High-Purity Glucaric Acid Produced by Microbes

Glucaric acid is a six-carbon diacid that has potential to serve as a platform chemical. In the past two decades, researchers and organizations have applied for several patents pertaining to glucaric acid. It has many uses, including:

- in the production of adipic acid for renewable nylon-6,6
- as a intermediate in the production of 2,5-furandicarboxylic acid (FDCA), a high-performance renewable replacement for polyethylene terephthalate (PET) in two-liter bottles
- as a polymer additive to increase the mechanical properties of several different classes of industrial fibers including polyacrylonitrile (PAN), a key precursor to carbon fibers
- as a replacement for phosphates to prevent corrosion in water treatment processes
- to strengthen recycled cotton so that it can be reused in clothing.

Each of these applications could help solve environmental challenges. Unfortunately, while the potential is significant, glucaric acid is primarily limited to pharmaceutical and nutraceutical uses because of its high cost.

Commercialization of glucaric acid has been hindered largely due to the lack of economically viable production methods. Traditional production methods such as nitric acid oxidation suffer from low yields and poor selectivity, which increases production costs. And, chemically synthesized glucaric acid requires extensive purification. A fermentation-based process could yield glucaric acid that is both low-cost and high-purity.

Researchers at the Massachusetts Institute of Technology (MIT) developed a high-theoretical-yield, short pathway to produce glucaric acid. The novel biosynthetic pathway, expressed in *E. coli*, consists of three enzymes from disparate organisms: yeast, mice, and bacteria.

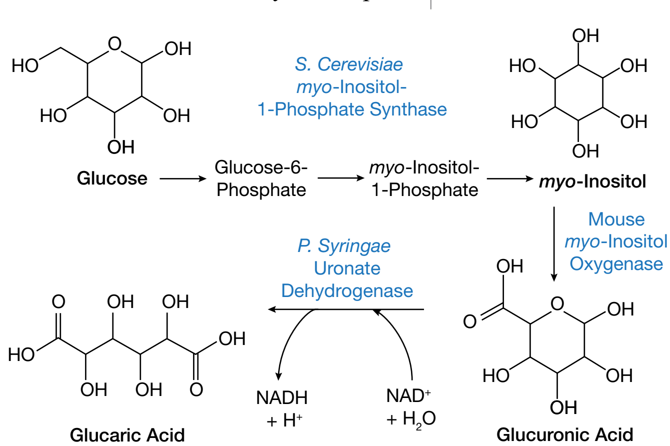
For producing large-scale commodity chemicals from biomass, a key concern is how much of the substrate (*i.e.,* the principal raw material) is lost on a mass basis. It is not uncommon for a bioconversion process to lose 60% or more of the substrate, which has significant cost implications at large scales. On a mass basis, the theoretical yield of the process created by MIT engineers is 1.17 — *i.e.,* it is actually gaining mass, not losing it.

Kalion, a Massachusetts-based biotechnology company, licensed this technology. With funding from NSF's Small Business Technology Transfer Program (STTR), Kalion improved the first-generation *E. coli* strain developed at MIT and is now developing an even lower-cost second-generation strain.

Kalion tweaked the first-generation strain to make it more industrially robust so that it can deal with challenges rarely seen at the lab scale, such as bacteriophage contamination. The company also developed an optimized downstream process that readily delivers multiple commercially relevant forms of glucaric acid with >99% purity. For example, the process can create an aqueous formulation for water treatment customers and a calcium salt form for pharmaceutical customers. Customers in water treatment and the pharmaceutical industry have tested and validated the final products for commercial production. And, the aqueous glucaric acid product has received Toxic Substances Control Act (TSCA)-related approvals, which are necessary for its use in water treatment applications.

In the initial process developed at MIT, product titers were high, but the feedstock, *myo*-inositol, was 10 times more expensive than glucose. This limited the profitability of the process, and made it cost-effective to use glucaric acid only in high-purity applications. Kalion made several key modifications in its second-generaton strain so that it can produce glucaric acid from the lower-cost substrate glucose.

The U.S. Environmental Protection Agency (EPA) recognized Kalion's work with a Green Chemistry Award for Small Business in 2019. Using the first-generation strain, the company will produce multi-ton volumes of glucaric acid by the first quarter of 2020. CEP



◀ Kalion is commercializing a fermentation process that transforms glucose to glucaric acid in five steps. The novel biosynthetic pathway, expressed in *E. coli*, consists of three enzymes (in blue) from disparate organisms: yeast, mice, and bacteria.

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