De novo Biosynthesis of Adipic Acid from Lignin-derived Aromatics

Howard Willett[1], Joshua Mueller[1], Xinyuan He[1], Bin Ma[2], Jiantao Guo[2], Wei Niu[1]*

1. Department of Chemical and Biomolecular Engineering, University of Nebraska – Lincoln, Lincoln, NE 68588
2. Department of Chemistry, University of Nebraska – Lincoln, Lincoln, NE 68588 *Corresponding Author, wniu2@unl.edu

Background

Lignin is the second most abundant organic polymer in nature. Its efficient utilization plays a critical role in the viability and profitability of biorefineries that process lignocellulosic biomass Adipic acid is the commercially most important dicarboxylic acid in petrochemical industry. It has a market volume of 2.6 million tons per year with an annual demand growth forecast of 3-3.5% globally. Adipic acid is used almost exclusively in the manufacturing of nylon 6,6, one of the most widely used thermoplastics. To sustain the production of adipic acid from renewable resources also eliminates the utilization of a carcinogen, benzene, as the starting material and the emission of green house gas, nitrous oxide (N_2O) (Figure 1).

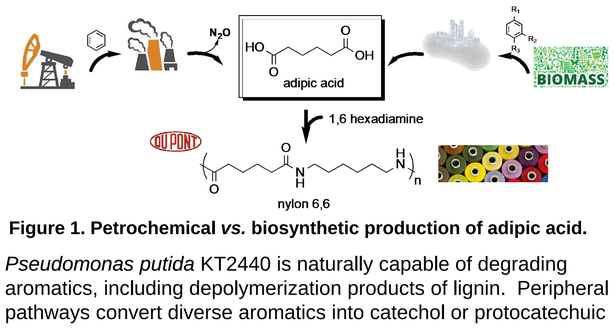


Figure 1. Petrochemical *vs.* biosynthetic production of adipic acid.

Pseudomonas putida KT2440 is naturally capable of degrading aromatics, including depolymerization products of lignin. Peripheral pathways convert diverse aromatics into catechol or protocatechuic acid intermediates, which are further funneled into the central metabolism through the β-ketoadipate pathway. We engineered an artificial adipic acid biosynthesis pathway into the KT2440 strain by hijacking the unique intermediate in aromatics degradation by KT2440.

Biosynthesis Design

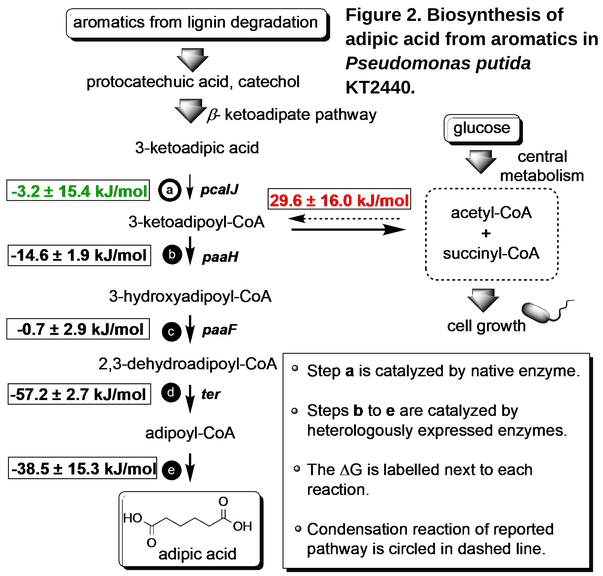


Figure 2. Biosynthesis of adipic acid from aromatics in *Pseudomonas putida* KT2440.

Probing Key Metabolic Node

β-Ketoadipoyl-CoA is the key metabolic node for directing carbon flux from the central metabolism into the biosynthesis of adipic acid. We studied its metabolism in KT2440 strains with genetic deletions.

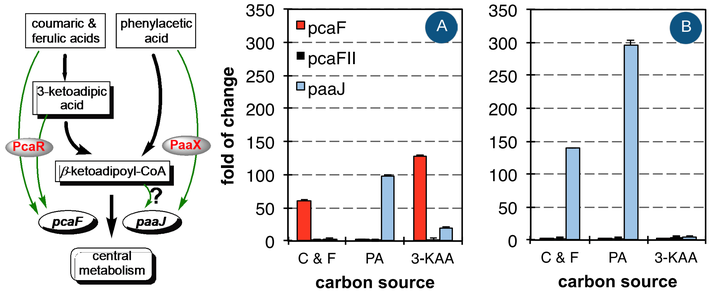


Figure 3. Gene expression analysis of KT2440 strains. A. wild-type. B. *ΔpcaF* strain. Abbreviations, C&F, coumaric acid and ferulic acid; PA, phenylacetic acid; 3-KAA, 3-ketoadipic acid.

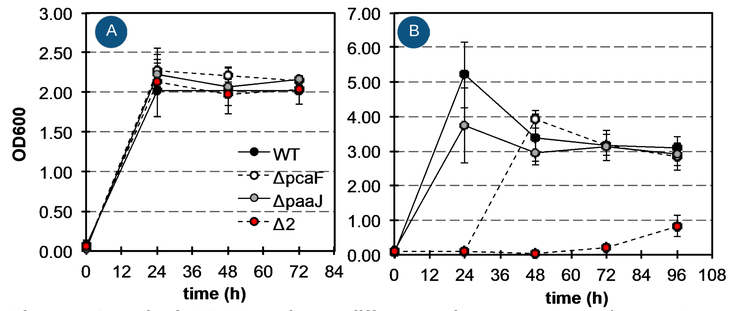


Figure 4. Growth of KT2440 strains on different carbon sources. A. glucose. B. coumaric and ferulic acids. Abbreviations, WT, wild-type; Δ2, KT2440 *ΔpcaFΔpaaJ*. All data are represented as the average of three trials with standard deviation as errors.

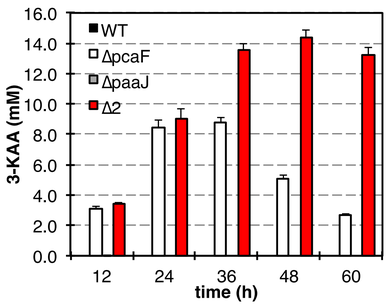


Figure 5. Accumulation of 3-ketoadipic acid (3-KAA) by KT2440 strains. Carbon source: glucose, coumaric and ferulic acids.

Engineering Carbon Source Utilization

Lignin hydrolysate is a mixture of sugar and aromatics. We examined effects of the deletion of global carbon utilization regulator, *crc*.

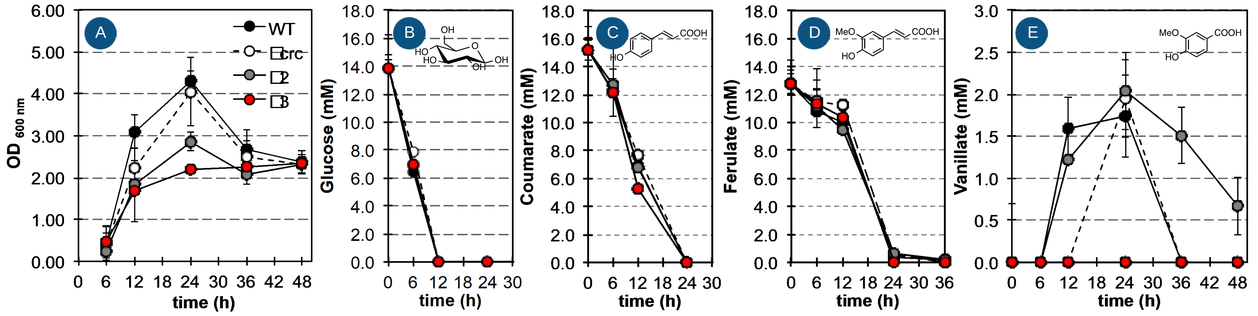


Figure 6. Carbon source utilization by KT2440 strains. Media contain glucose, coumaric acid and ferulic acid. A. cell growth. B-E, carbon source consumption. Abbreviations, Δcrc, KT2440 *Δcrc*; Δ2, KT2440 *ΔpcaFΔpaaJ* ; Δ3, KT2440 *ΔpcaFΔpaaJΔcrc*.

De novo Biosynthesis of Adipic Acid

The biosynthesis of adipic acid was studied using strains that expressed the heterologous pathway from plasmids or from the genome.

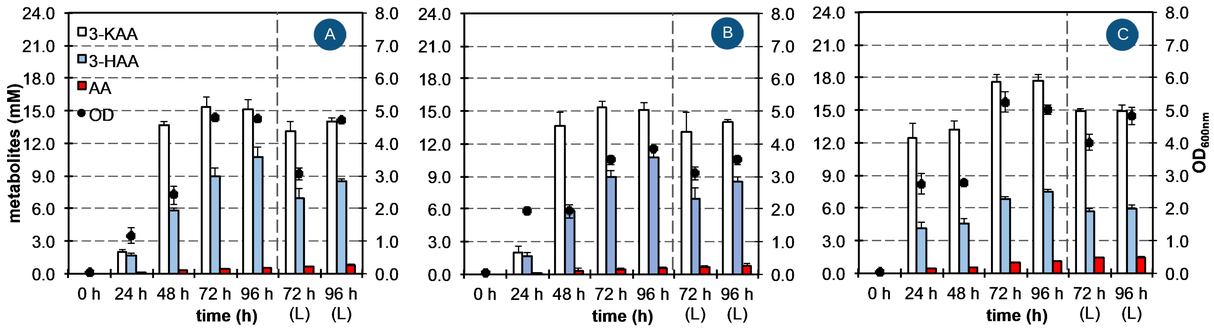
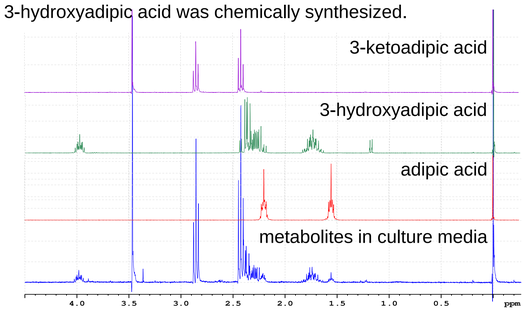


Figure 7. Metabolite accumulation by engineered KT2440 strains. A. Δ2/PT plasmid. B. Δ3/PT plasmid. C.KT2440 *ΔpcaF*::PT *ΔpaaJ*. Media contain glucose, coumaric and ferulic acids. Cells were cultured at 30 ° C, 250 rpm or 100 rpm (L) aeration rate. Abbreviation, AA, adipic acid.

^{1}H NMR Analysis

Identification and quantification of secreted metabolites were done by ^{1}H NMR analysis. The 3-ketoadipic acid was isolated from culture of Δ3 strain. The 3-hydroxyadipic acid was chemically synthesized.



Conclusions

- Pathway crosstalk is discovered in the metabolism of β-keto adipoyl-CoA. PaaJ enzyme in the phenylacetic acid degradation pathway also functions in the utilization of this CoA molecule.
- Elimination of the Crc regulator has minimal effect on the co-metabolism of sugar and aromatics, but reduces cell growth.
- Engineered KT2440 strains successfully produced adipic acid from model compounds of lignin degradation. The highest titer is 1.48 mM (6.4% yield) by using a pathway integration strain.
- Optimization of the carbon distribution at the β-ketoadipoyl-CoA node and the balancing of global currency molecules are the focus of further engineering efforts.

Acknowledgement

This research is supported by the Nebraska Center for Energy Science Research (NCESR).