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Reconstruction of a Genome-Scale Metabolic Model to Simulate Uncoupled Growth of *Zymomonas mobilis*

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Abstract*—Zymomonas mobilis* is known as an example of the uncoupled growth phenomenon. This microorganism also has a unique metabolism that degrades glucose by the Entner–Doudoroff (ED) pathway. In this paper, a genome-scale metabolic model including 434 genes, 757 reactions and 691 metabolites was reconstructed to simulate uncoupled growth and study its effect on flux distribution in the central metabolism. The model properly predicted that ATPase was activated in experimental growth yields of *Z. mobilis*. Flux distribution obtained from model indicates that the major carbon flux passed through ED pathway that resulted in the production of ethanol. Small amounts of carbon source were entered into pentose phosphate pathway and TCA cycle to produce biomass precursors. Predicted flux distribution was in good agreement with experimental data. The model results also indicated that *Z. mobilis* metabolism is able to produce biomass with maximum growth yield of 123.7 g (mol glucose)$^{-1}$ if ATP synthase is coupled with growth and produces 82 mmol ATP gDCW^{-1}h^{-1}. Coupling the growth and energy reduced ethanol secretion and changed the flux distribution to produce biomass precursors.

***Keywords*—**Genome-scale metabolic model, *Zymomonas mobilis*, uncoupled growth, flux distribution, ATP dissipation.

I. Introduction

ZYMOMONAS mobilis is a Gram-negative bacterium that can efficiently produce ethanol from glucose, fructose, and sucrose [1]. *Zymomonas mobilis* converts 95–98% of the substrate carbon into ethanol and carbon dioxide while only 3–5% of substrate carbon is converted into biomass. It results in a relatively low growth yield between 2.3 and 9 gDCW (mol glucose)$^{-1}$[2]. In addition to little cell mass production, *Z. mobilis* grows with a high glucose uptake rate (reaching 4-5.6 g gDCW^{-1}h^{-1}) and a low energetic efficiency and represents a typical example of uncoupled growth [3].

This microorganism has a unique metabolism that certainly plays an important role for its unusual physiology. It has high activity of the glycolytic enzymes and uses the Entner–Doudoroff (ED) pathway to metabolize glucose which results in only one mole of ATP being produced per mole of glucose [2]. Embden–Meyerhof–Parnas (EMP) pathway is not operating in this bacterium and the gene for phosphofructokinase is lacking in the genome. The TCA cycle is truncated, and consists of two branches and produces 2-oxoglutarate and fumarate as the end products. The genes for the 2-oxoglutarate dehydrogenase complex and malate dehydrogenase are not in the genome, and accordingly, ^{13}C-labeling patterns of 2-oxoglutarate and oxaloacetate do not confirm the cyclic function of this pathway in *Z. mobilis* [4]. Despite the absence of these enzymes, *Z. mobilis* still produce important building blocks including oxaloacetate, malic acid, and fumaric acid using alternative metabolic pathway [1]. Also, the pentose phosphate pathway is not complete, and transaldolase is inactive [4].

The mechanistic reason of uncoupled growth in *Z. mobilis* in not clear. The respiratory system does not appear to participate in ATP synthesis, and excess ATP for growth is produced in glycolysis. In fact, high catabolic rate and low growth yield of *Z. mobilis* lead to generate excess pyruvate, NADH and ATP with a considerably high specific rate. Pyruvate is converted into ethanol via the pyruvate decarboxylase (pdc) and alcohol dehydrognease (adh) enzymes [5]. ATP is wasted in *Z. mobilis* by ATP-hydrolyzing reaction. The membrane F_0F_1-type H^+-ATPase has been considered the most likely candidate for recycling ATP in *Z. mobilis* [2].

A high-quality genome-scale metabolic network reconstruction as a common denominator for systems biology studies could be applicable for understanding the characteristics of *Z. mobilis* with an interesting metabolism. Metabolic networks include information about stoichiometry of metabolic reactions, chemical formulas and charges of metabolites, and the associations between genes, proteins, and reactions [6]. Metabolic networks have become a useful tool for studying the metabolism of cells [7], [8]. Two genome-scale stoichiometric reconstructions of *Z. mobilis* ZM4 have been reported [1]-[5]. These stoichiometric models are based on the available genome annotation [9], [10] and present an overall view of *Z. mobilis* metabolism. However, they have not included some of the previously published biochemical data on this bacterium that are essential information for proper reconstruction and stoichiometric simulations, but which cannot be extracted from sequence data alone. Especially, their model was not redox balance, and aerobic catabolism was not considered [11].

In this research, a genome-scale metabolic model based on genome annotations and previously published information have been proposed for *Z. mobilis* ZM1(ATCC 10988). The model was validated with experimental data of growth and flux distribution presented by ^{13}C-metabolic flux analysis. It was used to simulate the un-coupled growth of *Z. mobilis*.

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II. Material and Methods

All intracellular reversible reaction fluxes were limited between -1000 and 1000 mmol $gDCW^{-1}h^{-1}$, while intracellular irreversible reaction fluxes had zero lower limits. For simulation of growth on minimal media, the upper bound of glucose uptake rate was set to 10 mmol $gDCW^{-1}h^{-1}$. In all in silico experiments, biomass formation was used as an objective function to be maximized. The equation for ATP synthesis or hydrolysis is:

adp[c] + pi[c] + 4 h[e]<=>atp[c] + 3 h[c] + h2o[c]

Positive and negative fluxes for this reaction represent ATP synthase and ATPase activity, respectively.

Calculations were made in MATLAB software using the SBML [12] and COBRA [13] toolboxes. The GLPK (GNU Linear Programming Kit) package [13] was used for solving LP problems.

III. Results and Discussion

A. Metabolic Network Reconstruction

The reconstruction procedure followed the approach outlined in Thiele *et al*. [7]. To reconstruct *Z. mobilis* ZM1 metabolic model, the KEGG [14], BIOCYC [15] and BRENDA [16] databases were used to create a draft reconstruction based on information of *Z. mobilis*'s genome sequence and its annotation. The transport DB [17] was applied to collect information about transport systems. Biochemical data collected by previous metabolic models were used to improve the model. An equation for biomass formation was developed to account for the drain of precursors and building blocks into biomass. Biomass synthesis was considered a combination of six macromolecular components (protein, DNA, RNA, small molecules, hapnoid, and cell wall components). The draft model was completed by adding biochemical data in the literature including information about central metabolism, fatty acids, phospholipids, hapnoids and respiratory chain.

Biosynthesis of fatty acids and phospholipids presented as a series of enzymatic reactions. This organism was found to include cardiolipin, phosphatidyl ethanol amine, phosphatidylglycerol, and phosphatidylcholine as main phospholipids. Vaccenic acid (C18:1) was the most plentiful fatty acid with fewer amounts of myristic (C14:0), palmitic (C16:0) and palmitoleic (C16:1) acids [18].

The lipid components of *Z. mobilis* have a unique feature compared to other organisms that permit for tolerance to higher alcohol concentrations. Because of the interaction between short-chain alcohols such as methanol and ethanol and the lipid bilayer, membrane fluidity enhances. The cell attempts to maintain its membrane fluidity by varying the components of the lipid layer and hence, increase the amount of long-chain fatty acids to create a strong cell wall. Hence, it syntheses hopanoid, a pentacyclic lipid compound,that can adjust the cell membrane permeability [1].Hopanoids play a significant role in the ethanol tolerance of *Z. mobilis* [19].

Five Hopanoids tetra hydroxyl bacterio hopane tetrol (THBH), tetra hydroxyl bacterio hopane-glucosamine (THBH-GA), tetra hydroxyl bacterio hopane-ether (THBH-ET), diplopterol, and dopene are presented in cell wall. However, genes involved in the biosynthesis of these hopanoids have not been identified [1]. Therefore, tetra hydroxyl bacterio hopane tetrol, tetra hydroxyl bacterio hopane-glucosamine and tetra hydroxyl bacterio hopane-ether biosynthesis pathways were constructed and incorporated into the metabolic model based on the results previously reported [20], [21].

Although the respiratory chain of *Z. mobilis*is not completely determined, this bacterium has a relatively simple respiratory chain, consisting of type-II NADH dehydrogenase, ubiquinone-10 (Q10), and a cytochrome bd-type ubiquinol oxidase. The NADH dehydrogenase showed relatively high enzyme activity in *Z. mobilis*. Furthermore, cytochrome bd-type ubiquinol oxidase is also the only functional terminal oxidase. Glucose dehydrogenase and D-lactate dehydrogenase also transfer electrons to Q10, but to with the lower rates compared to NADH dehydrogenase. Genes of the cytochrome bc1 complex (electron transport complex III) and cytochrome c are identified, while the genes of cytochrome c oxidase (electron transport complex IV) are not determined. Recently, cytochrome c peroxidase was considered a balancing respiratory chain enzyme instead of cytochrome c oxidase in *Z. mobilis* [22].

Z. mobilis has a type II NADH oxidoreductase that does not pump protons during electron transport in aerobic respiration, dissimilarto the more common type I NADH oxidoreductase. Therefore, type II NADH oxidoreductase does not carry out the proton gradient of the cellular membrane to generate ATP. While this type II NADH oxidoreductase does not pump protons, other membrane proteins, such as cytochrome bc1 complex, cytochrome bd-type ubiquinol oxidase are responsible for the proton gradient [1]. Furthermore, *Z. mobilis* is one of the few known bacteria that both NADH and NADPH can be used as electron donors for the respiratory type II NADH dehydrogenase [11].

The resulting metabolic model contains 757 intracellular reactions, 691 metabolites, and 434 genes.

B. Growth prediction and Model Validation

The model predicts that growth rate depends on the membrane F_0F_1-type H^+-ATPase/synthase activity. In fact, simulation of *Z. mobilis* growth is slightly different from other microorganisms such as *E. coli* and requires ATPase/synthase rate to be fixed. According to Fig. 1, ATP dissipation resulted in growth yield lower than 8.9 g mol^{-1} that is in accordance with experimentally measured maximum growth yield in the minimal glucose medium [23].

Furthermore, intracellular flux distribution obtained from the model was in good agreement with fluxes for glucose fermentation determined by ^{13}C NMR and fermentation data (Fig. 2). Thus, the model could properly simulate the uncoupled growth of *Zymomonas mobilis*. Relative reaction flux in Fig. 2 indicates reactions fluxes as a percentage of the glucose uptake rate.

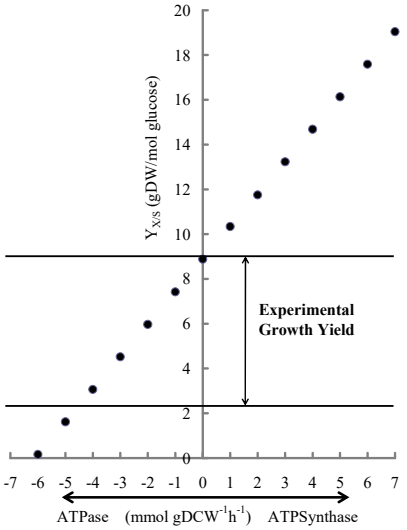

Fig. 1 Relationship between ATPase/synthase activity and growth yield predicted by the metabolic model. The predicts that ATPase is active

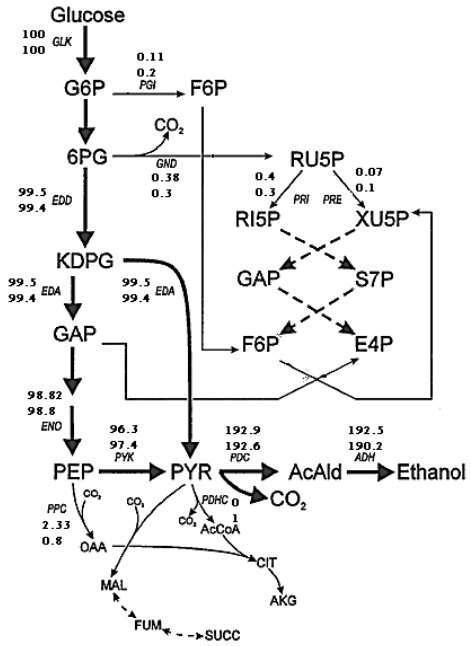

Fig. 2 Relative reaction fluxes predicted by metabolic model at ATPase rate of -3 mmol gDCW^{-1}h^{-1} (top) and 13C NMR-ferementation data (down) [24]Abbreviations: PGI (glucose-6-phosphate isomerase), GND (6-phosphogluconate dehydrogenase), EDD (6-phosphogluconate dehydratase), EDA (2-keto-3-deoxy-gluconate aldolase), ENO (enolase), PYK (pyruvate kinase), PPC (phosphoenol pyruvate carboxylase), PDC (pyruvate decarboxylase), ADH (alcohol dehydrogenases), PRE (ribulose-5-phosphate epimerase), PRI (phosphoribose isomerase), PDHC (pyruvate dehydrogenase complex)

Flux distribution indicates that the major carbon flux passed through ED pathway that resulted in the production pyruvate and ATP. Most of the pyruvate was converted into acetaldehyde and then, alcohol dehydrogenase (ADH) catalyzed acetaldehyde into ethanol. Small amounts of carbon source were entered into pentose phosphate pathway and TCA cycle.

C. Change in Flux Distribution

Fig. 1 demonstrates that increased ATP synthase activity results in more growth rate. The model predicts that ATP synthase rate can increase to 82 mmol gDCW^{-1}h^{-1}. This results in maximum growth yield of 123.7 g mol^{-1} glucose for *Z. mobilis*. This maximum yield is close to that was observed for *E. coli* and indicates the ability of *Z. mobilis* metabolism to produce biomass. Thus, the uncoupled growth of *Z. mobilis* and ATP dissipation is not due to its different metabolic characteristics. Fig. 3 presents change in flux distribution because of the increase in ATPase/synthase activity.

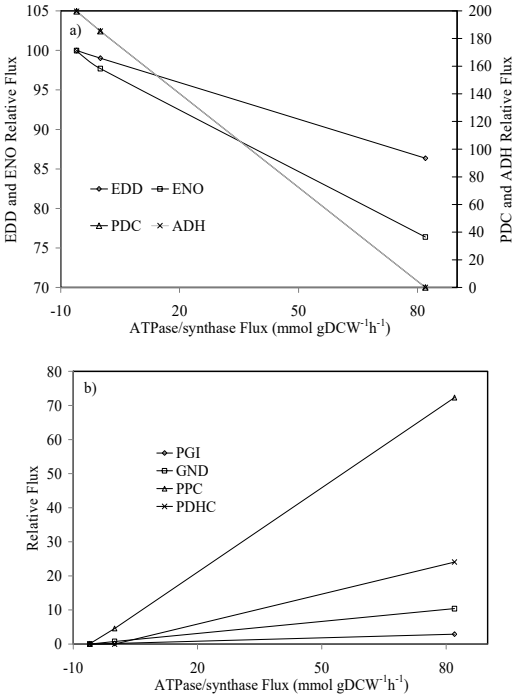

Fig. 3 Effect of ATPase/synthase flux on relative fluxes of a) EDD, ENO, PDC, and ADH, b) PGI, GND, and PPC reactions

It can be seen that relative fluxes of EDD and ENO reactions in ED pathway were decreased to 86.3 and 76.4, respectively, at a maximum rate of ATP synthase. Decrease in fluxes of ED pathway is associated with an increase in fluxes of reactions in TCA cycle and pentose phosphate pathway according to Fig. 3 (b). The increase of fluxes in TCA cycle is more than in pentose phosphate pathway. In fact, the model predicts that coupling growth and energy produced during electron transport chain changes the flux distribution to produce precursors of biomass further. Reduced rates of pyruvate decarboxylase and alcohol dehydrogenases to zero indicate a decrease in ethanol production in the coupled growth condition.

IV. Conclusion

A genome-scale metabolic model of *Zymomonas mobilis* ZM1 based on genome annotation, previously published model and accumulated biochemical data, is presented. The metabolic model was used to simulate uncoupled growth. The simulation demonstrated that ATPase in *Z. mobilis* is active and responsible for the uncoupled growth. The predicted flux distribution was in good agreement with the experimental data. Flux distribution indicated that the major carbon flux passed through ED pathway, and less carbon flux is diverted to pentose phosphate pathway and TCA cycle, thus leading to high ethanol production. The model predicted that increased ATP synthase activity results in a decrease in fluxes of ED pathway and increase in fluxes of pentose phosphate pathway and TCA cycle. In fact, the model predicts that coupling growth changes the flux distribution to produce precursors of biomass further.

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