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Metabolic engineering strategies to bio-adipic acid production

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Adipic acid is the most industrially important dicarboxylic acid as it is a key monomer in the synthesis of nylon. Today, adipic acid is obtained via a chemical process that relies on petrochemical precursors and releases large quantities of greenhouse gases. In the last two years, significant progress has been made in engineering microbes for the production of adipic acid and its immediate precursors, muconic acid and glucaric acid. Not only have the microbial substrates expanded beyond glucose and glycerol to include lignin monomers and hemicellulose components, but the number of microbial chassis now goes further than *Escherichia coli* and *Saccharomyces cerevisiae* to include microbes proficient in aromatic degradation, cellulose secretion and degradation of multiple carbon sources. Here, we review the metabolic engineering and nascent protein engineering strategies undertaken in each of these chassis to convert different feedstocks to adipic, muconic and glucaric acid. We also highlight near term prospects and challenges for each of the metabolic routes discussed.

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Introduction

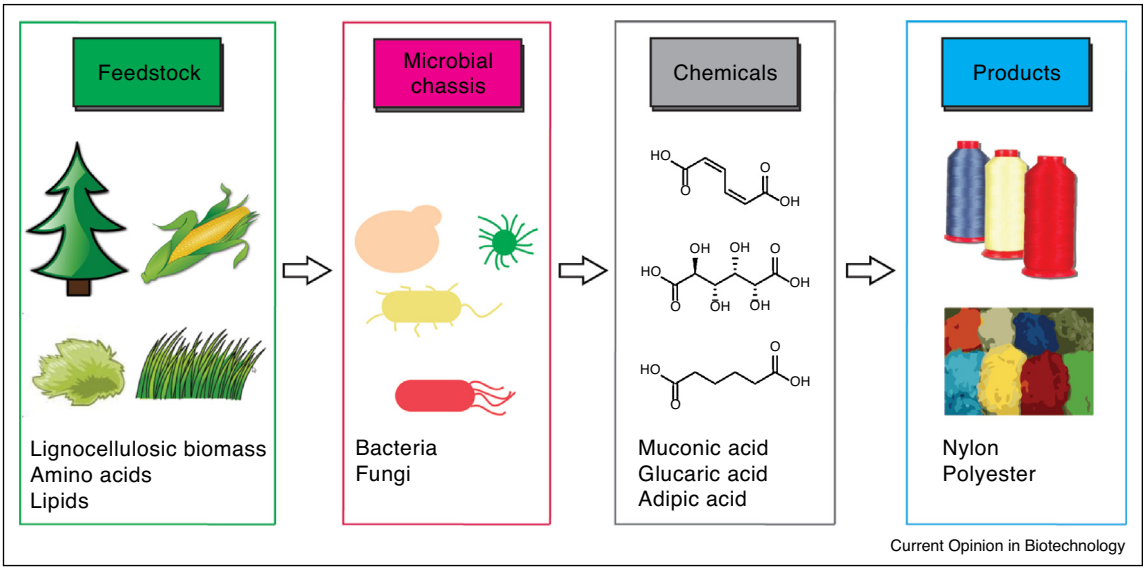
In 2010, global production of adipic acid was estimated at 2.6 million tons, with 65% going to the production of nylon-6,6 fibers [1,2]. Commercially, adipic acid is synthesized from petroleum-derived benzene, which is reduced to cyclohexane followed by oxidation to a mixture of cyclohexanol and cyclohexanone. This mixture is further oxidized using a vanadium or copper catalyst with nitric acid to produce adipic acid. The commercial synthesis of adipic acid not only generates ~10% of the world's man-made nitrous oxide [3], a greenhouse gas 300-times more potent than carbon dioxide, but it also uses benzene, a known carcinogen, as a starting material. Production of bio-adipic acid, wherein the carbons are derived from a renewable feedstock, has the potential to reduce greenhouse gas emissions and eliminate the need for fossil fuel precursors. Based on the 2.6 million tons of adipic acid produced annually from petroleum, renewable production of adipic acid could eliminate the use of benzene found in 6 billion barrels of crude oil.

Advances in metabolic engineering and synthetic biology now allow the engineering of microbes for the production of advanced biofuels [4], commodity chemicals and pharmaceuticals [5]. In the last two years, a number of different metabolic engineering strategies have been applied to the microbial synthesis of adipic acid and its immediate precursors muconic acid and glucaric acid (Figure 1, Table 1). In particular, there has been an expansion in the renewable feedstocks used as starting materials, as well as an increase in the number of metabolic pathways engineered for the microbial synthesis of these compounds. The number of chassis has also risen and now goes beyond the workhorse chassis of *Escherichia coli* and *Saccharomyces cerevisiae*, to include the naturally aromatic compound metabolizer *Pseudomonas putida* [6], the cellulolytic bacteria *Thermobifida fusca* [7], and *Klebsiella pneumonia* [8], which can metabolize a number of different carbon sources. In this review, we focus on these latest advances and direct the reader to recent comprehensive reviews on adipic acid and muconic acid production [2,9,10].

Metabolic pathways to muconic, glucaric, and adipic acid

A total of ten biosynthetic pathways from lignocellulosic biomass, lipids and amino acids have been leveraged for the production of adipic acid, muconic acid and glucaric acid, with pathways utilizing lignin, lipids and amino acids as precursors demonstrated since 2014 (Figure 2). In 1994, Frost and Draths developed the first production of bio-adipic acid by exploiting *E. coli* primary metabolism to convert glucose to 3-dehydroshikimic acid (DHS), and using three heterologous enzymes to produce protocatechuic acid (PCA), catechol and ultimately cis-cis muconic acid, which was in turn chemically hydrogenated to adipic acid [11]. More recent pathways to muconic acid

Figure 1



Bio-adipic acid production. In the last two years an increasing number of feedstocks and microbial chassis have been used for the production of adipic acid and its immediate precursors, muconic acid and glucaric acid. Adipic acid is one of the most industrially important dicarboxylic acids being used in the synthesis of nylon and polyesters.

include re-routing chorismate to anthranilic acid [12], 2,3 dihydroxybenzoic acid [13,14], salicylic acid [15,16], or *p*-hydroxybenzoic acid [17] to produce catechol, and a β-ketoadipate pathway modified to stop at PCA followed by a re-route to catechol [18••]. Glucaric acid has also been microbially produced from glucose via a synthetic five step pathway [19•]. Finally, adipic acid has been microbially synthesized directly from renewables by taking advantage of (1) the reversed adipate degradation pathway [20,21], (2) the reversed β-oxidation coupled to alkane degradation [22], and (3) the reversed phenylacetate degradation pathway coupled to reversed adipic acid degradation [23••].

Muconic acid from cellulose- and hemicellulose-derived feedstocks

Microbes can convert cellulose-derived glucose or hemicellulose-derived xylose to phosphoenolpyruvate (PEP) via glycolysis and erythrose-4-phosphate (E4P) via the pentose phosphate pathway. Aromatic amino acid biosynthesis takes PEP and E4P and eventually converts them to DHS, a key node in the biosynthesis of muconic acid. The concept of leveraging aromatic amino acid biosynthesis for muconic acid production is rooted in the successful engineering of *E. coli* for the production of aromatic amino acids at g/L levels [24].

In *E. coli*, DHS has been converted to muconic acid via PCA and catechol resulting in 36.8 g/L of muconic acid with a 22% yield from glucose [25•]. Key to achieving this yield was the introduction of a feedback resistant 3-deoxy-D-arabino-heptulosonic acid 7-phosphate (DAHP) synthase, which condenses PEP and E4P in the first committed step of muconic acid biosynthesis. Of note, in this strain the conversion of DHS to aromatic amino acids was blocked and cell growth required addition of aromatic amino acids and vitamins to the media. The same DHS to muconic acid pathway has been introduced in *S. cerevisiae* to produce 1.56 mg/L of muconic acid [26], and, in 2013, 141 mg/L of muconic acid with a 0.8% yield from glucose [27•]. Interestingly, the low mg/L titers were obtained despite successful implementation of similar metabolic engineering strategies as in *E. coli*, including relieving feedback inhibition of DAHP synthase, and deletion of glucose-6-phosphate dehydrogenase to force the carbon flux from glycolysis to the pentose phosphate pathway via the transketolase reaction, which is kinetically hindered *in vivo*, rather than glucose-6-phosphate dehydrogenase. More recently, the DHS to muconic acid pathway was introduced into a diploid *S. cerevisiae* strain carrying similar up- and down-regulations to increase the carbon flux to the shikimic acid pathway. Additionally, the diploid carried a 3-dehydroquinate dehydratase mutant (Aro: D1409A) that stopped conversion at DHS, reducing synthesis of the byproduct shikimate. When this strain was grown under anaerobic conditions to improve the activity of the oxygen sensitive protochatechuic decarboxylase, it produced 560 mg/L of muconic acid [28]. The *S. cerevisiae* results underscore the extensive regulation of aromatic amino acid biosynthesis in yeast, where limited pathway modifications do not result in the g/L titers seen in