# Biosynthesis of Glucaric Acid with Microbial Cell Factories

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## Short Commentary

**ABSTRACT**

Glucaric acid is a kind of organic acid and widely used in food, medicine and chemical industries. During the past decade, model microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* have been engineered for production of glucaric acid. Especially, *Pichia pastoris* has also been engineered for production of glucaric acid with the highest reported value. In this commentary, recent efforts and advances for glucaric acid biosynthesis were discussed.

# INTRODUCTION

Glucaric acid, a natural, high valuable organic acid, has been characterized as a "top value-added chemical" from biomass because of its various applications [1]. Due to the drawbacks of traditional chemical methods, production of glucaric acid with microbial cell factories as a potential clean and environmentally-friendly approach has attracted much attention [2,3]. Many efforts have been devoted to construct robust cell factories for glucaric acid biosynthesis [4-6]. Naturally, glucaric acid can be synthesized in mammalian and plant cells. However, the biosynthesis pathway is long and not fully recognized [7]. Thus, a short biosynthetic pathway from glucose or myo-inositol was designed and characterized. In 2009, Moon et al. firstly recruited the myo-inositol-1-phosphate synthase encoding gene INO1 from *Saccharomyces cerevisiae*, and the myo-inositol oxygenase encoding gene *mMIOX* from mouse and the urinate dehydrogenase encoding gene *udh* from *Pseudomonas syringae* and constructed a novel glucaric acid biosynthesis pathway in *Escherichia coli* [6]. Although glucaric acid was detected in cultures (about 1 g/L), the intermediates myo-inositol and glucuronic acid were accumulated because of the low activity of MIOX. Thus, improvement the stability and activity of MIOX is crucial for optimizing the flux towards glucaric acid. In this regard, the researchers from Prather group tried different strategies including directed evolution, synthetic scaffolds and fusion tags to increase the activity of MIOX to balance the flux towards glucaric acid [8,9]. As a result, 4.85 g/L glucaric acid was achieved from 10.8 g/L myo-inositol. However, the production failed to increase after further modifying pathways and optimizing feeding of supplemental carbon sources [10-12]. The results from *E. coli* suggested that achievement of high level expression of the rate-limiting MIOX and improvement of the tolerance towards pH-mediated toxicity should be the key points for high titer of glucaric acid. To this end, *Saccharomyces cerevisiae* was also investigated for production of glucaric acid because of its satisfactory acid-tolerance [4]. Accordingly, the constructed glucaric acid biosynthetic pathway in *E. coli* was ported into *S. cerevisiae* with codon-optimized MIOX. Applying a fed-batch fermentation strategy, the production was increased to 1.6 g/L from glucose supplemented with myo-inositol. The results indicated that pH-mediated toxicity is not an essential issue for *E. coli*.

In addition to *E. coli* and *S. cerevisiae*, the methylotrophic yeast *Pichia pastoris* (now reclassified as Komagataella phaffii), has also been widely used for production of heterologous proteins and valuable chemicals because of its many advantages, for instance, the ability to grow to high cell densities in simple media and the GRAS (generally recognizedas safe) status [13,14]. In particular, it has been demonstrated that *P. pastoris* possesses excellent performance in functional expression of cytochrome P450 related oxygenases [15-17]. Additionally, expression of the final enzyme *Udh* of the glucaric acid synthetic pathway is compatible with cell growth since *Udh* is thermally unstable and displays high activity at 30°C. In consideration of these points, *P. pastoris* should be a suitable candidate for production of glucaric acid. For this reason, Liu et al. firstly investigated and functionally validated an endogenous MIOX, and then successfully constructed the glucaric acid biosynthesis pathway in *P. pastoris* [5]. After optimization

of the expression of MIOX and *Udh* with a fusion expression strategy, and applying a fed-batch approach, the titer of glucaric acid was significantly increased to 6.61 g/L from glucose and myo-inositol. Compared with *E.coli* and *S. cerevisiae*, *P. pastoris* was much more appropriate and efficient for glucaric acid production. In addition, it could be found that in addition to the low activity of MIOX, the inefficient biosynthesis of myo-inositol is also a bottleneck for high level production of glucaric acid from glucose. Additionally, myo-inositol also serves as an essential precursor for synthesis of phosphatidyliositol, which plays an important role in signaling and lipid synthesis. Therefore, future efforts for *P. pastoris* should be focused on (I) engineer and overexpression of the upstream genes, especially INO1 for myo-inositol biosynthesis; (II) modification and improvement of MIOX expression and activity with inducible promoters; (III) knock-down of the competing essential pathway flux; (IV) exploration and construction of novel synthetic pathways towards glucaric acid **(Figure 1).**
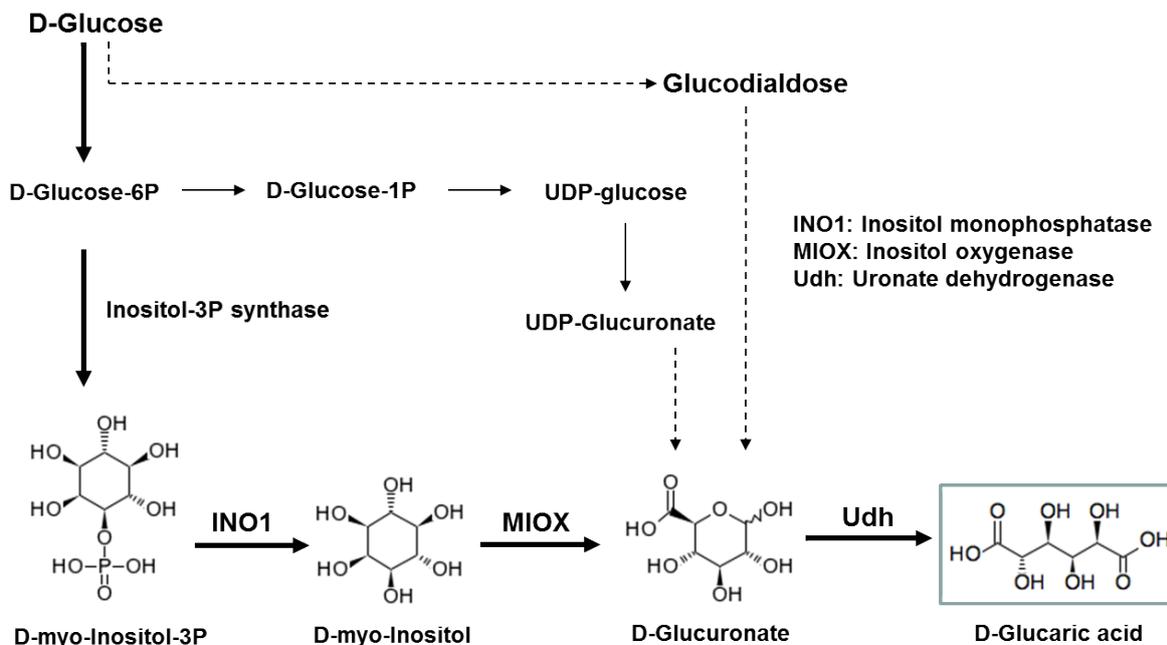


**Figure 1.** Potential pathways for glucaric acid biosynthesis. The dash lines means the corresponding enzymes are not identified or characterized.

## CONCLUSION

*Pichia pastoris* is a desirable host for glucaric acid biosynthesis. With the development of synthetic biology toolboxes and metabolic engineering strategies, the intact glucaric acid biosynthesis pathway from glucose could be systematically engineered and optimized in the context of global regulation. By combining cost effective fermentation strategies, efficient processes for production of glucaric acid from glucose should be established in near future.

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