

Title: Discovery of Bacterial Species and Molecular Mechanisms Driving Growth on the Plant Root Metabolite Sorgoleone

Authors: Ryan McClure^{1*}(Ryan.McClure@pnnl.gov), Ritu Shrestha¹, Aaron Ogden¹, Henri Baldino,¹ Andrew Wilson,¹ Andrew Frank¹, Jared Kroll¹, Vivian Lin¹, Yuliya Farris¹, Yasuhiro Oda², Bill Nelson¹, Joshua Elmore¹, Caroline Harwood², **Rob Egbert**¹

Institutions: ¹Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352. ²Department of Microbiology, University of Washington, Seattle, WA 98195

Website: <https://genomicscience.energy.gov/research/sfas/pnnlbiosystemsdesign.shtml>

Project Goals: The Pacific Northwest National Laboratory Persistence Control Scientific Focus Area aims to gain a fundamental understanding of factors governing the persistence of engineered microbial functions in rhizosphere environments. From this understanding, we will establish design principles to control the environmental niche of native rhizosphere microbes. In our first funding period, we are examining the efficacy of genome reduction and metabolic addition to plant root exudates in environmental isolates as persistence control strategies using the bioenergy crop sorghum and defined microbial communities as a model ecosystem. Effective persistence control will lead to secure plant–microbe biosystems that promote stress-tolerant and highly productive biomass crops.

Abstract

The safe and responsible deployment of engineered microbes to promote bioenergy crop health demands biocontainment strategies that precisely control the environmental niche of the microbe. To investigate the potential to control the persistence of an engineered microbe through metabolic addition to a plant root exudate compound, we have focused on identifying bacteria and genes responsible for use of the plant metabolite sorgoleone for growth. Sorgoleone is a hydrophobic allelochemical unique to the bioenergy crop sorghum [1]. To date, no studies have identified a microbe or catabolic pathway capable of degrading sorgoleone, though sorgoleone is clearly mineralized by the soil microbiome [2]. Here we report three bacteria from different genera that use sorgoleone as a sole carbon source (a *Burkholderia*, *Pseudomonas*, and *Acinetobacter* species). We discovered these microbes by purifying sorgoleone from seedling roots and enriching and isolating bacteria that use sorgoleone as a sole carbon source from sorghum field soil. This was followed by genome sequencing, and growth characterization in synthetic sorgoleone growth media. Each microbe has unique growth kinetics on sorgoleone and some also use other plant-derived compounds for growth. To identify genes associated with sorgoleone utilization, we performed differential transcriptomics experiments on each bacterial strain grown on sorgoleone and acetate. The three strains that we investigated have at least seven proteins in common that we hypothesize to be required for sorgoleone degradation. These are likely involved in the degradation of the quinone ring and alkene tail of sorgoleone. Mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy analysis of spent media from each bacterial strain indicated that no sorgoleone remained in the media after growth, though further investigation is required to identify possible strain-specific byproducts. The discovery of these sorgoleone degrading bacteria

opens up new avenues of research into how plant exudates shape their bacterial microbiomes. Further, we anticipate these findings will lead to the first demonstration and characterization of metabolic addiction of a microbe to a plant root exudate for biocontainment in a field setting.

References

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[2] Gimsing, A. L.; Bælum, J.; Dayan, F. E.; Locke, M. A.; Sejerø, L. H.; Jacobsen, C. S. Mineralization of the allelochemical sorgoleone in soil. *Chemosphere* 2009, 76, 1041-1047, <https://dx.doi.org/10.1016/j.chemosphere.2009.04.048>.

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