CRADA Final Report **Date:** May 2016
PI : Todd Pray

CRADA No. FP2152_____

LBNL Report Number: LBNL-1007111

1. Parties: Pareto Inc.. LBNL

2. Title of the Project: Production of chemical intermediate triacetic acid lactone (TAL) via bacterial fermentation of deconstructed lignin

3. Summary of the specific research and project accomplishments: (Were the goals of the CRADA achieved? Include relevant information but do not include proprietary or protected CRADA information.)

   The goals listed in this CRADA were achieved. We studied the feasibility of growing *A. baylyi* strain on lignin-based monomers as a feedstock with defined and complex media compositions. We performed Biolector studies for process optimization and toxicity studies. We also conducted shake flask fermentation to study monomeric lignin conversion to Triacetic acid lactone (TAL). At 2L bioreactor scale, fed-batch fermentation was implemented where lignin was used as a feed for TAL production and product toxicity studies.

4. Deliverables:

Deliverable Achieved	Party (LBNL, Participant, Both)	Delivered to Other Party?
Complete product toxicity study at shake flask scale, the study shows that Triacetic acid lactone (TAL) does not inhibit growth of *A. baylyi* at concentrations that are consistent with high-yielding fermentation	LBNL	Yes
Conducted at shake flask and 2L scale fermentation and the result showed that *A. baylyi* grow using monomers of deconstructed lignin as a carbon source	LBNL	Yes
Engineered the relevant yeast strains pathways and enzymes to generate TAL	Pareto	Yes

5. Identify publications or presentations at conferences directly related to the CRADA? None at the time of this report.

6. List of Subject Inventions and software developed under the CRADA:

(Please provide identifying numbers or other information.)

7. A final abstract suitable for public release:
(Very brief description of the project and accomplishments without inclusion of any proprietary information or protected CRADA information.)

Triacetic acid lactone (TAL) is demonstrated to be food preservatives additive, fragrances, plastics, and carbon neutral alternative to petroleum based chemicals. TAL can be further modified to drop-in replacement for petrochemicals using proprietary Pareto technology. This project examined the feasibility of growing of *A. Baylyi* strain using lignin based monomers as a feedstock, in defined and complex media. The growth of *A. Baylyi* was measured using biomass growth, sugar and lignin consumption, and TAL production. A high proportion of the overall cell growth was found to have taken place after the glucose was consumed, and lignin monomers were added. The quantities of TAL produced were markedly lower for defined media than for complex media. The process was successfully scaled up from 2 mL to 50mL and then 2L bioreactor.

8. Benefits to DOE, LBNL, Participant and/or the U.S. economy.

Developed *A. Baylyi* fermentation process at shake flask and 2L scales to produce any product, including TAL. Demonstrated the production of TAL, a versatile biorenewable molecule, by converting lignin based monomers. TAL can be used as a platform chemical for higher value, more specialized purposes such as piperylene, a component in synthetic plastics, and sorbic acid, a food preservative, without relying on comparatively expensive sugars.

9. Financial Contributions to the CRADA:

DOE Funding to LBNL	$20,000
Participant Funding to LBNL	$0
Participant In-Kind Contribution Value	$5,000
Total of all Contributions	$25,000

January 23, 2017

SUMMARY REPORT

Objective

The objective of this study was to test the feasibility of using *Acinetobacter baylyi* as a host strain for production of Triacetic acid lactone (TAL) molecules. *A. baylyi* was never used as a host strain for the production of an industrial chemical. Moreover, we wanted to test if *A. baylyi* can consume lignin-based monomers and then convert them to TAL as well. Process optimization in well plate, shake flask and 2L bioreactors along with TAL toxicity studies were planned.

ABPDU Project Team: Fre Tachea, Fabrice Masson, Chyi-Shin Chen, Qian He, Deepti Tanjore, Ning Sun, Todd Pray

Pareto Project Team: Jamie Bacher

Deliverables

1. TAL product toxicity study

Complete product toxicity study at shake flask scale, showed that TAL does not inhibit growth of *A. baylyi* up to 6.1 mg/L concentrations.

2. Fermentation

Shake flask and 2L scale fermentations indicated that *A. baylyi* can consume glucose, coumaric acid, and vanilic acid as the carbon source and produce 6.1 mg/L concentration TAL. The highest optical density 7.2, was observed in the LB reactor and 1.8 in the M9A reactor. A high proportion of the overall cell growth was found to have taken place after the glucose was consumed, and lignin monomers were added. The quantities of TAL produced were markedly lower for M9A than for LB, but showed improvement over the immeasurably small quantity produced in regular M9 media.

3. Generation of TAL

Engineered the relevant yeast strains pathways and enzymes to generate TAL.

Summary

Triacetic acid lactone (TAL) is demonstrated to be food preservatives additive, fragrances, plastics, and carbon neutral alternative to petroleum based chemicals. TAL can be further modified to drop-in replacement for petrochemicals using proprietary Pareto technology. This project examined the feasibility of growing of *A. Baylyi* strain using lignin based monomers as a feedstock, in defined and complex media. The growth of A. Baylyi was measured using biomass growth, sugar and lignin consumption, and TAL production. A high proportion of the overall cell growth was found to have taken place after the glucose was consumed, and lignin monomers were added. The quantities of TAL produced were markedly lower for defined media than for complex media. The process was successfully scaled up from 2 mL to 50mL and then 2L bioreactor.