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Q4 Target: Report on capabilities that could accelerate the design and testing of microbial isolates with new beneficial functions.

Executive Summary

As PNNL's contribution to the Secure Biosystems Design program, the Persistence Control Science Focus Area (PerCon SFA) aims to use high-throughput genetic and bioinformatic tools to understand, predict, and control plant-microbe interactions in the rhizosphere to benefit the bioenergy crop sorghum. Specifically, we investigate how microbes survive in the rhizosphere to engineer bacterial isolates native to the sorghum rhizosphere for metabolic dependence on root exudate compounds. These metabolic addiction phenotypes will promote growth and activity in the plant rhizosphere and prevent proliferation away from the plant. Pursuing the secure biocontainment aims of our research will position the US to responsibly harness the full potential of rhizosphere microbiomes to support ecosystems that fulfill national goals for a resilient bioeconomy through sustainable bioenergy cropping systems with potential to durably deposit atmospheric carbon in soils.

In the inaugural funding cycle of the SFA, we have discovered genes responsible for bacterial catabolism of two root exudate compounds that inhibit biological nitrification for the bioenergy crop sorghum. Full elucidation of these pathways for sorgoleone and methyl 3-(4-hydroxyphenyl) propionate (MHPP) will serve two aims: (1) provide better understanding of mechanisms and dynamics of biological nitrification inhibition (BNI) in soil and rhizosphere environments, and (2) establish means to better control the persistence of engineered microbes in the rhizosphere by creating tunable metabolic niches for plant-specific metabolites. Our advances will accelerate the design and testing of microbial isolates with beneficial functions in the rhizosphere of bioenergy crops. Specifically, two major SFA advances towards understanding BNI degradation and controlled rhizosphere persistence are summarized below:

1. We transferred a novel route for catabolism of phenylpropanoid methyl esters (PPME), a family of BNI compounds that includes MHPP, to sorghum rhizosphere bacteria [1].

2. We discovered a conserved gene cluster that is essential for the catabolism of sorgoleone and shared by three new sorghum rhizosphere isolates from distinct genera [2].

Establishing capabilities for catabolism of plant-specific root exudates could accelerate the design and testing of microbial isolates with new beneficial functions in multiple ways. First, the ability to tune rhizosphere-specific metabolic niches for bacteria will likely enable us to boost the relative abundance of microbes that provide beneficial functions only in the presence of target bioenergy crops. Further, a better understanding of biological nitrification dynamics in soil and rhizosphere environments may inform strategies to control the nitrogen cycle and reduce greenhouse emissions. One potential way to alter nitrogen cycling dynamics is to outcompete or promote the growth of native microbiome members by eliminating or augmenting BNI compounds in the rhizosphere.

Background

The potential to employ emerging genetic and computational tools to design and securely deploy synthetic rhizosphere microbiomes, defined communities of microbes that colonize plant roots and benefit plant growth, offers tremendous opportunity to realize highly productive and stress-tolerant biomass cropping systems. A critical obstacle to realizing this vision is understanding fundamental principles of microbiome persistence in complex environments, notably amid the dynamics of root exudation and microbial colonization. Further, we need predictive tools to assess the risks associated with the deployment or unintended release of engineered microbes in plant and soil ecosystems and to mitigate those risks through effective strategies to control persistence. To meet these challenges, high-throughput genetic manipulation and bioinformatic tools provide a platform to elucidate the genetic elements underpinning rhizosphere community function, to create beneficial communities, and to control their environmental persistence.

The Persistence Control SFA investigates approaches to reshape the environmental niche of native sorghum rhizosphere bacterial isolates by creating an engineered metabolic addiction to root exudate compounds. We aim to understand the genes and networks that underpin environmental persistence of microbiomes to create dependencies of engineered microbes on plants such that the microbes can no longer persist in the environment in the absence of the target plant (**Fig. 1**). Our strategy to control the environmental persistence of engineered microbes is to establish metabolic addiction to plant root exudate compounds by installing compound-specific catabolic pathways while removing pathways used for scavenging nutrients in soil microbiome environments. This strategy requires exquisite control of genome content to, for instance, eliminate metabolic niches that allow survival cross-feeding of nutrients by other microbiome members as well as to introduce complete pathways to thrive in the plant rhizosphere, but not in the rhizosphere of other plants. It also demands a deep understanding of plant or microbe interactions that could overcome control through complementation.

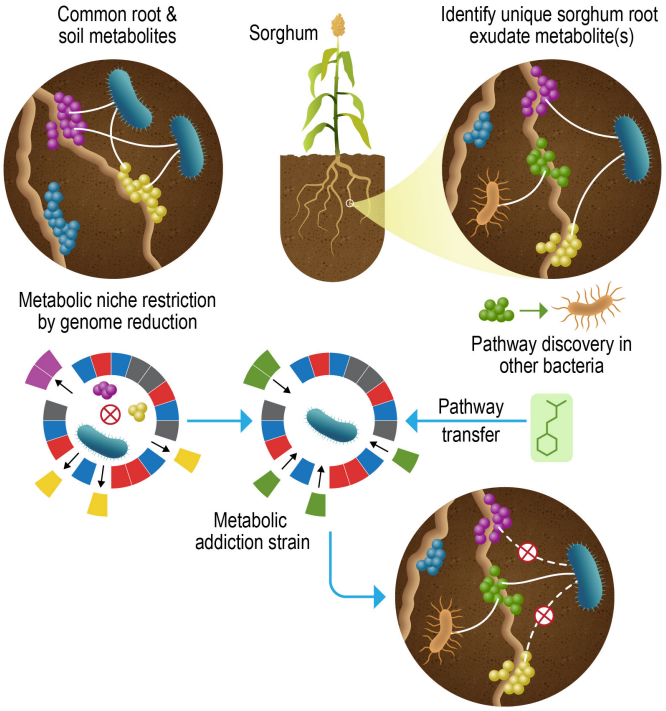

Fig. 1. The Persistence Control SFA aims to control the proliferation of engineered microbes by establishing metabolic addiction of microbe to a bioenergy crop supported by the engineered microbiome.

To help accomplish the goals of the Secure Biosystems Design program, the PerCon SFA team draws expertise from across three DOE Office of Science National Laboratories – Pacific Northwest National Laboratory, Lawrence Berkeley National Laboratory, and Oak Ridge National Laboratory – and three Universities: the University of California Berkely, the University of California Santa Barbara, and the University of Washington. To understand the genes and networks that control environmental fitness we have developed and integrated research strategies that draw from our team's expertise in functional genomics, synthetic biology, microbial ecology, chemical biology, bioinformatics, machine learning, and plant-microbe interactions. In this report, we share a selection of capabilities developed for persistence control that accelerate the design and testing of microbial isolates with new beneficial functions.

Transferring a novel root exudate catabolism pathway to sorghum rhizosphere isolates

Agriculture is a major source of greenhouse gases (GHG) on the planet [3]. Although application of N-fertilizers increases crop yields [4], a large proportion of applied N-fertilizer is ultimately lost to the atmosphere as N_2O, a potent GHG (**Fig. 2**) [5, 6]. Nitrifying microbes catalyze the oxidation of reduced forms of nitrogen (NH_3, NH_4^+) into nitrate (NO_3^-) and NO_3^- is converted to the potent greenhouse gas N_2O by denitrifying bacteria that are widely prevalent in soil [7]. One approach to reduce nitrification in agriculture is the co-application of chemical nitrification inhibitors with fertilizers [8, 9]. These chemicals generally inhibit activity of the highly conserved ammonium oxidase (AMO) and hydroxylamine oxidoreductase (HAO) enzymes that perform biological nitrification. These enzymatic functions are limited to a small collection of archaeal (AOA) and bacterial (AOB) lineages, and blocking these activities inhibits the growth of these microbes. Another approach is to limit the application of chemical fertilizers. For this, researchers and several companies are developing engineered microbial and plant [10] systems that can be deployed in agricultural soils to enhance fixation of elemental nitrogen (N_2) into biologically available nitrogen (NH_3) [10-13]. Supplementing soils with these engineered N_2-fixing bacteria can substantially reduce the amounts of chemical N-fertilizer required for robust crop yields. While each approach is effective, a deeper understanding of the mechanisms driving biological nitrogen cycles is necessary to address the complex challenge of reducing nitrification.

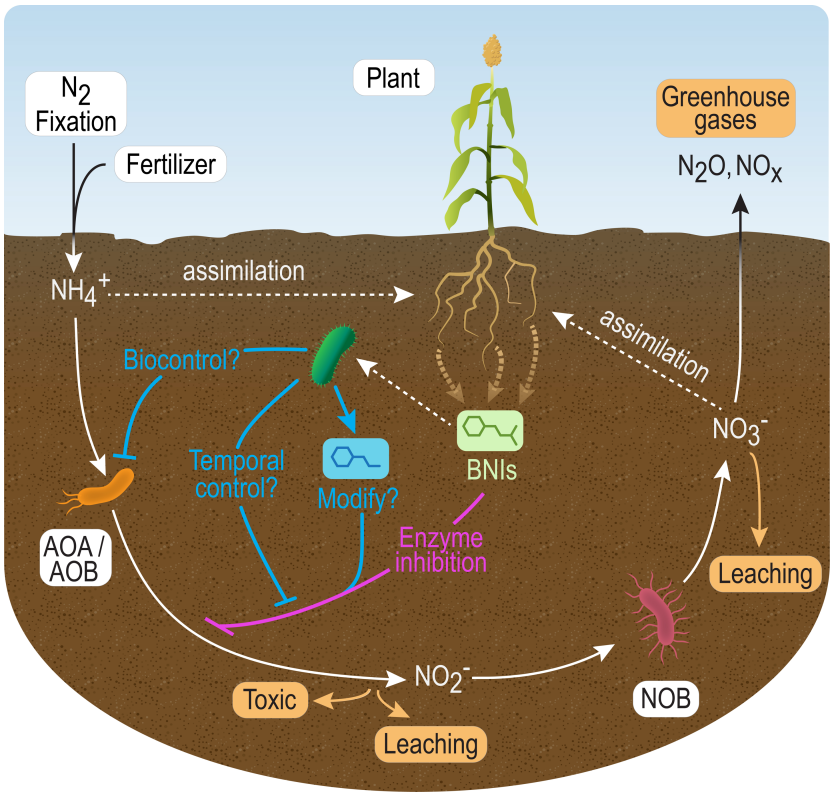

Fig. 2. Biological inhibition of the nitrification cycle. Ammonium oxidizing archaea (AOA) and bacteria (AOB) convert excess N in the soil into forms that leach into soils and are released as nitrogenous greenhouse gases. Plant roots exude biological nitrification inhibitor chemicals (BNIs) to prevent this loss of soil nitrogen. To date the main known BNI mode-of-action is the inhibition of ammonia oxidizing enzymes. A more complete understanding of the nitrogen cycle will require investigation of microbes capable of consuming BNIs. For example, enriched microbes may enhance inhibition through biocontrol of AOA/AOB growth or exert temporal control over enzyme inhibition through mineralization or modification of BNI compounds.

Currently, biological nitrification inhibitors (BNIs) are primarily identified using *in vitro* enzyme activity assays [14] or measurements of ammonium and nitrate in soils supplemented with BNI compounds [15]. However, these and microbial abundance assays (e.g., 16s rRNA sequencing) have been suggested to be insufficient to understand the driving mechanisms of BNI [15] and have led to potentially incorrect conclusions that BNI compounds function exclusively through inhibition of AMO or HAO enzyme activity. The diversity of BNI compounds and knowledge that these compounds have other functions in the environment [16, 17] (e.g., herbicidal activity of the BNI sorgoleone [17]) suggests that BNI compounds may have other targets [18].Plants respond to various stress conditions by exuding

compounds into the rhizosphere that encourage growth of microbes with beneficial functions (e.g., N-fixation, antifungal activity) [19-21]. Similarly, exuded BNIs may perform secondary functions, such as encouraging growth of microbes that directly or indirectly inhibit growth of ammonium oxidizing microbes (**Fig. 2**).

The Persistence Control SFA has identified microbes that can use phenylpropanoid methyl esters (PPMEs), a class of BNIs, as growth substrates. This class of phenolic BNI is produced by a number of plants [16, 22, 23].We then characterized a metabolic pathway for PPME degradation and identified a novel enzyme that performs the first step in PPME catabolism. This step permits PPME to flow into a known pathway for phenylpropanoid metabolism. Finally, we show this metabolic pathway can be transferred into heterologous hosts.

Phenylpropanoid methyl esters (PPME) function as biological nitrification inhibitors [22, 23], but their mechanisms of action and how they are ultimately removed from the environment are not fully understood. Pseudomonads can use phenylpropanoids as carbon sources to support growth [24-26], and so we reasoned that some may also be able to use PPMEs as carbon sources. To identify organisms capable of degrading PPMEs we evaluated the ability of several *Pseudomonas* environmental isolates to grow on these compounds (**Fig. 3**). These isolates include three recently isolated endophytes of *Sorghum bicolor*, a well-established producer of multiple BNIs, including methyl 3-(4-hydroxyphenyl) propionate (MHPP) – the first PPME found to inhibit biological nitrification [22]. We used the Genome Taxonomy Database toolkit to assign taxonomic classifications for each endophyte [27]. Strain TBS10 was previously classified as *Pseudomonas frederiksbergensis* [28]. Strains TBS28 and TBS49 lacked sufficient similarity with existing species to assign a classification at the species level. TBS49 is related to the *P. putida* group, and we refer to it as *Pseudomonas sp.* TBS49. We assigned strain TBS28 the provisional species name *Pseudomonas facilor*.

When we evaluated each strain for use of either glucose or *trans*-*p*-coumarate as a sole carbon source (**Fig. 3a**), we found that all strains grew robustly with glucose, but only KT2440, SBW25, TBS10, and TBS28 were able to use *p*-coumarate. We found that each of these strains harbor genes encoding an established pathway for *p*-coumarate degradation. A phenylpropanoid commonly observed in plant root exudates, *p*-coumarate is structurally similar to PPMEs.

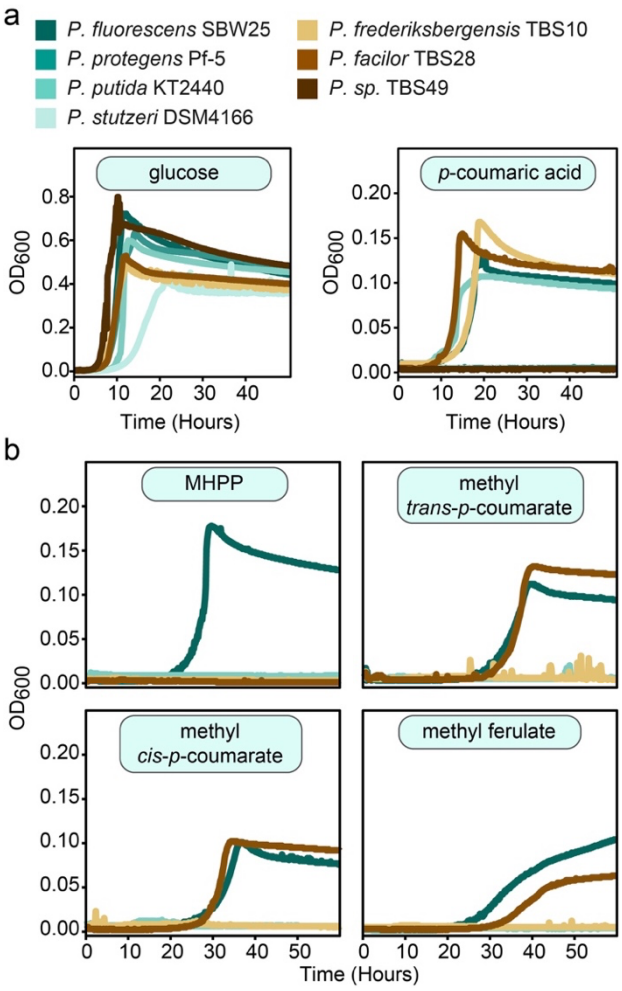

Fig. 3. A subset of environmental Pseudomonas isolates can utilize phenylpropanoid methyl esters as carbon sources. Microtiter plate cultivation assays comparing growth of environmental Pseudomonads using (a) two control carbon sources and (b) four phenylpropanoid methyl esters. Experiments in panel b only include the four Pseudomonads that grew with *p*-coumaric acid in panel a. Assays were performed with MME medium containing either 10 mM (glucose) or 2.5 mM (all others) of the indicated carbon sources. Each panel contains a single representative curve from one of three biological replicates.

The four strains that used *p*-coumarate were further evaluated for their ability to grow with four different PPMEs (**Fig. 3b**) and phloretic acid as sole carbon sources. The four PPMEs that we used were methyl *trans-p*-coumarate, methyl *cis-p*-coumarate, methyl ferulate, and MHPP. Phloretic acid is a potential intermediate of MHPP catabolism. SBW25 used all four PPMEs as carbon sources, TBS28 used all except MHPP, and the remaining two Pseudomonads were unable to use any of the PPMEs. While unable to use any of the PPMEs, KT2440 grew with phloretic acid. Because it could use all tested PPMEs for growth, we used SBW25 to investigate the metabolic pathway.

To identify genes involved in catabolism of PPMEs, we used high-throughput random barcode transposon-site sequencing (RB-TnSeq). By measuring the change in relative abundance of barcoded transposon mutants within a pooled population, each gene's contribution to fitness can be quantified in parallel. Negative gene-level fitness values of -1.8 or lower indicate that the gene is important for growth in the test condition [29-31] and positive fitness values of 1.8 or greater indicate that abolishing expression of the encoded protein improves growth in the test condition [32, 33]. For this work, we generated a barcoded transposon mutant library in *P. fluorescens* JE4621, which is a SBW25-derivative containing a genome integrated poly-*attB* cassette that enables use of serine recombinase-assisted genome engineering (SAGE) [28].

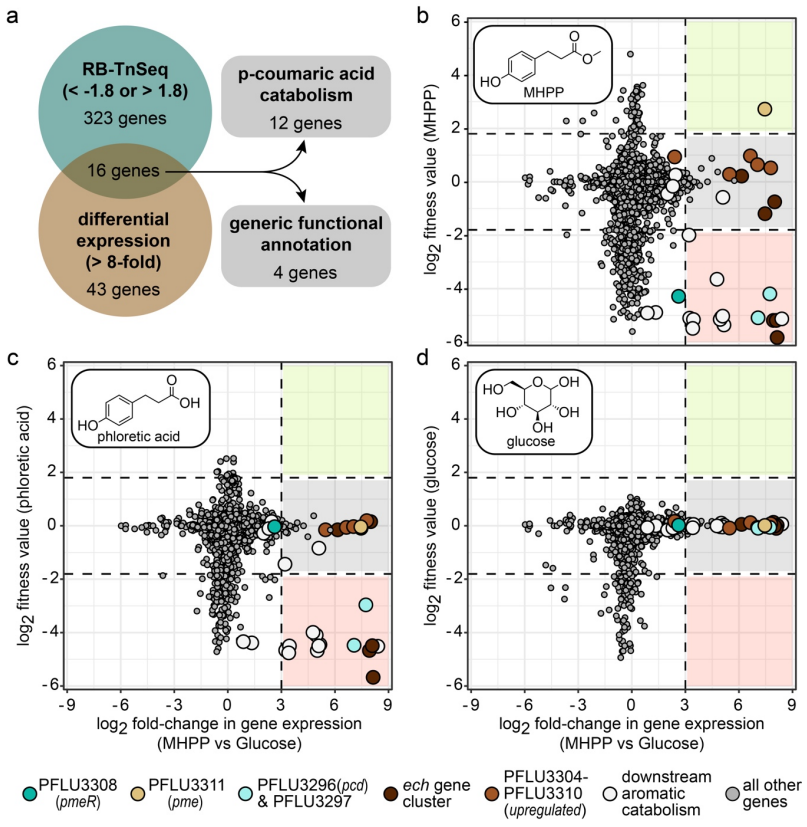

Fig. 4. Co-evaluation of genome-wide fitness and differential gene expression reduces search space for novel pathway genes. (a) Venn diagram showing overlap between genes with substantial fitness values when SBW25 is grown using MHPP and genes that are more highly expressed when SBW25 is grown with MHPP compared to glucose. (b-d) Plots comparing differential expression versus mean RB-TnSeq fitness values, with fitness values from cultures grown with (b) MHPP, (c) phloretic acid, or (d) glucose. Positive and negative differential expression values indicate higher expression during growth using MHPP and glucose as carbon sources, respectively. Dots indicate genes encoding the putative PPME-sensitive *pmeR* transcription factor (dark teal), phenylpropanoid methyl esterase (light brown), putative phloretoyl-CoA dehydrogenase and putative β-ketothiolase (light teal), other genes in the *ech* gene cluster (dark brown), other genes in the MHPP-upregulated gene cluster (medium brown), downstream aromatic catabolic pathway gene clusters (white), and all other genes (dark gray).

Genome-wide profiling of cellular fitness on MHPP compared to glucose revealed many genes with substantial positive and negative fitness impacts. In fact, too many genes contributed to fitness during growth on this compound to reasonably evaluate on an individual basis for their potential roles in MHPP degradation. Overall, the exceptionally high number of genes with either positive or negative fitness

impacts on MHPP catabolism suggests that in addition to serving as a carbon source, this compound may impact other aspects of cellular physiology (e.g., through the formation of toxic intermediates). Thus, we sought to further clarify the set of target genes important for MHPP catabolism by co-evaluating genes that contribute to fitness with those that are differentially expressed in transcriptomics experiments.

We measured transcriptomic profiles of SBW25 cells growth with glucose or MHPP as carbon sources. We identified 43 genes that were upregulated by at least 8-fold in MHPP cultures versus glucose cultures. Genes in the *ech* gene cluster (PFLU3296-3303), which includes genes encoding enzymes involved in *p*-coumaric acid catabolism, were expressed at very high levels during growth on MHPP. We also identified a second large cluster of genes (PFLU3304-3311) that was highly expressed in MHPP-grown cells. While most of the *ech* gene cluster is highly conserved between our four *p*-coumarate catabolizing Pseudomonads at both the level of protein sequence and gene synteny, the second gene cluster is only partially represented in the sorghum isolates and completely absent in KT2440.

The combination of genome-wide functional genomics data, differential transcriptomics data, and the generation of single-gene mutants in SBW25 allowed us to devise a putative catabolic pathway for PPMEs (**Fig. 5**). First, phenylpropanoid methyl esterase, encoded by the PFLU3311, hydrolyzes the PPME, releasing an aromatic acid and methanol. The aromatic acids are then ligated with CoA by feruloyl-CoA synthetase and processed through parallel pathways (**Fig. 5** – brown highlight). Ferulic acid and *p*-coumaric acid are subsequently metabolized into TCA cycle intermediates via established pathways. In our proposed pathway phloretoyl-CoA, the product of CoA ligation to phloretic acid, is subsequently oxidized by phloretoyl-CoA dehydrogenase, which is the product of *pcd* (PFLU3296), into *p*-coumaroyl-CoA where it enters the pathway of *p*-coumaric acid catabolism.

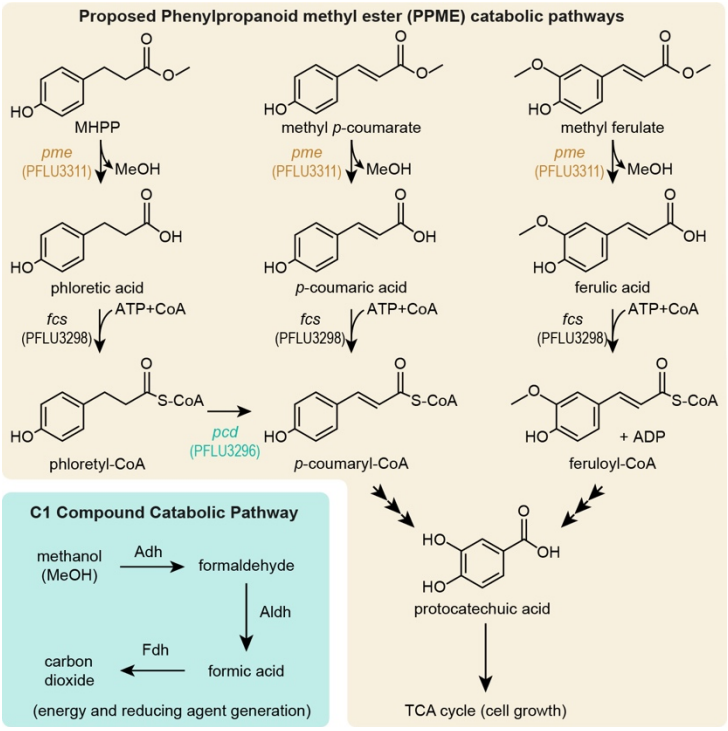

Fig. 5. A proposed pathway for PPME catabolism. Proposed pathways for catabolism of several different PPMEs. Brown highlighted pathways follow catabolism of the phenylpropanoid moeity following an initial de-esterification step, and the teal highlighted pathway is a standard C1 metabolic pathway that follows the MeOH moeity released upon de-esterification. Unknown Adh (alcohol dehydrogenase), aldehyde dehydrogenase (Aldh), and formate dehydrogenase (Fhd) enzymes are indicated in the teal highlighted C1 pathway.

To confirm the proposed pathway and demonstrate the transfer of a BNI catabolism pathway to a naïve microbial host, we introduced PPME pathway genes into *Pseudomonas* strains KT2440, TBS28, and TBS10, each of which contain a subset of the genes we identified contribute to PPME catabolism. We found that the genes PFLU3311 and PFLU3296 indeed provide these strains with a PPME catabolic capability (**Fig. 6**).

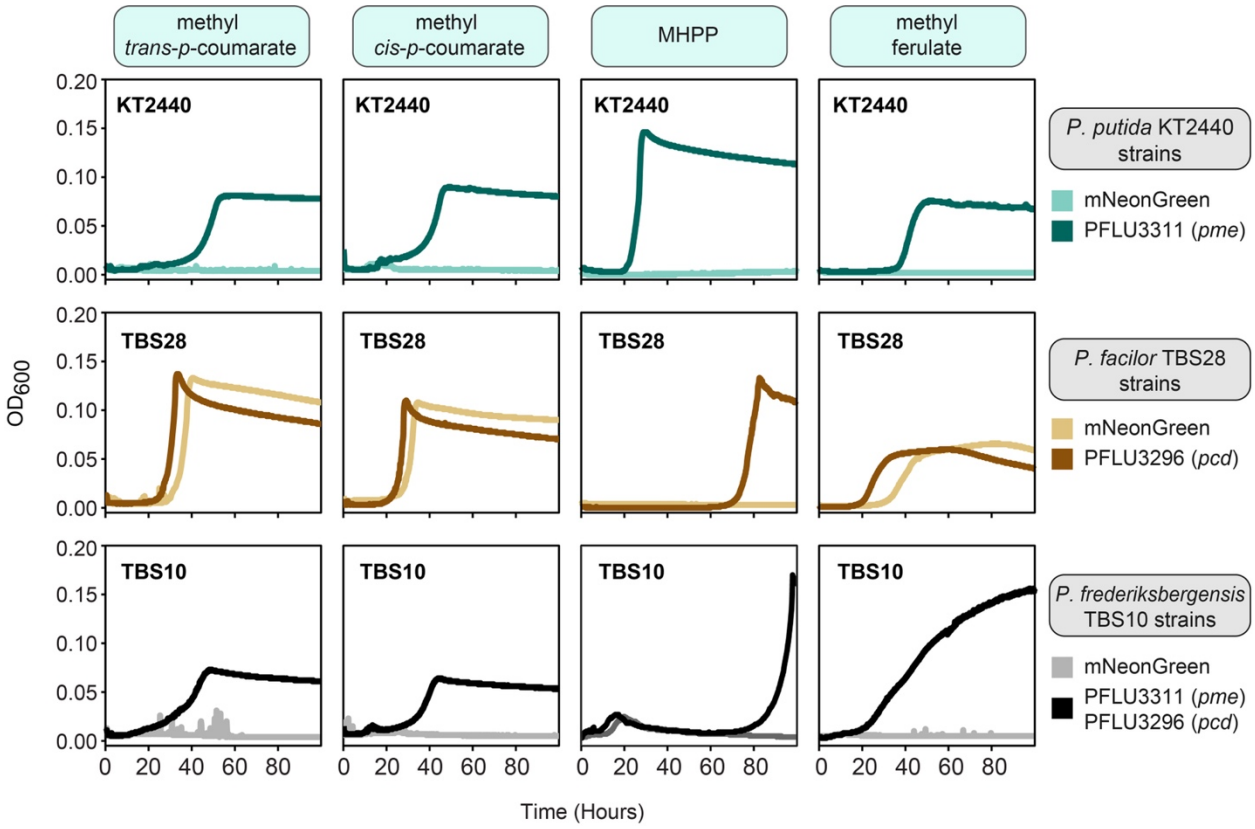

Fig. 6. Transfer of *pme* and *pcd* genes enables heterologous hosts to use PPMEs as carbon sources. Microtiter plate cultivation assays comparing growth of Pseudomonads expressing either the fluorescent protein mNeonGreen or the Pme/Pcd enzymes from SBW25. Assays were performed with MME medium containing 2.5 mM of the indicated carbon source. Each panel contains a single representative curve from one of three biological replicates.

Elucidating bacterial catabolism of the important sorghum allelochemical sorgoleone

The secondary metabolite sorgoleone, 2-hydroxy-5-methoxy-3-[(8'*Z*,11'*Z*)-8',11',14'-pentadecatriene]-*p*-benzoquinone (**Fig. 7**), is a major component of exudates from root seedlings of *Sorghum bicolor* [34, 35]. Sorgoleone has drawn substantial attention from the research community and the agriculture industry because of its allelochemical properties [36, 37]. It has a variety of impacts on soil ecology and has been used in integrated weed management [38]. It can also reduce loss of nitrogen fertilizers in soil by inhibiting biological nitrification [39]. Previous work has shown that sorgoleone is slowly mineralized to carbon dioxide in soils and that microbial activities are responsible for this process [40]. Sorgoleone also influences the composition and network structure of soil and rhizosphere microbial communities [41].

The PerCon SFA team has enriched and isolated sorgoleone-degrading bacteria from soil that had been planted with sorghum by using sorgoleone as a sole carbon source. Whole genome sequencing of three sorgoleone-utilizing isolates followed by transcriptome and random bar code transposon-site sequencing (RB-TnSeq) analyses identified genes likely to be involved in sorgoleone degradation. Mutational analysis confirmed that a four gene cluster conserved among our isolates was required for growth on sorgoleone. A phylogenetic survey further revealed that

these genes were enriched in *Streptomyces* species associated with sorghum. These results are a first step to determine if sorgoleone might be harnessed to control persistence of plant beneficial bacteria in root rhizospheres.

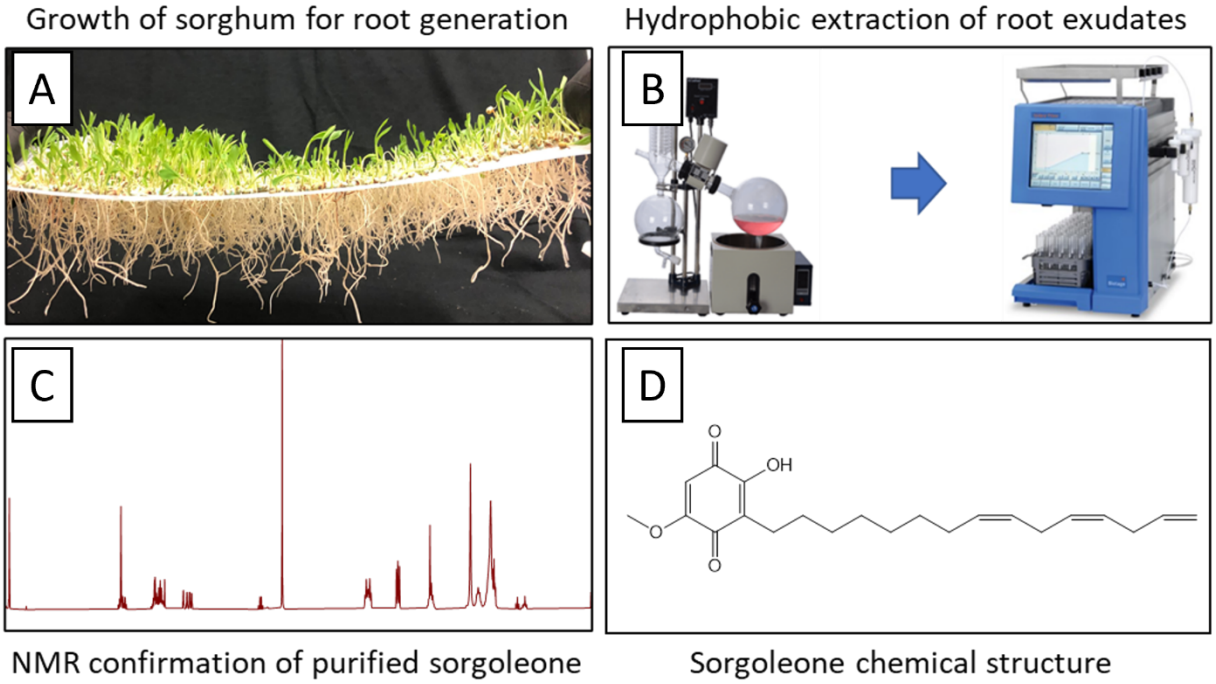

Fig. 7. Summary of sorgoleone isolation. (A) Germination of sorghum seeds. (B) Extraction of sorgoleone from germinated sorghum roots using MeOH/dichloromethane and fractionation by thin layer chromatography. (C) Qualification of purified sorgoleone by NMR analysis. (D) Chemical structure of sorgoleone.

Sorgoleone was purified from sorghum seedlings [35] as summarized in **Fig. 7**, and used as a sole carbon source to enrich and isolate three sorgoleone-degrading strains from soli collected from a sorghum-growing field site in Kearney, CA, USA. Two strains, SO1 and SO82 grew with 2 mM sorgoleone to a final yield of about 10^9 CFU/mL. The third strain, SO81, grew to lower yields on the same concentration of sorgoleone. A combination of whole genome sequencing and Genome Taxonomy Database (GTDB) analysis (https://gtdb.ecogenomic.org/) designated strains SO1 and SO82 as *Acinetobacter pitii* and *Burkholderia anthina*, respectively. Strain SO81 was a novel *Pseudomonas* species. Based upon its ability to use sorgoleone as a carbon source, we named this strain *Pseudomonas sorgoleonovorans* SO81.

To identify sorgoleone degradation genes, we determined the transcriptomes of each strain grown with either sorgoleone or acetate as sole carbon sources. We found that 228 genes from *A. pitti* SO1 and 156 genes from *B. anthina* SO82 were expressed at ≥8-fold higher levels in cells grown with sorgoleone as compared to acetate. By contrast just 33 genes were expressed at ≥8-fold higher levels in *P. sorgoleonovorans* SO81 grown with sorgoleone as compared to acetate. The three strains shared seven genes in common that were expressed at high levels during growth with sorgoleone. These included four genes predicted to encode a monooxygenase, two α/β hydrolases, and a cytosine deaminase. Each of these genes was expressed at greater than 40-fold higher levels during growth on sorgoleone compared to acetate. Except for *P. sorgoleonovorans* SO81, which has a permease gene (SO81_17470) between its monooxygenase

and cytosine deaminase genes, the four genes are adjacent to each other on the genomes. We therefore refer to these four genes as the *srg* (sorgoleone degradation) cluster (**Fig. 8**).

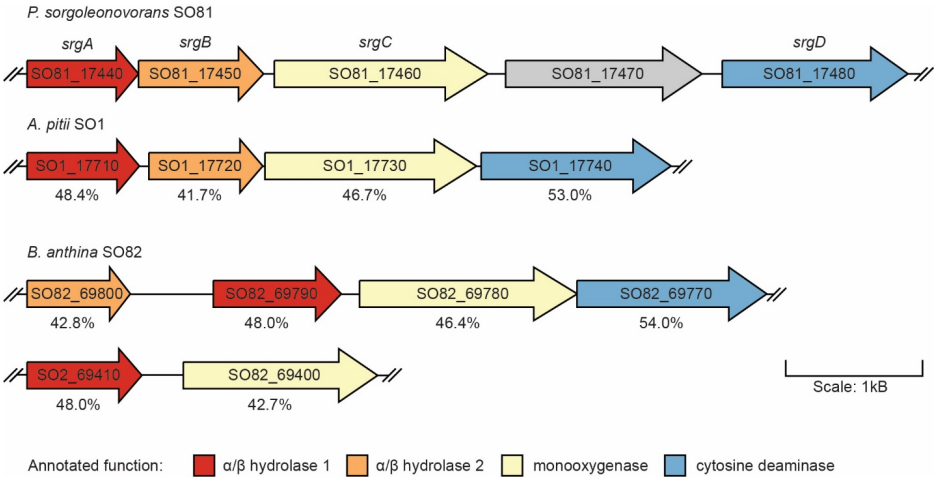

Fig. 8. The *srg* cluster for sorgoleone degradation. The color code indicates similar functions among strains. Percentages indicates the protein identity to *P. sorgoleonovorans* SO81 genes.

We used the functional genomics-based approach of RB-TnSeq to screen directly for genes involved in sorgoleone catabolism [42]. Because of the ease of genetic manipulation of *Pseudomonas* species, we generated an RB-TnSeq library in *P. sorgoleonovorans* SO81. The library had 367,775 unique barcoded transposon insertion mutants with insertions in 3,924 of the 4,649 predicted genes (84%), with an average coverage of 60.3 transposon insertion mutants per gene. Using this library, we performed fitness assays that evaluated the relative growth of mutants in the library on sorgoleone and a series of other carbon sources (acetate, citrate, glucose, or octanoate). We identified 14 genes with strong negative fitness scores exclusive to growth on sorgoleone. Of these, eight genes were induced by sorgoleone in our transcriptome analysis (**Fig. 9**). Included in this set were the *srg* cluster (SO81_17440 to 17480) and two genes adjacent

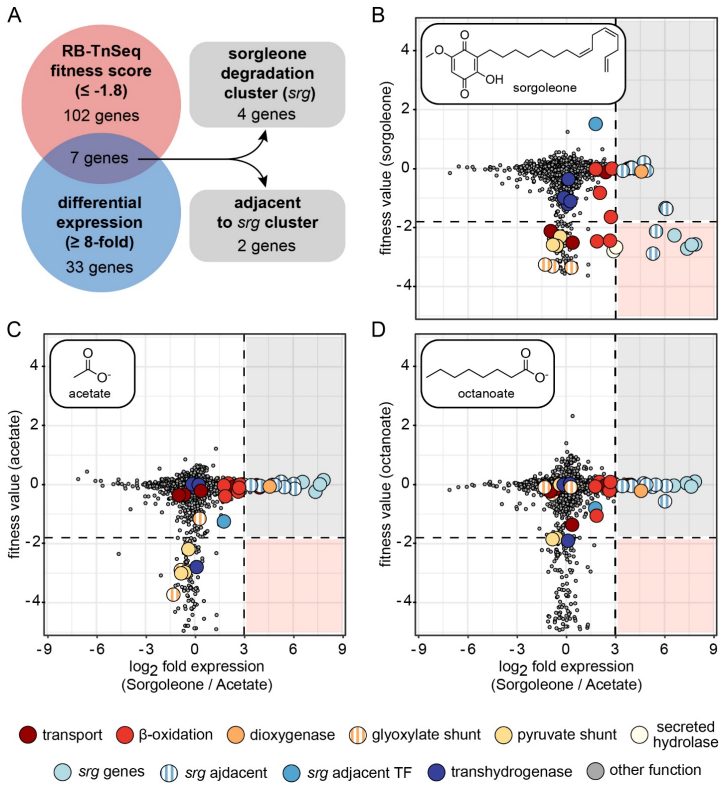

Fig. 9. Summary of RB-TnSeq and RNA-Seq data from *P. sorgoleonovorans* SO81. (A) Venn diagram of overlap between genes with substantial fitness values for SO81 grown on sorgoleone and genes that are more highly expressed when grown on sorgoleone versus acetate. Plots comparing differential expression versus mean fitness values are displayed from growth on (B) sorgoleone, (C) acetate, or (D) octanoate. Markers as indicated represent *srg* genes and other genes important for related to transporters (maroon), β-oxidation enzymes (red), dioxygenase (orange), glyoxylate shunt enzymes (orange stripes), pyruvate shunt enzymes (yellow), a putative secreted hydrolase (light yellow), the *srg* cluster (light blue), genes adjacent to the *srg* cluster (light blue stripes), transcription factor adjacent to the *srg* cluster (blue), NAD(P)H transhydrogenase (dark blue), and all other genes (gray).

to the *srg* cluster. Our RB-TnSeq experiments identified several genes involved in β-oxidation (SO81_19340, SO81_19860, SO81_39590, SO81_42070, and SO81_42080) that conferred substantially reduced fitness during growth on either sorgoleone or ocanoate when disrupted.

To confirm that the *srg* cluster is essential for sorgoleone degradation, we constructed a *P. sorgoleonovorans* SO81 *srg* deletion mutant (SO81Δ*17440-17460*). Both the wild type and the deletion mutant grew well on glucose, but the mutant strain failed to grow on sorgoleone. Integration of a single copy of the *srg* cluster (*SO81_17440-17460*) into the *attTn7* site of SO81Δ*17440-17460* complemented this phenotype.

Our observation that the *srg* cluster is conserved among several species of our soil isolates prompted an investigation of the distribution of this gene cluster across sequenced organisms and its suitability as a genetic marker for sorgoleone catabolism. Our approach involved cluster construction of Snekmer protein family models [43] for the *srg* genes using sequences from our three isolates and data from the UniProt Reference Proteome. We used the *srg* protein models to search the GFOBAP collection of 3,837 genomes derived from soil- and plant-associated environments [44], and identified hits for the complete cluster in one *Acinetobacter* and 21 Actinobacteria, most of which are *Streptomyces* sp.

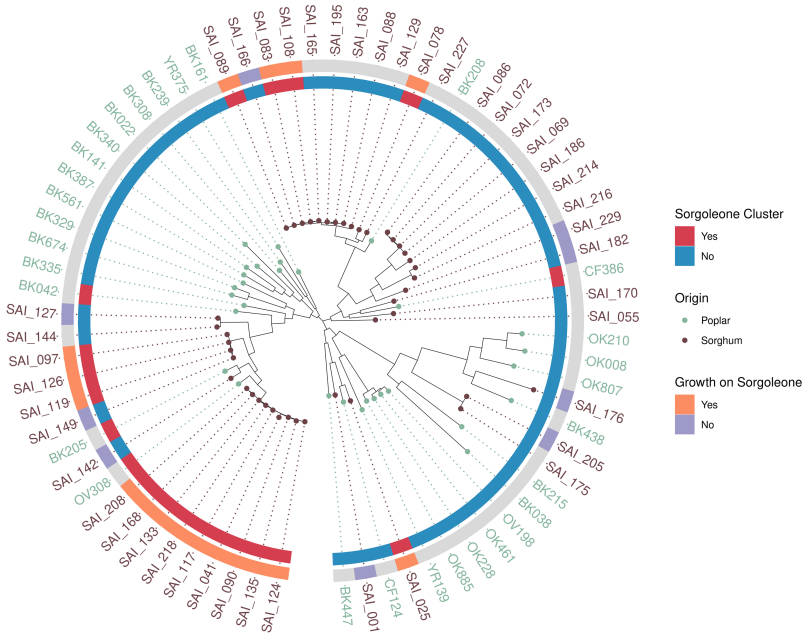

Fig. 10. Phylogenetic analysis and growth of selected *Streptomyces* strains on sorgoleone. Outside to inside, the rings represent strain-plant association, growth phenotype on sorgoleone for a subset of strains, and the presence/absence of the *srg* gene cluster from whole-genome sequence data. The tree at the center represents phylogenetic distance.

To investigate the prevalence of the *srg* cluster among *Streptomyces* isolates associated with different plants we analyzed publicly available whole genome sequences of 44 *Streptomyces* strains isolated from sorghum-cultivated fields and 30 *Streptomyces* strains isolated from soils near poplar trees. We found a significant enrichment of the *srg* cluster among the strains associated with sorghum (17/44) over poplar (4/30) (two-proportion z-test, p=0.012) (**Fig. 10**). To assess whether the presence of the *srg* cluster is required for *Streptomyces* to grow on sorgoleone, we selected a taxonomically diverse collection of sorghum-associated *Streptomyces* strains, including strains with and without the *srg* cluster, and tested for growth in minimal medium containing sorgoleone as the sole carbon source. We found that only the strains encoding the *srg* cluster were able to grow on sorgoleone (**Fig. 10**). An ortholog profiling analysis of *Streptomyces* strains revealed general conservation of the gene neighborhood of the *srg* cluster with several strains encoding a TetR family transcriptional regulator adjacent to the *srg* cluster. These results suggest that the *srg* cluster provides the critical steps to break down sorgoleone in *Streptomyces* and can be used as a biomarker for sorgoleone catabolism.

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