the seven strains.

Table 1 shows functional categories of the genes whose deletion showed decreased ethanol production in *S. cerevisiae*. Many functional categories were found, but statistical overrepresentation of some of these functional categories was not detected (hypergeometric test, $p<0.01$; data not shown).

(2) Identification of metabolic pathway whose disruption exhibited decreased ethanol production by simulation using a genome-scale model

Next, the metabolic pathways whose disruption led to the decreased ethanol production by *S. cerevisiae* were determined by simulating the effect of disruption of each meta-

Table 1 Functional categories of the genes whose deletion showed decreased ethanol production by *S. cerevisiae.*

GO term	Gene
Transport	*PHO88, NUP84*, *ATG9*, *PEX7*, *CUP5*, *ECM10*, *FTR1*, *ATP7*, *MRS4*, *PEP3*, *VRP1*, *NPL6*, *YDJ1*, *TOM70*, *SLG1*, *VTC3*
Response to chemical stimulus	*PRX1*, *GRX7*, *BRE1*, *DHH1*, *MNI1*, *HSP104*, *PEP3*, *STE11*, *ASC1*, *YDJ1*
RNA metabolic process	*BRE1*, *DHH1*, *DEG1*, *TAD1*, *CTK2*, *DLS1*, *MRS4*, *DCS1*, *IKI3*, *NPL6*
Membrane organization	*NUP84*, *ATG9*, *CUP5*, *PEP3*, *VRP1*, *TOM70*, *SLG1*, *VTC3*
Response to stress	*PRX1*, *GRX7*, *NUP84*, *ATG9*, *HSP104*, *STE11*, *NPL6*, *SLG1*
Transcription	*BRE1*, *CTK2*, *DLS1*, *IKI3*, *NPL6*
Protein modification process	*BRE1*, *RUB1*, *UBP6*, *CTK2*, *STE11*
Cellular homeostasis	*PRX1*, *FYV5*, *CUP5*, *FTR1*, *VMA13*
Protein folding	*CNE1*, *ECM10*, *HSP104*, *YDJ1*
Generation of precursor metabolites and energy	*CYC7*, *YVH1*, *ATP7*, *GLG1*
Cell cycle	*SAP4*, *YVH1*, *BFA1*, *VRP1*
Protein complex biogenesis	*ATG9*, *PEX7*, *ATP7*, *PKR1*
Vesicle-mediated transport	*CUP5*, *PEP3*, *VRP1*, *SLG1*
Cytoskeleton organization	*VRP1*, *SLG1*, *CTF19*
Vacuole organization	*CUP5*, *PEP3*, *VTC3*
Chromosome organization	*HHT1*, *BRE1*, *NPL6*
Cellular carbohydrate metabolic process	*YVH1*, *GLG1*, *HSP104*
Mitochondrion organization	*ECM10*, *YDJ1*, *TOM70*
Cellular component morphogenesis	*DHH1*, *VRP1*, *SLG1*
Transposition	*HHT1*, *BRE1*, *ASC1*
Signal transduction	*STE11*, *ASC1*, *SLG1*
Heterocycle metabolic process	*GUD1*, *HIS1*, *ATP7*
Conjugation	*DHH1*, *STE11*
Translation	*CTK2*, *ASC1*
Cell wall organization	*UTR2*, *SLG1*
Peroxisome organization	*PEX32*, *PEX7*
Cellular protein catabolic process	*CNE1*, *YDJ1*
Cytokinesis	*VRP1*
Cellular respiration	*CYC7*
Chromosome segregation	*CTF19*
Meiosis	YVH1
Vesicle organization	*ATG9*
Sporulation resulting in formation of a cellular spore	*YVH1*
Cellular amino acid and derivative metabolic process	*HIS1*
Nucleus organization	*NUP84*
Pseudohyphal growth	*STE11*
Ribosome biogenesis	*NUP84*
Cellular lipid metabolic process	*LRO1*
Cell budding	*VRP1*
Cellular aromatic compound metabolic process	*GUD1*
Biological process unknown	*YBL083C*, *YBR013C*, *YDL071C*, *YDL094C*, *YDR010C*, *YDR015C*, *OMS1*, *AIM8*, *YEL028W*, *MTC7*, *YEL068C*, *EMC4*, *YIL024C*, *YKR012C*, *YKR075C*, *YMR082C*, *JLP2*, *YMR279C*, *YNL043C*, *YOR059C*

Gene ontology (GO) slim terms (process) were used as functional categories. Analysis was performed by using *Saccharomyces* genome database (SGD) gene ontology slim mapper (http://www.yeastgenome.org/cgi-bin/GO/goSlimMapper.pl).

bolic reaction on flux distribution using a genome-scale metabolic model of *S. cerevisiae*. Good agreement of the flux distribution previously reported by Paula *et al.*[3] with that predicted by our genome-scale model was confirmed (data not shown). In this simulation, the specific glucose and oxygen uptake rates were set to 15 and 1 mmol/g dry cell/h, respectively. As a result of simulation, disruption of the reaction catalyzed by triosephosphate isomerase in glycolysis as well as those responsible for ethanol formation was found to decrease ethanol production. However, increased glycerol production was also predicted to reoxidize NADH to NAD^+. In addition, other metabolic pathways whose disruption led to decreased ethanol production could not be found. These results of our simulation analysis suggest that the ethanol production by *S. cerevisiae* might be robust against perturbations to maintain redox balance.

Conclusion

In this study, the metabolic pathway(s) whose disruption leads to the decreased ethanol production was identified by screening of a single gene deletion strain library for mutants exhibiting low ethanol production and by *in silico* simulation using a genome-scale metabolic model. The results suggest that some gene disruptions might be effective in decreasing ethanol production by *S. cerevisiae*. Investigation of effectiveness of such gene disruptions on the production of useful metabolites other than ethanol is necessary. However, in both analyses, i.e. cultivation of gene deletion strains and *in silico* simulation, the number of candidates of gene disruption or metabolic pathway disruption that exhibit decreased ethanol production is low. These suggest that the ethanol production by *S. cerevisiae* might be robust against perturbations. For effective production of

useful metabolites other than ethanol using yeast cells, genetic modification and optimization of culture conditions that give an extraordinary impact on this robustness (i.e. ethanol production) would be necessary.

References

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