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QUALITATIVE METABOLITE PROFILING OF GENETICALLY MODIFIED *Escherichia coli* DURING XYLITOL PRODUCTION USING GC-MS

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ABSTRACT: Many studies have been done by metabolically modifying *Escherichia coli* to produce elevated levels of xylitol. While there have been some positive results, the xylitol yield is still not at par with that produced via the chemical route. This study employed GC-MS, combined with multivariate analysis to qualitatively profile the metabolites found in genetically modified *E. coli* (Δ*pgi*+*xpdh*) for xylitol production with glucose as substrate. It was found that over time, xylitol level increased, with the peak intensity at 24h being the highest. This was parallel with the optical density (OD) value, which also increased over time, indicating that as the cell grew, more xylitol was generated. Top contributing metabolites were identified and subjected to pathway analysis, revealing that amino acids may have a significant role in xylitol production. The findings from this study provide initial metabolomics input for enhancing recombinant *E. coli* strains in the biotechnological production of xylitol, whereby glucose is employed as primary substrate.

KEYWORDS: *Metabolomics, GC-MS, Escherichia Coli, Xylitol, Amino Acid.*

1. INTRODUCTION

Xylitol is a naturally occurring 5-carbon polyol mainly used in the food industry as sugar substitute due to its similar sweetening power to sucrose but with lower caloric content among other benefits [1]. Current commercial production of xylitol via the chemical route is an extensive and unsustainable process requiring refining treatment for xylose which also makes it costly [2]. As such, continuous research has been done to produce xylitol biotechnologically by genetic modification of microorganisms, including *Escherichia coli*, which is a model microorganism for producing target compounds in laboratory settings [3] and resulted in positive outcomes [4, 5]. In terms of substrate, both xylose and glucose had been studied for xylitol production with the latter being much preferable due its price and availability [6]. Nonetheless, despite various approaches taken to increase xylitol yield including improvisation of immobilisation strategy and process parameters optimisation [7, 8], the amount of xylitol yielded is still incomparable to that of the chemical route. The metabolomics approach has proven to be successful in improving target compound production, specifically 1-butanol [9, 10, 11]. So far, none of the studies on recombinant *E. coli* producing xylitol has taken the metabolomics approach to increase xylitol yield. Therefore, this study seeks to qualitatively profile the metabolites present in recombinant *E. coli* (Δ*pgi*+*xpdh*) during xylitol production using glucose as a substrate by using GC-MS and multivariate analysis, as the first step in the metabolomics approach to improve xylitol production.

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2. MATERIALS AND METHODS

2.1. Bacterial Strain

E. coli BL21 strain from Novagen was deleted for phosphoglucose isomerase (Δ*pgi*) gene to redirect the glucose catabolism from glycolysis to pentose phosphate pathway (PPP), and xylitol phosphate dehydrogenase (*xpdh*) gene from *Clostridium difficile* was cloned into the strain by using plasmid pET21 as vector. Insertion of *xpdh* gene was to enable the conversion of D-xylulose-5-phopshate to xylitol in PPP.

2.2. Bacterial Culture

Xylitol production took place for 24 h in a 250 mL shake flask with 50 mL LB medium (5 g/L yeast extract, 10 g/L NaCl, 10 g/L tryptone) added with 34 μg/mL chloramphenicol, induced by adding 0.1 mM isopropyl β-d-1-thiogalactopyranoside (IPTG) and 10 g/L glucose and incubated in an orbital shaker at 200 rpm and 37 ℃.

2.3. Cell Sampling and Metabolites Extraction

Cell sampling and metabolites extraction was done according to Hashim and Fukusaki [12] with slight modification. 5 mL of culture media was transferred to a fresh tube at 0, 2, 5, and 24 h. The sample was centrifuged at 4 ℃, 5000 rpm for 5 min to separate the cell pellet from the media. The media which contained extracellular metabolites was transferred to a new tube and kept at -20℃ for further analysis. Meanwhile, the cell pellet was immediately added with 2 mL of pre-cooled (-20℃) single-phase extraction solvent (methanol/chloroform/water = 5/2/2 v/v/v%) with 60 μL ribitol (0.2 mg/mL) as internal standards [12, 13]. The mixture was then left for 30 min in an incubator shaker at 200 rpm, 4 ℃. Afterwards, 1 mL of distilled water was added into the mixture, vortexed and centrifuged at 4 ℃, 16100 rcf for 3 min. Next, the upper polar phase was transferred to a new tube via syringe filtration (0.2 μm PTFE hydrophilic membrane, Hawach Scientific, Shaanxi, China) and freeze dried. Derivatisation was done by adding 75 μL of methoxyamine hydrochloride (Sigma-Aldrich, MO, USA) dissolved in pyridine (Merck, Darmstadt, Germany) to the lyophilised extracts and left in thermomixer to react at 30 ℃, 1200 rpm for 90 min in a process called oximation, followed by silylation by adding 50 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) (Sigma-Aldrich, MO, USA) and the reaction was performed at 37 ℃, 1200 rpm for 30 min [13]. The derivatised samples were transferred to glass vials and analysed with GC-MS within 24 h.

2.4. Analytical Methods

2.4.1. Cell Growth

The cell growth was observed by measuring the optical density at 600 nm (OD_{600}) using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) at 0, 1, 2, 3, 4, 5, and 24 h. Measured values of OD_{600} were plotted on the growth curve.

2.4.2. GC-MS Analysis

GC-MS analysis was performed using GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan). The samples were separated using a BP5MS capillary column coated with 5% phenylpolysilphenylene-siloxane (30 m x 0.25 mm i.d. x 0.25 μm film thickness). One μL of sample was injected in split mode with a split ratio of 1:25. The injector, transfer line, and ion source temperature were set at 230, 250, and 200 ℃, respectively. The column flow rate was 1.12 mL/min (linear velocity 39 cm/s). The column temperature was held at 80 ℃ for 2 min,

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raised by 15 ℃/min to 330 ℃ for 12 min. Electron ionisation (EI) was performed at 70 eV. The mass range of the detector was set to *m/z* 35 to 800 and the detector voltage (set by auto-tuning) was at 0.80 kV.

2.5. Multivariate Analysis and Statistical Analysis

The amount of each metabolite (peak intensity) was normalised to the internal standard (ribitol), mean centred and scaled to unit variance. Multivariate data analysis, such as principal component analysis (PCA), and pathway analysis was performed using MetaboAnalyst 5.0 [14].

3. RESULTS AND DISCUSSION

3.1. Bacterial Cell Growth

To determine bacterial cell growth, OD_{600} was measured using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). Fig. 1(a) shows the plotted growth curve for *E. coli* (Δ*pgi*+*xpdh*). It was observed that the exponential growth phase of the cell was between 1 to 4 h, with the maximum specific growth rate (μ_{max}) of 0.0.0459 h^{-1}. The cell continued to grow until the 24th hour albeit slower, indicating that the cell was starting to enter the stationary phase past 24 h.

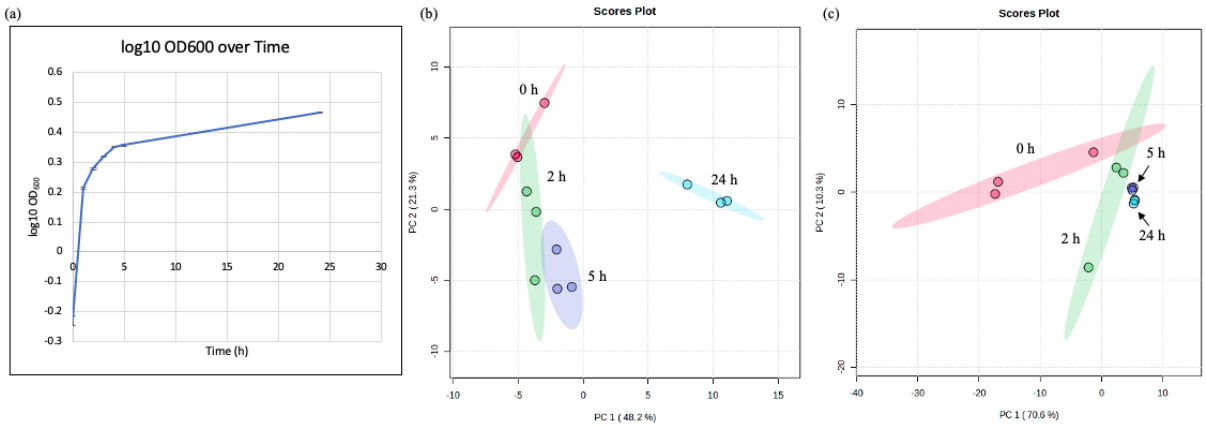

Fig. 1. (a) Growth curve of modified *E. coli* during 24 h xylitol production, and PCA score plot showing metabolite profile in (b) intracellular extracts, (c) extracellular extracts collected at 0, 2, 5, and 24 h.

3.2. Time-course Metabolite Profiling

Fig. 1(b) and 1(c) show time-course metabolite profiles of *E. coli* (Δ*pgi*+*xpdh*) at 0, 2, 5, and 24 h post-induction for intracellular and extracellular extracts respectively. It can be seen that the metabolites at 24 h are distinctly separated from the rest especially in the intracellular samples. This pattern corresponds to xylitol production which reach its peak at 24 h post-induction as can be observed in Fig. 2(a) and 2(b). The increase of xylitol is parallel with the decrease of glucose in the extracellular extracts in Figure 2(c), which tallies with the information in Fig. 1(a). It can be concluded that the genetically modified *E. coli* consumed glucose for growth and generated more xylitol as it grew.

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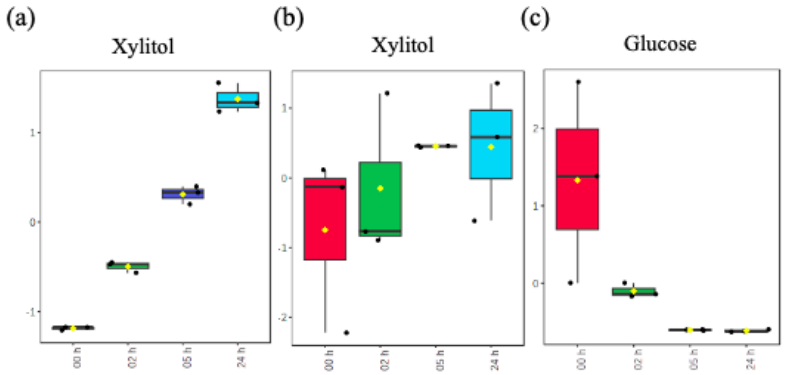

Fig. 2. Peak intensity of (a) xylitol in intracellular extracts, (b) xylitol in extracellular extracts, and (c) glucose in extracellular extracts

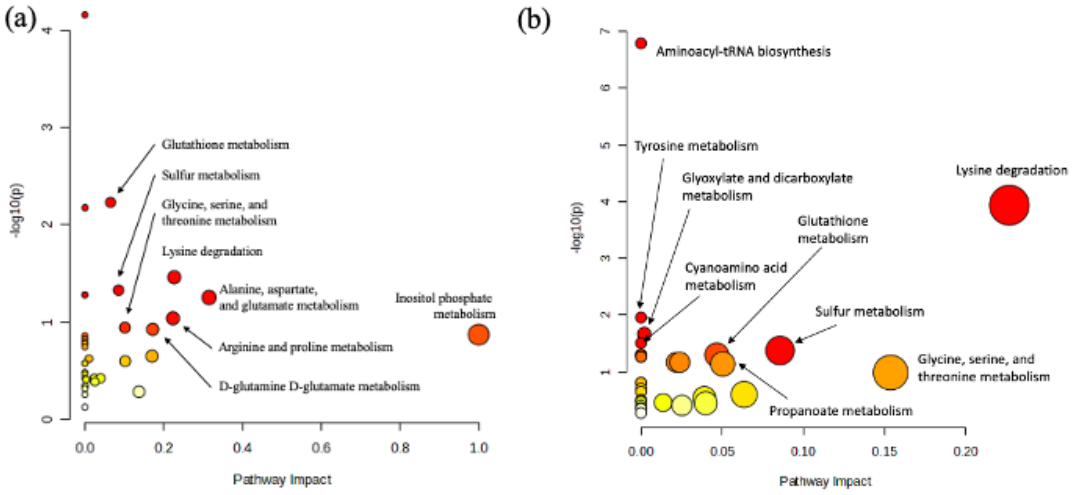

Fig. 3. Pathway analysis for top 20 most contributing metabolites in (a) intracellular samples, (b) extracellular samples

3.3. Metabolome Analysis Reveals Impacted Amino Acids Metabolisms

The most contributing metabolites in the separation of the samples were determined from the list of metabolites identified using GC-MS library and subjected to pathway analysis. The results show that in both intracellular and extracellular samples, amino acid metabolisms are mainly affected (Figure 3). This result suggested that amino acids may play a prominent role in xylitol production. This outcome is supported by a study done by Fuzi et al. [15] which demonstrated that individual supplementation of certain amino acids was able to increase the xylitol titer by nearly 50% in genetically modified *E. coli* (Δxy1AB+P21XR) whereby xylose was the main substrate and glycerol was used as carbon source. The study also concluded that amino acids may play a critical role in the production of xylitol.

4. RESULTS AND DISCUSSION

From this study, it was found that amino acid metabolisms were mainly affected in xylitol production. Further research should be done to understand the relationship between amino acids and xylitol production by comparing the metabolic profile of *E. coli* (Δ*pgi*+*xpdh*) when different amino acids were to be added to the fermentation media to learn how the amino acids affect xylitol production.

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REFERENCES

[1] Queiroz, S. S., Jofre, F. M., Mussatto, S. I., & Maria das Graças, A. F. (2022). Scaling up xylitol bioproduction: challenges to achieve a profitable bioprocess. Renewable and Sustainable Energy Reviews, 154, 111789.

[2] Rafiqul, I. S. M., & Sakinah, A. M. (2013). Processes for the production of xylitol—a review. Food Reviews International, 29, 2, 127-156.

[3] Yu, C., Cao, Y., Zou, H., & Xian, M. (2011). Metabolic engineering of Escherichia coli for biotechnological production of high-value organic acids and alcohols. Applied microbiology and biotechnology, 89, 573-583.

[4] Sakakibara, Y., Saha, B. C., & Taylor, P. (2009). Microbial production of xylitol from L-arabinose by metabolically engineered Escherichia coli. Journal of bioscience and bioengineering, 107, 5, 506-511.

[5] Su, B., Wu, M., Zhang, Z., Lin, J., & Yang, L. (2015). Efficient production of xylitol from hemicellulosic hydrolysate using engineered Escherichia coli. Metabolic engineering, 31, 112-122.

[6] Granström, T. B., Izumori, K., & Leisola, M. (2007). A rare sugar xylitol. Part II: biotechnological production and future applications of xylitol. Applied microbiology and biotechnology, 74, 273-276.

[7] Abd Rahman, N. H., Jahim, J. M., Munaim, M. S. A., Rahman, R. A., Fuzi, S. F. Z., & Illias, R. M. (2020). Immobilization of recombinant Escherichia coli on multi-walled carbon nanotubes for xylitol production. Enzyme and microbial technology, 135, 109495.

[8] Bedő, S., Fehér, A., Khunnonkwao, P., Jantama, K., & Fehér, C. (2021). Optimized bioconversion of xylose derived from pre-treated crop residues into xylitol by using Candida boidinii. Agronomy, 11, 1, 79.

[9] Nitta, K., Laviña, W. A., Pontrelli, S., Liao, J. C., Putri, S. P., & Fukusaki, E. (2017). Orthogonal partial least squares/projections to latent structures regression-based metabolomics approach for identification of gene targets for improvement of 1-butanol production in Escherichia coli. Journal of bioscience and bioengineering, 124, 5, 498-505.

[10] Ohtake, T., Pontrelli, S., Laviña, W. A., Liao, J. C., Putri, S. P., & Fukusaki, E. (2017). Metabolomics-driven approach to solving a CoA imbalance for improved 1-butanol production in Escherichia coli. Metabolic engineering, 41, 135-143.

[11] Nitta, K., Laviña, W. A., Pontrelli, S., Liao, J. C., Putri, S. P., & Fukusaki, E. (2019). Metabolome analysis revealed the knockout of glyoxylate shunt as an effective strategy for improvement of 1-butanol production in transgenic Escherichia coli. Journal of bioscience and bioengineering, 127, 3, 301-308.

[12] Hashim, Z., & Fukusaki, E. (2016, June). Metabolomics-based prediction models of yeast strains for screening of metabolites contributing to ethanol stress tolerance. In IOP Conference Series: Earth and Environmental Science (Vol. 36, No. 1, p. 012046). IOP Publishing.

[13] Yoshida, R., Tamura, T., Takaoka, C., Harada, K., Kobayashi, A., Mukai, Y., & Fukusaki, E. (2010). Metabolomics-based systematic prediction of yeast lifespan and its application for semi-rational screening of ageing-related mutants. Aging cell, 9, 4, 616-625.

[14] Pang, Z., Zhou, G., Ewald, J., Chang, L., Hacariz, O., Basu, N., & Xia, J. (2022). Using MetaboAnalyst 5.0 for LC–HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. Nature protocols, 17, 8, 1735-1761.

[15] Fuzi, S. F. Z. M., Zahari, F. A., Puay, O. H., Oon, L. K., & Abdullah, I. (2023). Screening Effect of Amino Acid on Xylitol Production by Recombinant Escherichia coli System. Journal of Bioprocessing and Biomass Technology, 2, 1, 43-47.