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Construction of an Ethanol-Tolerant Yeast Model using the E-cell System

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Abstract

In brewing Japanese sake, since ethanol reaches to a high concentration by using a peculiar manufacturing method, yeast with strong alcohol tolerance is needed. Such yeast can be obtained through huge number of trial and error processes with huge cost and long time. To avoid this problem, we try to model the response mechanism of alcohol-tolerant yeast by computer simulation based on known parameters from existing literatures. We hope new insight will be obtained by our model. In this paper, we model a simple metabolism of 1,3-β glucan synthesis, which is the major components of cell well, and a signaling pathway of the Pkc1-MAPK cascade using the E-Cell system.

E-Cell System を用いたアルコール耐性酵母に関するモデル構築

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概要

清酒醸造では特有の醸造手法を用いるため酵母菌の生成するエタノールは高濃度に達する．そのため醸造工程においてアルコールに強い耐性を持つものが求められる．このような酵母菌は時間，コストのかかる試行錯誤的な実験によって取得される．そこで既知の文献などを元にアルコール耐性酵母菌に関して計算機上でシミュレーションモデルの構築を試み，そのモデルを用いて新たな知見獲得を試みる．本稿では，汎用細胞シミュレーションシステムである *E-Cell System* を用いて酵素菌の細胞壁の主成分である *1,3-β* グルカンの簡潔な合成代謝経路，シグナル伝達として *Pkc1-MAPK* カスケードに関してモデルの構築を試みる．

1 Introduction

In brewing Japanese sake, the flavor is dominated by both alcohol fermentation of yeast and the by-product flavor compound that is generated at the fermentation process. Therefore, the flavor of sake totally depends on the yeast. "Flower yeast" which is extracted from flowers is known as attractive flavor yeast. We have double-flowered cherry tree of Nara, which is one of natural monument, in our campus.

Since alcohol concentration reaches about 20% through a parallel-double fermentation method, in brewing sake, alcohol-intolerant yeast has no way to survive. Therefore, good yeast must have tolerance to high concentration of alcohol for the good brewing of sake. It is not an easy task to obtain alcohol-tolerant yeast by mutation breeding because alcohol tolerance is deeply influenced with many genes. Therefore, valid experiments with various alcohol concentrations are needed to obtain such yeast. It takes excessive amount of time and unrealistically high cost.

To avoid this problem, the response mechanism of alcohol-tolerant yeast should be studied by constructing a model in silico. In this paper, we propose a preliminary model for the analysis of alcohol-tolerant yeast. Using such computer simulation, it is expected to obtain alcohol-tolerant yeast with ease.

We propose several sub-models for our study in section 2. In section 3, we describe experimental results and discuss the simulation results.

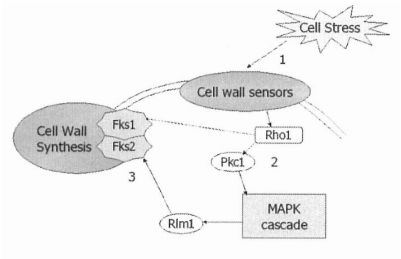

Figure 1: Model image

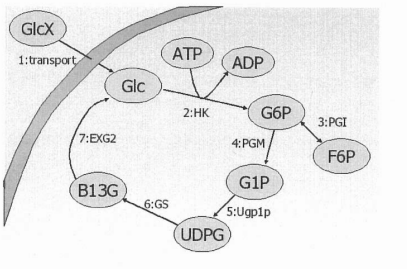

Figure 2: metabolic pathway of 1,3-β glucan synthesis

2 Proposed model

In our study, we propose a simulation model for the response mechanism of alcohol-tolerant yeast as figure 1. In 1 of figure 1, cell wall sensor (Mid2 or Wsc1) senses cell wall stress caused by ethanol. At 2, once signal of senescing at 1 conveys through Rho1 and the Pkc1-MAPK pathway, then Rho1 influences to Fks1. Fks1 is one of the genes to encode synthase of 1,3-β glucan that is the main component of cell wall. At 3, the transferred signal causes synthetic advance of cell wall such as activation of Fks2, which is the other genes to encode 1,3-β glucan synthase, to positive [1].

Alcohol-tolerant yeast has a number of genes, which receive stress signal by ethanol. These genes express in their own alcohol concentration. In this study, we focus on Mid and Wsc families as a cell wall sensor with well-study and many literatures.

2.1 Cell wall synthesis

As already explained, there is a relationship between 1,3-β glucan synthesis and stress-response signaling. In this paper, we construct the model about this synthesis. Figure 2 shows a system for a metabolic pathway of 1.3-β glucan synthesis. This system is constructed from minimum elements and reaction, which are based on a pathway database KEGG [2]. Each element is given in table 1.

Actual reaction appears at various places such as cell membrane and cell cytoplasm of yeast. In this paper, we assume all reactions occur in cell cytoplasm for simplification.

The reaction rate equations respectively deceide by literature searching. Reaction of Transport, HK, PGI, PGM, and UGP1 are refered to [5], [6][7], [3], [3], and [4], respectively. Reaction of GS and EXG2 is based on Michaelis-Menten equations.

2.2 Signaling

In our study, we focus the MAPK cascade of the Pkc1-MAPK pathway. For this pathway, we also refer to KEGG. Figure 3 shows a pathway of the generated MAPK cascade. Phosphorylated element is expressed as the element name followed by P. Let an input and an output be Stimu and Rlm_P, respectively.

Table 1: Abbreviations of elements

Abbreviation	Appellation
GlcX	Extracellular Glucose
Glc	α -D-Glucose
G6P	α -D-Glucose-phosphat
F6P	β -D-Fructose6-phosphat
G1P	α -D-Glucose-phosphat
UTP	UTP
UDPG	UDP-Glucose
B13G	1,3- β -Glucan
PP	Disphosphat
HK	Hexokinase
PGI	Glucose-6-phosphate isomerase
PGM	phosphoglucomutase
UGP1	UDP-Glucose-pyrophorylase
GS	1,3- β -glucan synthase
EXG2	glucan 1,3- β glucosidase

When the MAPK cascade is an autocatalyst, this reaction is described as follows:

$$
\begin{aligned}
(react1)\quad \alpha &: S + E \overset{a_1/d_1}{\longleftrightarrow} S \times E \\
\beta &: S \times E \xrightarrow{k_1} S\_P + E \\
(react2)\quad \alpha &: S\_P + E\_ase \overset{a_2/d_2}{\longleftrightarrow} S\_P \times E\_ase \\
\beta &: S\_P \times E\_ase \xrightarrow{k_2} S + E\_ase
\end{aligned}
$$

The amount of change per unit time of each material is expressed in differential equations as follows:

$$
\begin{aligned}
\alpha \quad & \frac{dS \times E}{d} = a[S][E] - d[S \times E] \\
\beta \quad & \frac{dS\_P}{d} = k[S \times E]
\end{aligned}
$$

Reaction of the MAPK cascade is progressive in each stage. Therefore this simple rate equation is adaptable to all reactions in the pathways of figure 3.

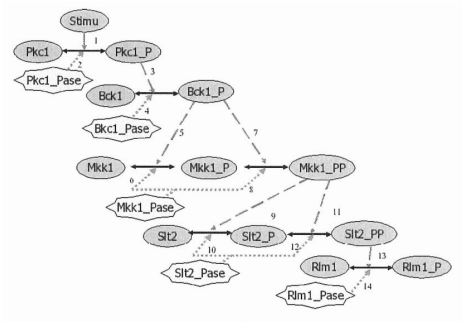

Figure 3: pathway of MAPK Cascasde

3 Result and discussion

To validate the proposed model, we perform simulation experiments. In this simulation, we use the E-Cell System (version 3.1.106) for constructing the model.

3.1 1,3-β glucan synthesis

In this subsection, we describe a validation experiment of the proposal model for the 1,3-β glucan synthesis. Each material, which is defined as substance or product, has various values against each reaction, expect ATP concentration. Since ATP concentration is constant for the yeast in a steady state, ATP concentration is considered as a fixed value in this experiment. Table 2 shows parameters for this experiment. Each parameter is obtained by existing literatures and BRENDA [9].

Figure 4 shows change in number of molecules for a period of about 600,000 sec. Each material simply increases and decreases. Since the system for 1,3-β glucan synthesis in figure 2 is a closed metabolic pathway, simple increase and decrease by synthesis and degradation are observed. Hence, our model replicates the phenomenon.

3.2 Pkc1-MAPK cascade

In this subsection, we describe an experiment to validate our model for signaling pathways. In this experiment, the kinetic constants are set to $ax = 1000$, $dx = 150$, and $kx = 150$ [13]. Since the MAPK cascade model is constructed from simple enzyme reaction for just phosphorylation and dephosphorylation, there is no need to change kinetic constants. They should be static on each reaction step.

Figure 5 shows the relationship between time and concentration of Rlm1_P at the three-staged stimulus (Stimu) concentration. Rlm1_P plots a reaction curve with a time rug. Hence, the proposed model might be according with scientific reason. When amount of stimulus increases, response start time is earlier. In signaling reaction that delivers extracellular stress to cell interior, this phenomenon is reasonable response.

Table 2: Parameters of the model

reaction	Parameter		model
Trasport	V_{max}	17.203	[5][6][10]
	K_{Glc}	1.1	
	K_{IG6P}	1.76	
	K_{IIG6P}	7.5	
	P	1.0	
HK	V_{max}	51.750	[6][11]
	K_{ATP}	0.10	
	K_{Glc}	0.11	
	K_{dGlc}	0.37	
PGI	V_{max}	496.04	[6][9]
	K_{mG6P}	0.8	
	K_{mF6P}	0.15	
	K_{eq}	0.2	
PGM	V_{max1}	11.274	[12]
	V_{max6}	3.5935	
	K_{mG1P}	0.0154	
	K_{mG6P}	0.057	
	K_{eq}	17.8	
Ugp1p	V_{max}	1.002	[14]
	K_{mUTP}	0.24	
	K_{mG1P}	0.19	
	K_{mUDP}	0.40	
	K_{mPP}	0.26	
	K_{eq}	0.15	
GS	K_{cat}	0.01048	[15]
	K_m	0.00037	
EXG2	K_{cat}	12.936	[9]
	K_m	0.000407	

4 Conclusions

We proposed a simulation model for the response mechanism of alcohol-tolerant yeast. In this paper, we constructed the model for the metabolic pathway of 1,3-β glucan synthesis and for the MAPK cascade of the Pkc1-MAPK pathway. We perform several experiments to validate these models.

In future work, parameters in the adjust cell wall synthesis model should be improved using measured data. The proposed model should be expanded for idiosyncratic expression mechanism. A model for a G protein molecular switch at upstream of signaling is needed.

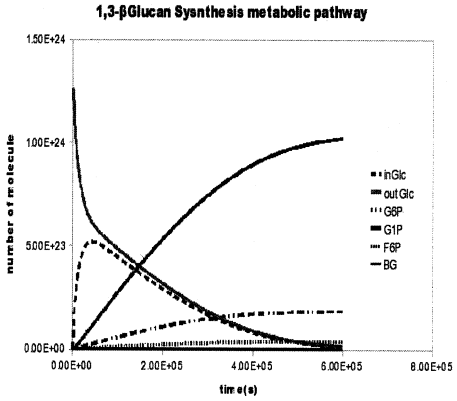

Figure 4: Change in number of moleculars

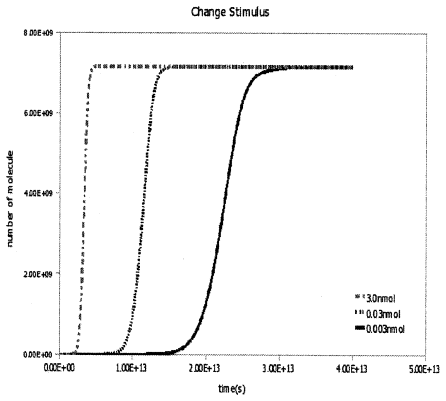

Figure 5: Change in number of molecular of Rlm1_P

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