METABOLIC NETWORK ANALYSIS OF XYLOSE METABOLISM BY PICHIA STIPITIS

Meng Liang¹, Min Hea Kim¹, Qinghua He², Jin Wang⁰

Abstract
The conversion of pentoses to ethanol is one of the major barriers of industrializing lignocellulosic ethanol processes. As the most promising native strain for pentoses fermentation, Pichia stipitis has been widely studied for its xylose fermentation. In spite of the abundant experimental evidence regarding ethanol and by-products production under various aeration conditions, the mathematical descriptions of the processes are relatively rare. In this work, the constraint-based metabolic network model for central carbon metabolism of P. stipitis was reconstructed by integrating genomic (P. stipitis v2.0, KEGG), biochemical (ChEBI, KEGG) and physiological information available for this microorganism and other related yeast. The stoichiometry of the metabolic reactions was used in combination with biosynthetic requirements for growth and pseudo-steady state mass balances over intracellular metabolites for the quantification of metabolic fluxes using metabolite balancing. This model was employed to perform an in silico characterization of the phenotypic behavior of P. stipitis grown on xylose and the model predictions are in general agreement with published experimental results. The effects of single reaction deletions on growth were assessed. In the process, essential biochemical reactions for growth were identified. In addition, flux balance analysis has been applied to the model to elucidate the redox balance of P. stipitis for xylose fermentation. The results revealed key metabolic constraints related to redox homeostasis. A comparison of flux distribution involved in redox balance with different oxygenation conditions provides important insights on the role of redox balance in the metabolism of xylose utilization, ethanol and xylitol production.

Keywords: xylose metabolism; Pichia (Scheffersomyces) stipitis; Flux Balance Analysis (FBA); metabolic network model; redox balance; Principal Component Analysis (PCA); ethanol; xylitol; systems biology

Introduction
High efficient utilization of xylose is one of the biggest obstacles for commercial production of lignocellulosic ethanol. Pichia stipitis (P. stipitis, now renamed as Scheffersomyces stipitis) has a set of physiological traits that make it potentially valuable candidate for the lignocellulosic ethanol production. Oxygen availability plays a critical role in xylose metabolism of P. stipitis due to redox balance (Jeffries & Van Vleet 2009). In spite of the abundant experimental evidence, little is known about the mechanism and metabolic flux distributions of how redox balance affects xylose metabolism. In this work we developed central carbon metabolic model for P. stipitis and analyzed xylose metabolism using Flux Balance Analysis (FBA) and Principal Component Analysis (PCA). The combination of FBA and PCA revealed details of metabolism shifts.

Methods
Construction of metabolic model
The metabolic model of P. stipitis was constructed following the published protocol (Thiele & Palsson 2010) and was built based on the genomic and biochemical information of the organism available in its

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genome project (Jeffries & Van Vleet 2009), KEGG, ChEBI and PubChem. An overview of the metabolic model is shown in Box 1. Cell mass reaction in the model was assembled from the macromolecular components (Senger 2010). The contribution of each component to cell mass and the appropriate coefficients for every building block were estimated from literature data.

**Flux Balance Analysis (FBA)**
Flux balance analysis was performed using a publicly available COBRA toolbox for Matlab version 2.04 (Schellenberger et al. 2011). The output of the toolbox includes the values of metabolic fluxes. The simulated results were used for further analysis to reveal the intracellular mechanism of xylose metabolism.

**Principal Component Analysis (PCA)**
Principal component analysis (PCA) is a simple, non-parametric method for extracting relevant information from confusing data sets (Jolliffe 2002). In this work, PCA was carried out with PLS toolbox for Matlab version 3.0 (Eigenvector Research, Inc.). The simulated metabolic fluxes were used as the input for PCA to identify different phenotypes caused by various oxygen supplies. The scores generated by PCA were used to distinguish the distributions of different reactions to the metabolic status changes.

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**Box 1. Overview of the metabolic network model**

The model represented cell growth on glucose and xylose. 117 reactions (66 reversible and 51 irreversible) were included. 15 compounds were allowed to exchange with external environment. Reactions were constituted from glycolysis, pentose phosphate pathway, tricarboxylic acid (TCA) cycle, glyoxylate and dicarboxylate metabolism, oxidative phosphorylation, nitrogen metabolism, nicotinate and nicotinamide metabolism, cell mass formation, and synthesis pathways of common byproducts of *P. stipitis*: ethanol, glycerol, xylitol and acetate. Some linear reactions in the model were lumped together for simplification. Transport reactions, including passive diffusion, facilitated diffusion and active transport, were also incorporated. Non-growth associated maintenance energy was set to 3.5 mmol/gDCW/h. The metabolic map (left) represents a brief overview of the metabolic network.
Analysis of *P. stipitis* metabolic network

After the construction of the model, FBA was applied to study the intracellular metabolic flux distributions under various conditions. In this work, the model was constrained to grow on minimal defined medium (Jeffries et al. 2007). The reaction essentiality to cell growth was evaluated as whether its removal was fatal. The results (excluding transport reactions and cell mass reaction) showed differences with different cultivation conditions: 14 and 18 reactions for aerobic and oxygen-limited glucose culture respectively while 16 and 26 for aerobic and oxygen-limited xylose cultivation. 10 reactions in glycolysis, pentose phosphate pathway (PPP) and urea metabolism are essential under all conditions. The bigger differences in xylose metabolism under different aeration conditions confirmed that xylose metabolism is more sensitive to oxygen. The predictions of model with glucose or xylose under aerobic or oxygen-limited condition were shown in Error! Reference source not found.. Under aerobic condition the carbon has been used for cell growth and energy generation. Under oxygen-limited condition the cell growth was inhibited and ethanol was produced with by-products (acetic acid, glycerol or xylitol). These results were in good agreement with experimental observation and indicated the constructed model could capture metabolic changes caused by different carbon source and oxygen supply. The carbon flux distribution through PPP was also studied. The result under aerobic glucose culture was 61.66%, which was consistent with the reported value of 57±9% (Fiaux et al. 2003).

Analysis of redox balance in xylose metabolism

Oxygen plays an important role in cell growth, redox balance, functioning of the mitochondria and generation of energy for xylose transport in *P. stipitis*. But how oxygen influences the intracellular flux distribution and redox balance and which reactions would be most important for redox balance have not been studied systematically. In this work, the oxygen influences, especially on the redox balance, was studied by combining FBA and PCA. The intracellular flux data for PCA were generated by FBA through changing oxygen supply within [0, 20] mmol/gDCW/h with a step of 0.001. The ratio of fluxes through NADPH- and NADH-dependent XR and the ratio through NADP+- and NAD+-dependent XDH were set to 1.5 and 0.02 respectively. By PCA, six phenotypes have been identified with the constraint on xylose uptake rate of maximal 10 mmol/gDCW/h. The results were shown in Fig. 1. The main characteristics of the six phenotypes identified were summarized in Table 1.
Fig. 1. Phenotypes identified with PCA when oxygen supply changes within [0, 20] mmol/gDCW/h.

### Table 1. Characteristics summary of phenotypes.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Growth limitation</th>
<th>Metabolic product(s)</th>
<th>Main metabolic characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Xylose</td>
<td>Cell mass</td>
<td>Aerobic growth</td>
</tr>
<tr>
<td>2</td>
<td>Xylose, Oxygen</td>
<td>Cell mass, acetic acid</td>
<td>Oxygen-limited and acetic acid production</td>
</tr>
<tr>
<td>3</td>
<td>Xylose, Oxygen</td>
<td>Cell mass, ethanol, acetic acid</td>
<td>Ethanol production and declined acetic acid production</td>
</tr>
<tr>
<td>4</td>
<td>Xylose, Oxygen</td>
<td>Cell mass, ethanol, xylitol</td>
<td>Declined ethanol production and increasing xylitol production</td>
</tr>
<tr>
<td>5</td>
<td>Oxygen</td>
<td>Cell mass, ethanol, xylitol</td>
<td>Declined ethanol and xylitol production.</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>Cannot maintain metabolism</td>
</tr>
</tbody>
</table>

With the scores for fluxes by PCA, the critical reactions for redox balance and the fluxes most influenced by oxygen supply change for each phenotype were also identified. The importance of identified reactions to redox balance in each phenotype was confirmed by comparison of the NAD(P)H consumption and generation fluxes. The involvement of key reactions to ethanol, xylitol, glycerol and acetic acid in the identified reactions indicated their roles to keep intracellular redox balance.

**Conclusion**

In this work the central carbon metabolic network of *P. stipitis* has been reconstructed. The validation of the model with experimental observation and flux distribution through PPP showed that the constructed model could capture most of the behaviors of central carbon metabolism. The essential reactions under different conditions were identified. Xylose metabolism by *P. stipitis*, especially redox balance, was studied by combining FBA and PCA. Six phenotypes have been identified and the reactions that satisfied the redox balance also been established. The results showed PCA could be a powerful tool for analyzing output of metabolic model.

**References**


