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# Engineering *Pseudomonas putida* KT2440 to produce adipic acid from lignocellulosic biomass

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## Abstract

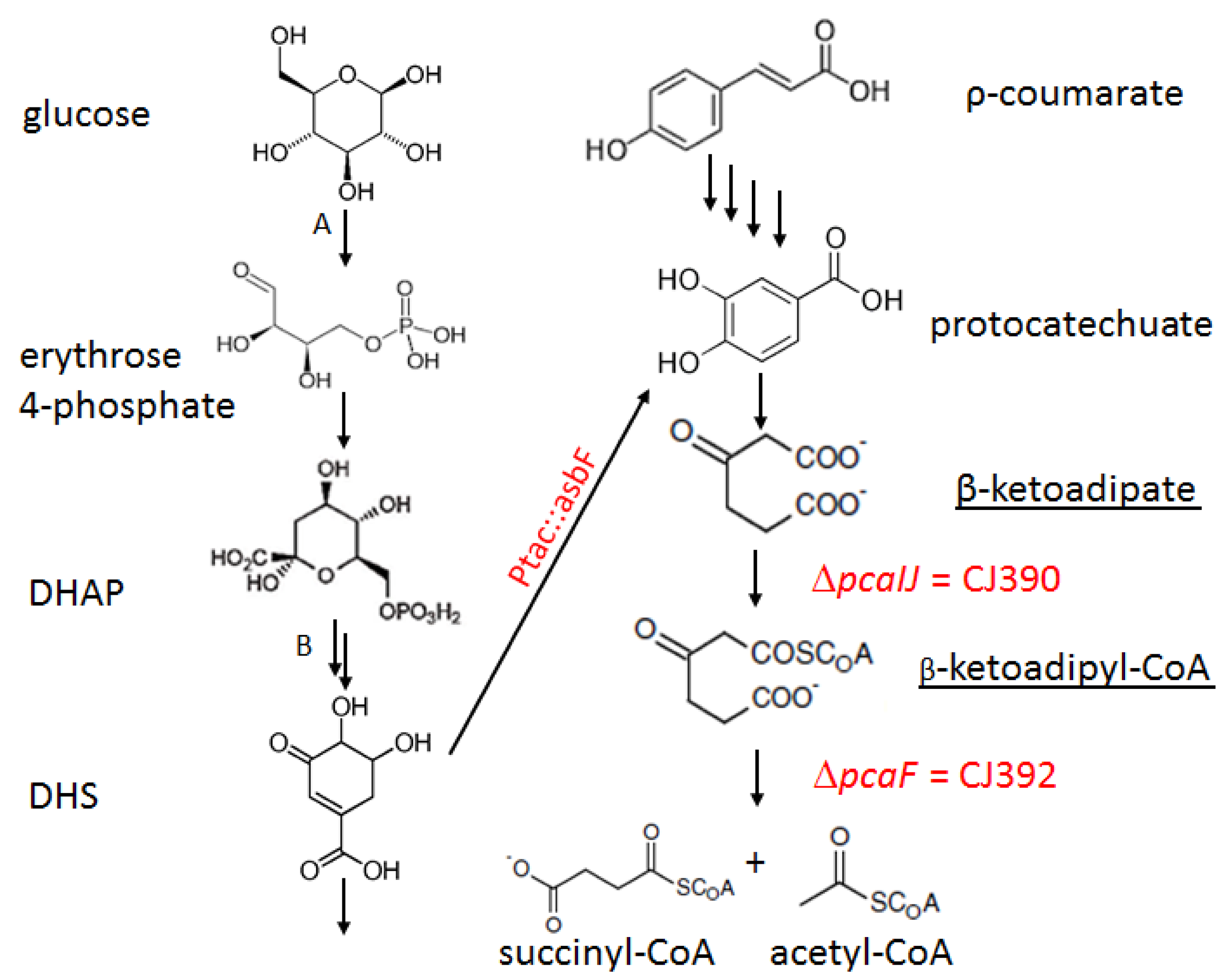
Adipic acid is an important industrial dicarboxylic acid that is primarily used as a precursor in the manufacturing of nylon, as well as plasticizers, food additives, and polyurethane resins, to name a few. With there being no significant occurrence in nature, 2.5 billion kilograms is manufactured annually from petroleum derived chemicals[1], an environmentally harmful process. By using plant components as starting material and engineering an industrially relevant bacterial host, we have fashioned an approach that is ecofriendly for making adipic acid. *Pseudomonas putida* KT2440 was metabolically engineered to produce adipic acid from chemically defined media containing sugar or aromatic compounds derived from lignocellulose. Using a multi-omic approach, we will be able to assess the metabolic, transcript, and protein profiles to identify bottlenecks in the engineered pathways. This allows for targeted modifications to the pathways to increase adipic acid titers. This work demonstrates that *P. putida* is a viable bacterial host for producing adipic acid from biomass and is a potential alternative to manufacturing adipic acid that does not rely on petroleum based feedstocks.

## Objective

Primary production of adipic acid involves reacting cyclohexane, a product generated from crude oil, with oxygen and then subsequently nitric oxide. This process generates the GHG nitrous oxide, which depletes the ozone layer. Bio-derived chemicals and fuels can offer cheaper and less green house gas-intensive alternatives to chemicals currently produced from petroleum. The primary objective is to establish and demonstrate the production of adipic acid in two industrially relevant hosts: *Pseudomonas putida* and *Streptomyces venezuelae*. The focus will be to optimize the production of adipic acid with a goal of demonstrating significantly improved titers and yields.

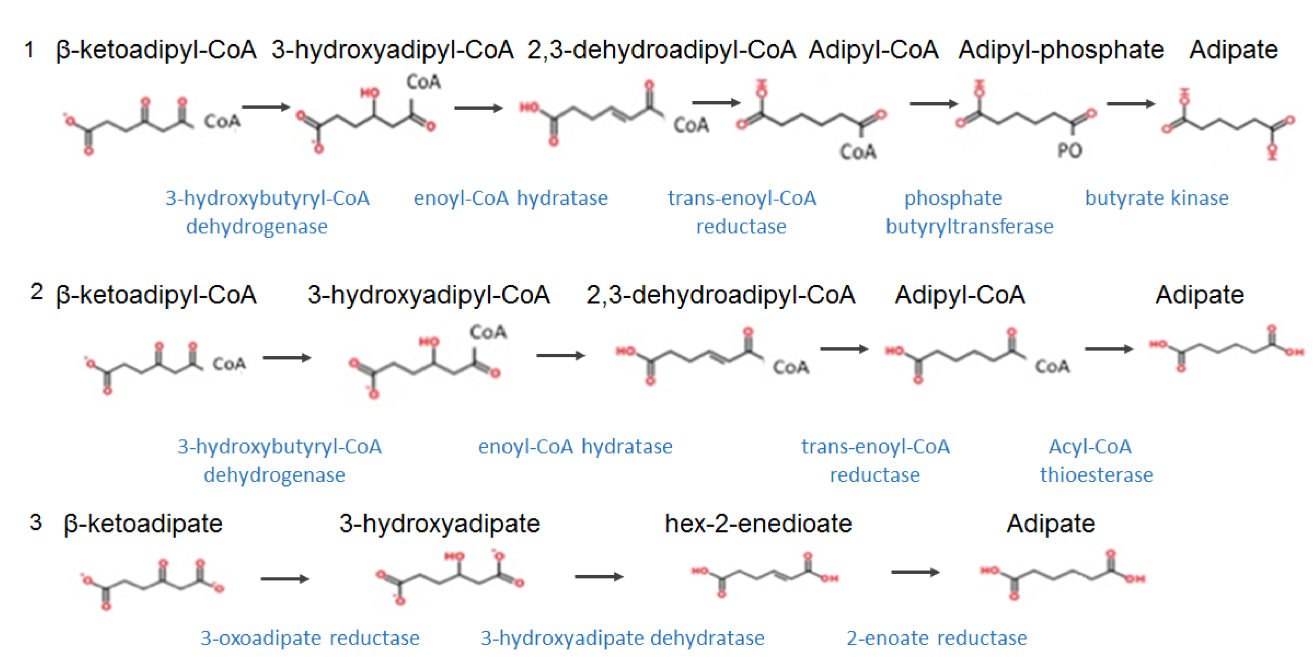
## Results

### Metabolism of sugar/aromatics to the precursors β-ketoadipate or β-ketoadipyl-CoA




A: pentose phosphate pathway, B: shikimate pathway. CJ390 Δ*pcalJ* ::Ptac *asbF*, CJ392 Δ*pcaF*::Ptac *asbF.*

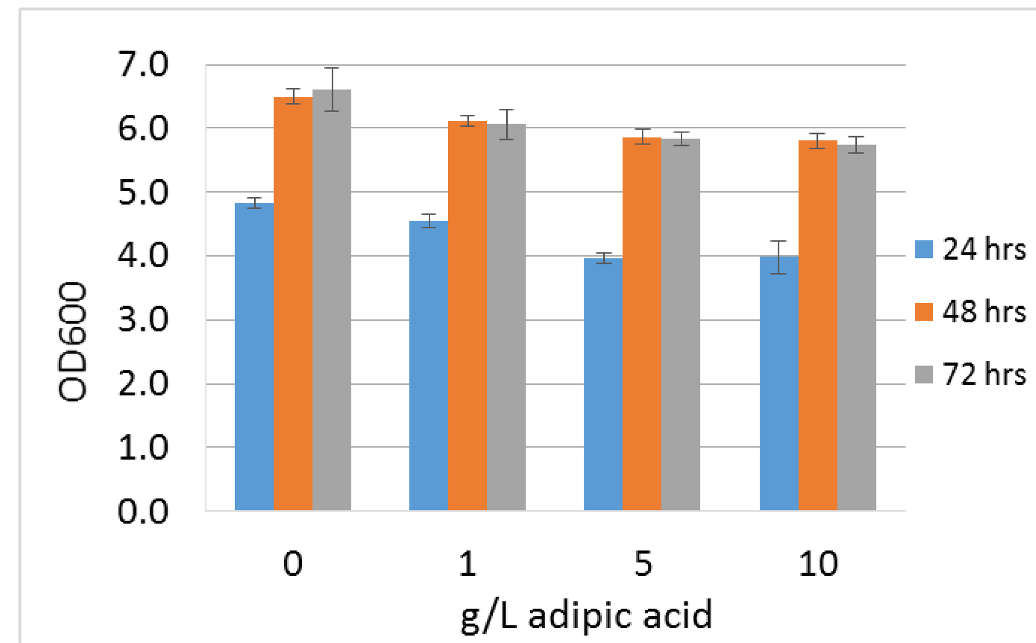
### Engineered pathways to produce adipic acid

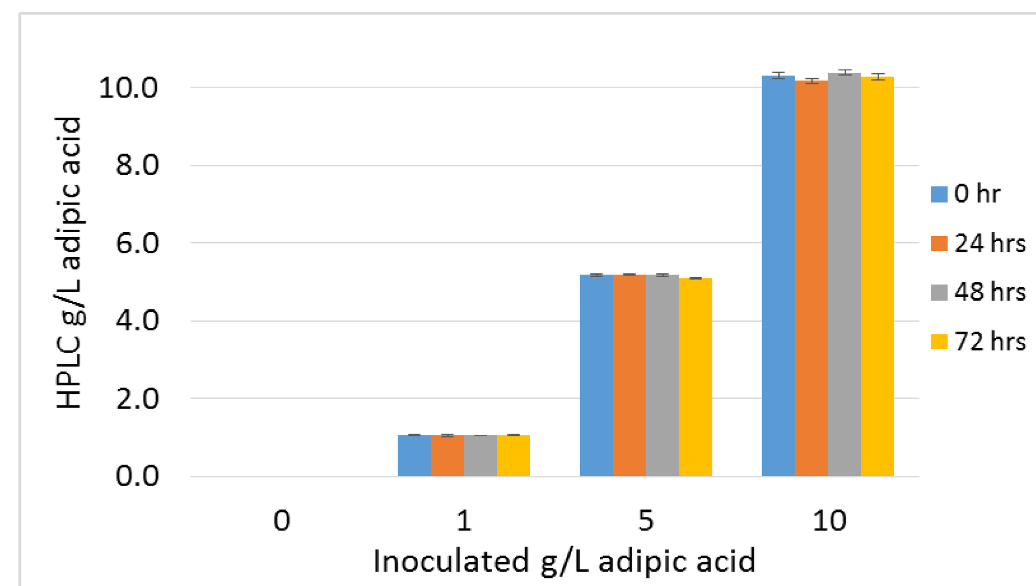



Three adipic acid pathways were designed to utilize intermediates from the native β-ketoadipate pathway. Strains were developed that can generate these intermediates from either aromatics or sugars derived from lignocellulose.

## Results (continued)

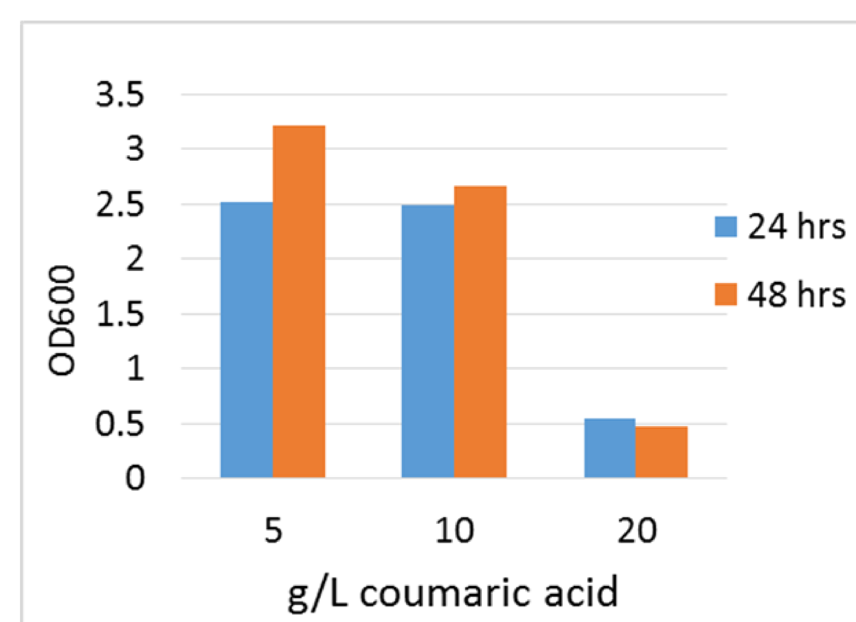
### *P. putida* does not consume and growth is not inhibited by adipic acid




*P. putida* was grown in test tubes with 10 ml of MOPS minimal media and 25 g/L glucose with varying adipic acid concentrations at 30°C and 200 rpm.

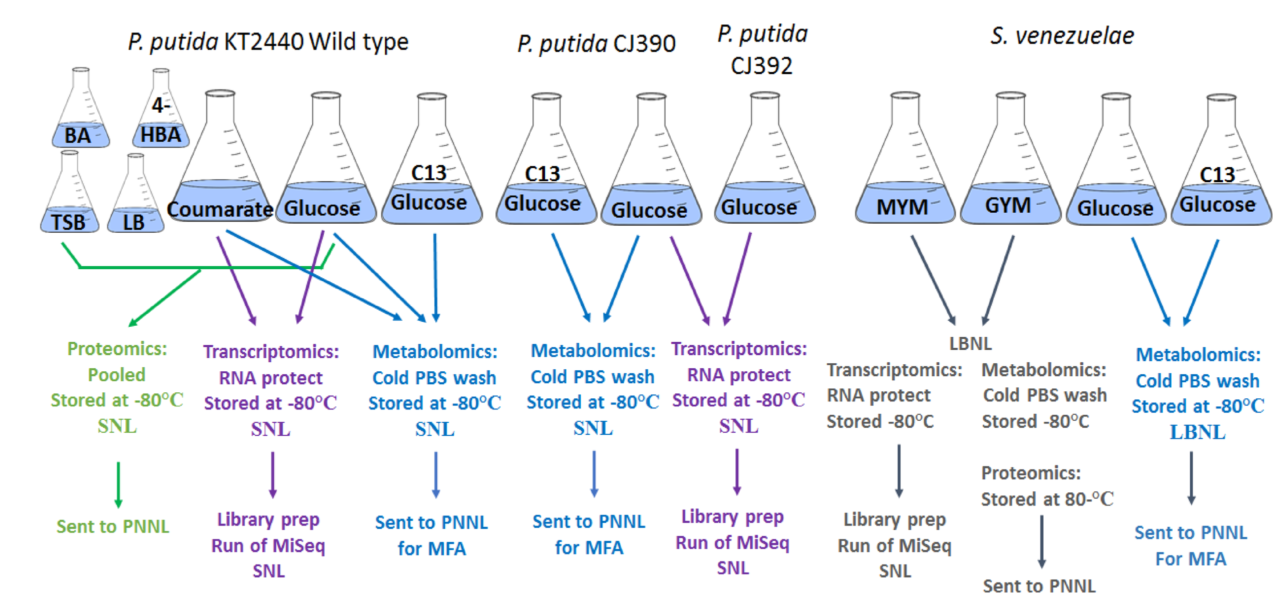
### *P. putida* can grow on coumarate as the sole carbon source




*P. putida* was grown in test tubes with 10 ml MOPS minimal media with varying concentration of coumarate at 30° C at 200 rpm.

## Results (continued)

### Sample preparation for transcriptomics, metabolomics and proteomics




## Conclusions

*P. putida* KT2440 can not use adipic acid as a sole carbon source (data not shown) and it does not consume adipic acid. *P. putida* growth is not inhibited by 10 g/L adipic acid, the desired titer. Coumarate and other lignin derived aromatic compounds (data not shown) can be used as sole carbon sources. These lignin derived aromatic compounds are degraded through the β-ketoadipate pathway, making them additional potential compounds in the adipic acid production process. To date, transcriptomics have been processed and analyzed for the wild type P. putida KT2440 strain and the modified parent strains CJ390 and CJ392. Proteomics, metabolomics, and labeled glucose metabolomic flux analysis have been processed at our collaborators facilities (PNNL).

## References

1. The Chemical Company, thechemco.com

## Acknowledgements

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