# Kalion's Fermentation for the Production of High-purity Glucaric Acid


## Abstract

Glucaric acid has diverse market potential. It has uses ranging from detergents to polymers and food additives. It enhances sustainability and improves the mechanical properties of several products, such as textiles, polymers, pharmaceuticals, and detergents. It is a cancer prevention agent. Its low-yield nitric acid-based production method has several limitations. Scientists have developed several biobased processes of glucaric acid production to overcome the limitations. In this article, we will see the advantages of biosynthetic pathways of glucaric acid over low-yield nitric acid-based glucaric acid production.


## Introduction

Glucaric acid, also called saccharic acid, is a member of oxidized sugars[1]. A German chemist Heinrich Kiliani first described its production reaction in 1925. This process produces low yields of glucaric acid [1]. There was a need to develop an efficient technology to eliminate nitric acid as the oxidizing agent. The biobased processes of glucaric acid production are environmentally friendly and cheaper. Kalion, a biotechnology company, conducted research and development work improving the biobased production of glucaric acid. It has been awarded a National Science Foundation Phase II Small Business Research grant for $746,822. In this mini-review, we will discuss new green methods of glucaric acid production vs. previous methods.

## Nitric acid-based glucose oxidation for gularic acid production

This method involves the oxidation of sugar with nitric acid. It is a low-yield method of glucaric acid production. Several collateral reactions also occur due to the use of nitric acid as an oxidizing agent. This reaction generates several oxidation products other than glucaric acid [1].

## Biosynthetic pathway of Glucaric acid in *E.coli*

A group of scientists tried to develop the biological production of glucaric acid in E. coli to enhance the production yields and eliminate the limitations of the traditional nitric acid-based glucose oxidation method. They coexpressed the genes that encode myo-inositol oxygenase (MIOX) isolated from mice and myo-inositol-1-phosphate synthase (Ino1) isolated from *Saccharomyces cerevisiae* in *E.coli*. Ino1 uses glucose-6-phosphate to produce myo-inositol-1-phosphate in yeast [2]. These enzymes produced glucuronic acid with myo-inositol. Both started to accumulate due to the rate-limiting activity of MIOX. Then they expressed the gene that encodes uronate dehydrogenase (Udh) isolated from *Pseudomonas syringae* in *E.coli*. This enzyme increased the conversion of glucuronic acid to glucaric acid thus, increasing the yields of glucaric acid production [3].

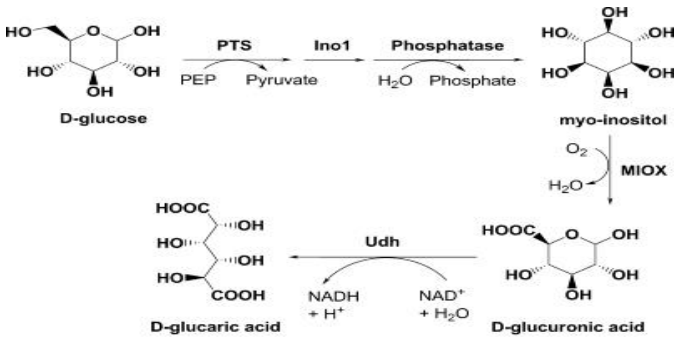


**Figure 1:** Glucaric acid production pathway in *E. coli*.

Another group of scientists used a modified IB1486-GA *E. coli* strain. They specifically controlled the activity of phosphofructokinase (Pfk) in the modified *E.coli* to increase the production of glucaric acid. The timed knockdown of Pfk activity improved the glucaric acid titer up to 42% [4]. Kalion used this method to produce D-glucaric acid in recombinant E. coli.

## Biosynthetic pathway of Glucaric acid in *Pseudomonas putida*

The glucaric acid production pathway was also developed in *Pichia pastoris.* The scientists first coexpressed the urinate dehydrogenase (Udh) isolated from *Pseudomonas putida* and native ppMIOX. The glucaric acid was accumulated from myo-inositol, but no glucaric acid production was detected from glucose. Then they coexpressed the heterologous MIOX isolated from the mouse and the urinate dehydrogenase (Udh) isolated from *Pseudomonas putida* in *Pichia pastoris.* The coexpression of mMIOX and Udh improved glucaric acid titer up to 6.61 g/L [5].

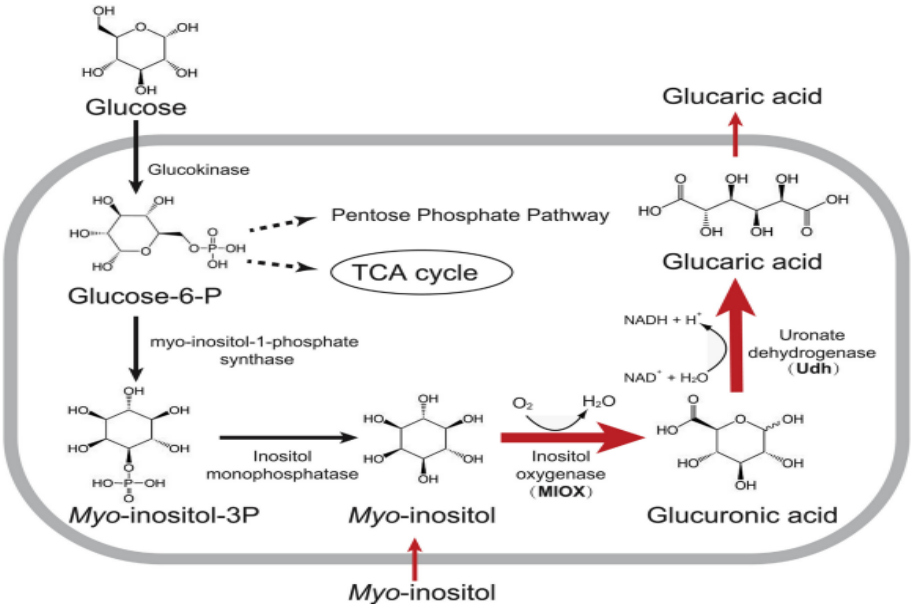


**Figure 2:** Glucaric acid production pathway in engineered *Pichia pastoris.*

## Biosynthetic pathway of Glucaric acid Production in *Saccharomyces cerevisiae*

The biosynthetic pathway of glucaric acid production was also developed in *Saccharomyces cerevisiae* using codon-optimized MIOX. The yield was increased up to 1.6 g/L applying a fed-batch fermentation [6]. The availability of myo-inositol and MIOX activity was not only rate-limiting in *E. coli* but also in *S. cerevisiae.* A group of scientists developed another way to solve the problem. They expressed stable MIOX4, isolated from *A. thaliana* in *S. cerevisiae.* The target genes were integrated into the delta sequence to increase the expression of stable MIOX4. The glucaric acid titer was increased in *S. cerevisiae* up to 6.0 g/L [7].

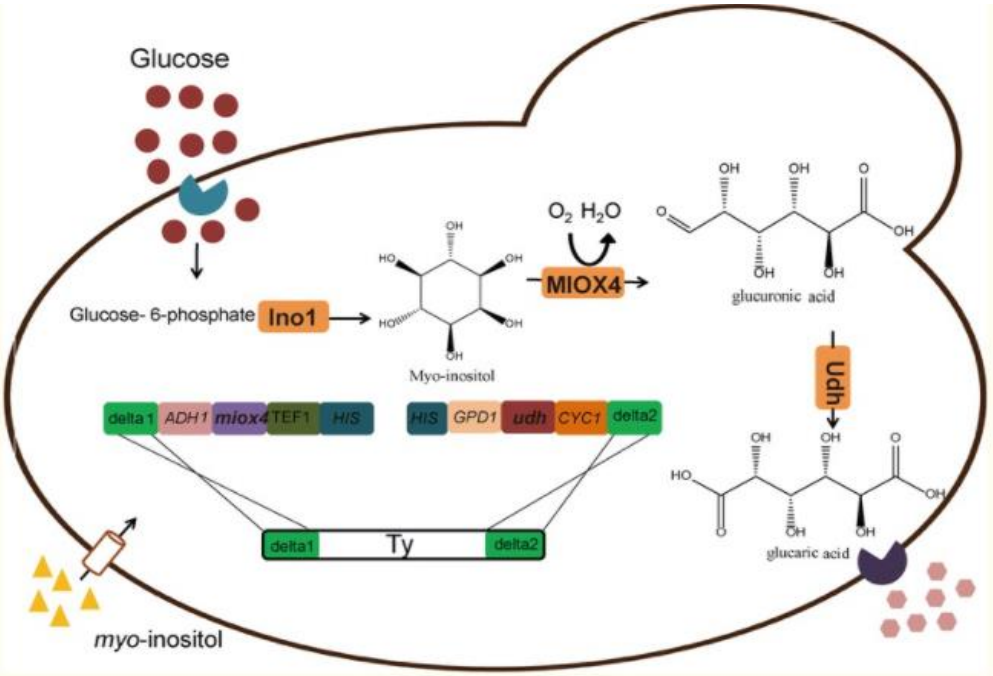


**Figure 3:** Use of miox4 and udh to produce glucaric acid in *Saccharomyces cerevisiae*.

## Conclusion

The biobased processes of glucaric acid production are environment friendly, cheaper, and produce high yields compared to the low-yield nitric acid-based production method. Kalion's research to improve the biobased production of glucaric acid in *E.coli* is highly appreciable.

## References


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