World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:15, No:5, 2021

Modified Genome-Scale Metabolic Model of *Escherichia coli* by Adding Hyaluronic Acid Biosynthesis-Related Enzymes (GLMU2 and HYAD) from *Pasteurella multocida*

P. Pasomboon, P. Chumnanpuen, T. E-kobon

***Abstract*—**Hyaluronic acid (HA) consists of linear heteropolysaccharides repeat of D-glucuronic acid and N-acetyl-D-glucosamine. HA has various useful properties to maintain skin elasticity and moisture, reduce inflammation, and lubricate the movement of various body parts without causing immunogenic allergy. HA can be found in several animal tissues as well as in the capsule component of some bacteria including *Pasteurella multocida*. This study aimed to modify a genome-scale metabolic model of *Escherichia coli* using computational simulation and flux analysis methods to predict HA productivity under different carbon sources and nitrogen supplement by the addition of two enzymes (GLMU2 and HYAD) from *P. multocida* to improve the HA production under the specified amount of carbon sources and nitrogen supplements. Result revealed that threonine and aspartate supplement raised the HA production by 12.186%. Our analyses proposed the genome-scale metabolic model is useful for improving the HA production and narrows the number of conditions to be tested further.

Keywords*—Pasteurella multocida*, *Escherichia coli*, hyaluronic acid, genome-scale metabolic model, bioinformatics.

I. Introduction

HA is a biomaterial that has several biomedical applications including treating rheumatoid arthritis, reducing inflammation of the wound, diagnostic maker for cancer, rheumatoid arthritis, liver pathologies ophthalmological, ontological surgeries, cosmetic regeneration, reconstruction of soft tissues as biocompatibility, angiogenesis, cell differentiation, tumor cell migration, and apoptosis [1]-[4]. Cosmetic products use HA for reducing wrinkles, moisturizing the skin, and used as a carrier to deliver drugs into target cells [5]. References [6]-[8] reported on microbial production of HA generated recombinant strains of *Bacillus subtilis*, *Escherichia coli*, and *Lactococcus lactis* and optimized the culture conditions for these bacterial growth and HA production which involved the increment of the glycolysis pathway and cell wall synthesis compared to cellular metabolism. *P. multocida*, especially serotype A strain, has Ha as a component of the capsular layer and its HA biosynthesis-related genes are usually candidates chosen for the HA recombinant bacterial production. However, molecular understanding of the modified metabolic pathways remains not fully explained. Therefore, this study used bioinformatic simulation methods to predict metabolic changes after modification of the *E. coli* genome-scale metabolic model by adding genes from the HA pathway of *P. multocida*. Here, we presented the reconstructed metabolic models of the *Escherichia coli* K-12 MG1655 (iAF1260 model) that contained GLMU2 and HYAD genes from *P. multocida*, and the model application on various carbon and nitrogen supplements. The modified models would provide suggestions for the experimental optimization and help to improve the HA production.

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II. Material and Methods

A. Reconstructing Genome-Scale Metabolic Model of HA Biosynthetic Pathway from P. multocida in the Context of Escherichia coli K-12 MG1655 Genome

The genome of *Escherichia coli* K-12 MG1655 was used as a template model for reconstructing genome-scale metabolic models of the HA biosynthetic pathway. The iAF1260 metabolic model of *E. coli* was obtained from the BiGG models database [9], [10]. The HA biosynthesis genes of *P. multocida* were obtained by comparing metabolic reactions of *P. multocida* with corresponding to those of *E. coli* K-12 MG1655 from KEGG, Uniprot, and MetaCys databases. Reconstruction of the modified genome-scale metabolic model was performed by using the cobra toolbox version 3.0. and the raven toolbox version 2.0 on the MATLAB program version R2019a (MATLAB 9.6) [11]-[14].

B. Prediction of HA Biosynthesis Using the Modified Genomic-Scale Metabolic Model

The modified IAF1260 metabolic model was investigated for the ability to simulate the *E. coli* HA biosynthesis on defined different conditions to maximize the biomass reactions. Adjusted amount of carbon sources (glucose and galactose for C6, xylose and arabinose for C5, and sucrose for C12), and nitrogen supplements (glutamate and glutamine for C5, threonine and aspartate for C4, alanine for C3, and lysine for C6) were applied to the modified model by Flux Balance

Analysis (FBA) to check the reactions that could carry fluxes within the whole metabolism pathway to predict the biomass of an organism or the production rate of the important metabolite. The conditions that maximize the HA production from the growth simulations or biomass synthesis were selected as the goal conditions.

III. Results

A. Reconstruction Genome-Scale Metabolic Model of HA Biosynthetic Pathway from P. multocida in the Context of Escherichia coli K-12 MG1655 Genome

Two enzymes of *P. multocida* (from eight HA-biosynthesis enzymes) were chosen and added to the metabolic model of *E. coli*. The key enzyme of the HA biosynthesis in *P. multocida* is HyaD (hyaluronan synthase) which polymerizes two precursors of the HA polymer. This enzyme does not appear in the biosynthesis of the capsular component of *E. coli*. The second enzyme, GlmU or GLMU2, catalyzes the transfer of uridyl group from UTP to glucosamine-1-P to produce UDP-*N*-acetylglucosamine as a precursor for the HA capsule. This enzyme is different between *E. coli* and *P. multocida* in terms of the H^+ usage in the reaction as described in the BiGG model, BRENDA, Uniprot, and ExPASy databases. Therefore, the GLMU2 and HYAD reactions of *P. multocida* were added to the BiGG model of *E. coli*, resulting in the modified metabolic model used for the prediction of biomass or HA product.

B. Prediction of HA Biosynthesis Using the Genomic-Scale Metabolic Model

The modified metabolic model of *E. coli* was reconstructed by adding two reactions related to the HA biosynthesis of *P. multocida*, including GLMU2 (UDP-N-acetylglucosamine diphosphorylase) and HYAD (Hyaluronan synthase). The original and modified models were compared in terms of growth and the HA flux. Biomass of *E. coli* after adding two enzymes from *P. multocida* raised to 1.101 μmol/gDW from 0.737 μmol/gDW while the HA flux of the biomass decreased to 1 μmol/gDW in every carbon and nitrogen supplements. Comparison of carbon flux in the central metabolism and HA flux on various carbon sources, including glucose, galactose (C6), xylose, arabinose (C5), sucrose (C12), showed that the flux changed in each process of the pathway depending on the carbon sources and enzymes in the carbon catabolism, however no effect was observed on the HA flux (0.366 μmol/gDW) (see Fig. 1). Due to the unchanged of HA flux on different carbon sources, Flux Scanning based on Enforced Objective Flux (FSEOF) analysis identified the higher flux reactions when the HA flux had increased. The modified model was later optimized by varying the nitrogen supplements, including glutamate and glutamine (C5), threonine and aspartate (C4), alanine (C3), and lysine (C6), together with g5lucose as the same carbon source. Glutamate and glutamine increased the HA flux (3.658 μmol/gDW, 9.999%), while decreased the flux of tricarboxylic (TCA) cycle and glycolysis. Alanine and lysine raised the HA flux to 4.058 μmol/gDW (11.092%) and 2.212 μmol/gDW (6.046%). In addition, threonine and aspartate had tendency to enhance the HA flux better than other nitrogen sources (4.458 μmol/ gDW, 12.186%) as shown in Fig. 2. Alanine was the third highly effective supplement involved in the generation of pyruvate. FSEOF identified another reaction with higher flux which was PPCK (phosphoenolpyruvate carboxykinase), involved in creating phosphoenolpyruvate in the glycolysis pathway. Taken together, this study found that changing the carbon source did not affect the HA flux, while the threonine and aspartate supplement had the most supportive effect on the increase of the HA flux. The minimum amount of aspartate was between 6 to 8 moles to initially enhance the HA flux. The glycolysis and TCA cycle fluxes reduced when the amount of aspartate increased except the SUCDi, FUM, and MDH reactions in the TCA cycle, which involved in the formation of malate, fumarate, and oxaloacetate, respectively. The changes of these three enzymes in the TCA cycle, PPCK, and HA flux were observed when the aspartate increased between 0-12 moles, while glycolysis and most enzymes in the TCA cycle flux reduced the flux. Reconstruction metabolic genome-scale model by adding two enzymes (GLMU2 and HYAD) of *P. multocida* did not affect the reduction of biomass and might not interfere with the growth of *E. coli*.

IV. Discussion

Reconstruction of the metabolic genome-scale for the HA production in *E. coli* by adding two reactions (GLMU2 and HYAD) from *P. multocida* could increase the biomass and HA yield in the simulation. GLMU2 produces UDP-*N*-acetylglucosamine, which is used to polymerize with UDP-glucuronic acid to become the HA molecule. The increase of HA biosynthesis could enhance the biomass because these genes are part of the cell wall synthesis [15], [16]. The reaction of GLMU2 with UAGDP in *E. coli* involves the hydrogen ion or proton uptake and affects the HA production. Normally, *E. coli* lacks HYAD because of the lacking of HA in the capsular component and instead of having colonic acid which shared first few enzymes with the HA biosynthetic pathway before modifying to the colonic acid [17], [18]. Therefore, HYAD from *P. multocida* could be used to develop the HA production in *E. coli* by adding new sugars to non-reducing end of the HA chain [19], [20]. Glucose uptake into glycolysis pathway of *E. coli* without adding the *P. multocida* enzymes was different when compared with after the addition. The *E. coli* model without *P. multocida* enzymes utilized the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) to uptake carbohydrates, especially hexoses, which is the energy source through the formation of phosphoenolpyruvate. In comparison, the modified model of *E. coli* with the enzymes from *P. multocida* would uptake the glucose by the reaction of HEX1 (Hexokinase).

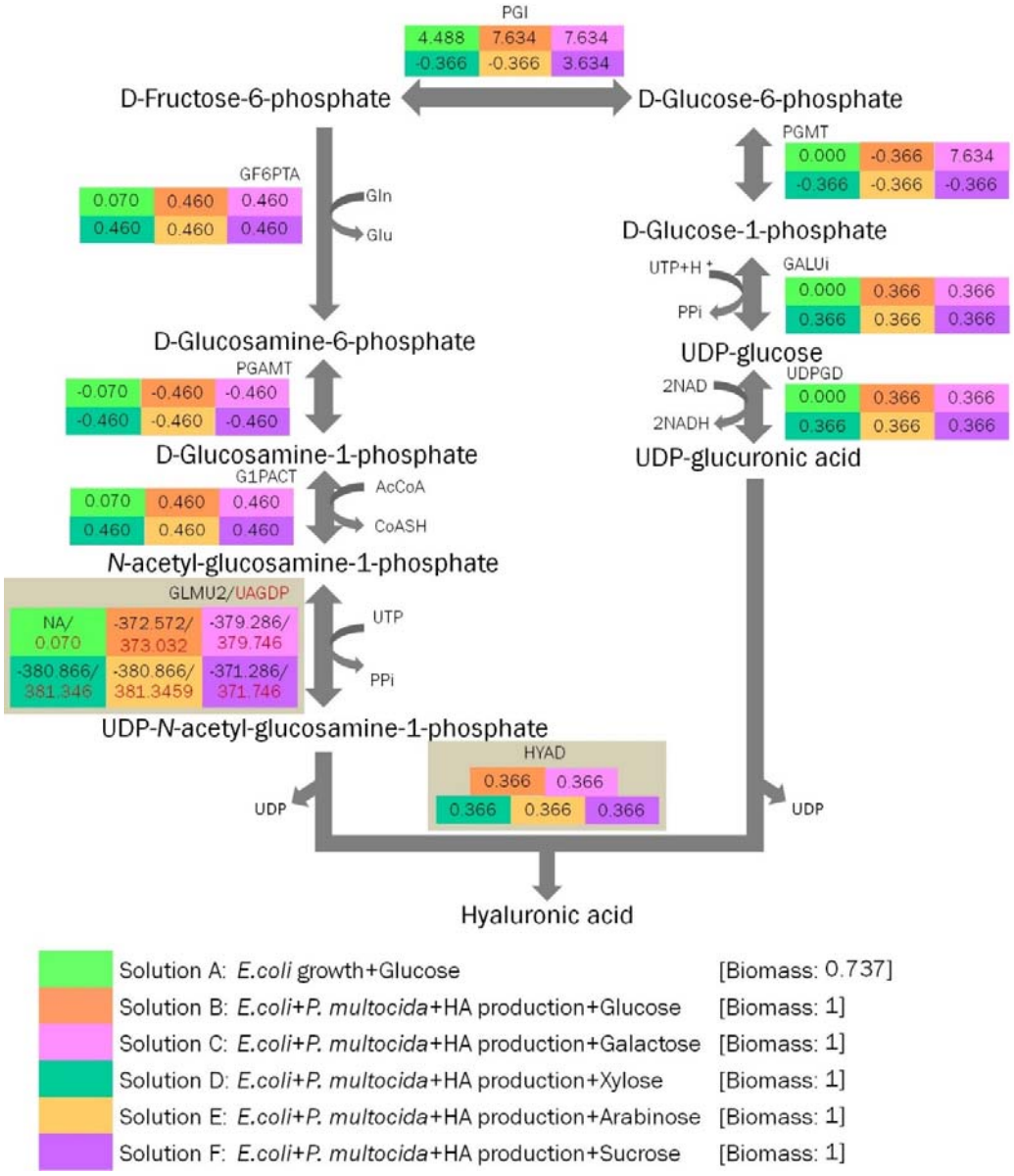

Fig. 1 Examination of the metabolic flux of the modified metabolic model of *E. coli* central carbon metabolism with the addition of HA biosynthesis genes from *P. multocida* with various carbon sources. Color boxes mean different objective functions (*E. coli* growth and HA production) and various carbon sources. Numbers showed the carbon flux value (μmol/gDW)

Various carbon sources including glucose, galactose, xylose, arabinose, and sucrose did not affect the HA production flux, as the same HA flux was returned in every carbon source (0.366 μmol/gDW) because these molecules were converted by various enzymes into the same central carbon metabolism. While various nitrogen supplements i.e., glutamate, glutamine, threonine, aspartate, alanine, and lysine, increased HA production better than the use of carbon source alone, particularly threonine and aspartate gave the highest HA flux (4.458 μmol/gDW). This could cause the decreased flux of the central carbon metabolism except those of SUCDi, FUM, and MDH in the TCA cycle. In this case, threonine could be converted to pyruvate and aspartate that separate to generate oxaloacetate and L-glutamate, while glutamine could also be converted to L-glutamate for the creation of glucosamine-6-P which was one of the N-acetyl-glucosamine-1-P precursors. Moreover, it was interesting to observe direct effect on the HA production after varying the amount of aspartate. Thus, this simulative analysis on the various carbon and nitrogen supplements could be used to optimize the actual HA production experimentally and cost effectively. Further efficient optimizations of other factors such as metal ions, vitamins, adenosine triphosphate (ATP), and other nutritional supplements together with the culture condition will benefit the improvement of the central metabolism and the HA biosynthetic pathway for future inventing of the value-added bio-products [21], [22].

V. Conclusion

The metabolic genome-scale model of *E. coli* (IAF1260 metabolic model) by adding two reactions (GLMU2 and HYAD) from *P. multocida* could propose the conditions to increase the HA production particularly the nitrogen supplements. Flux intensity analysis in the presence of different carbon and nitrogen supplements revealed that threonine and aspartate supplement were beneficial for the improved HA production (4.458 µmol/gDW), while variation of the carbon sources did not alter the flux (0.366 µmol/gDW). Therefore, this modified genome-scale metabolic model could be used to cost-effectively identify the optimal bacterial growth condition for the HA production. Further experiments would help to confirm these results and could be applied for the production of other useful compounds.

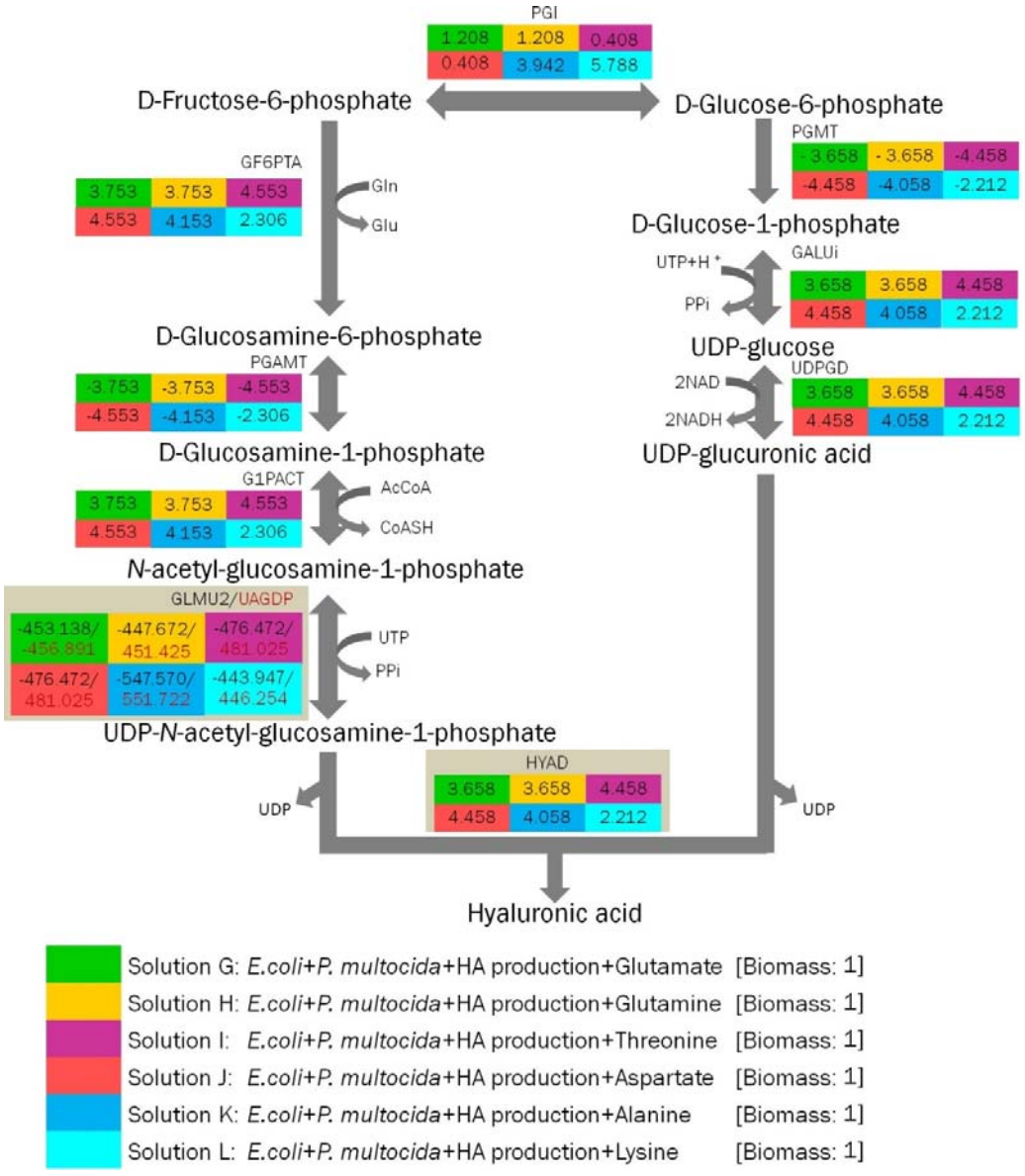

Fig. 2 Examination of the metabolic flux of the modified metabolic model of *E. coli* central carbon metabolism with the addition of HA biosynthesis genes with various nitrogen supplements. Color boxes mean different nitrogen supplements (amino acids) and the effect on the HA production. Numbers showed the carbon flux value (µmol/gDW)

Acknowledgment

We greatly thank the Department of Genetics and Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand, and the Omics Center for Agriculture, Bioresources, Food and Health, Kasetsart University (OmiKU), Kasetsart University, Thailand.

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