Engineering *Pseudomonas putida* as a Chassis for the Bio-production of Small Molecules from Renewable Feedstocks

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Why *Pseudomonas putida* and lignin?

P. putida KT2440 is a metabolically diverse, BSL1 host for the cloning of foreign genes.[1] It has been shown to be highly tolerant to organic solvents and is amenable to genetic manipulations. *P. putida* also has metabolic pathways to utilize typical "waste-products" such as lignin-derived biomass or pollutants.[2,3] With appropriate genetic tools, *P. putida* can become a useful chassis for the production of chemicals from renewable feedstocks.

Lignin as a polymer comprises 15-30% of plant biomass. It is mainly burned for energy due to its complex structure that is recalcitrant to depolymerization. Various methods are in development to overcome this hurdle. We seek to build synthetic biology and systems biology platforms to enable the engineering of *P. putida* for further valorization of lignin-derived compounds. This will allow the synthesis of valuable chemicals from a lignin source (Figure 1).

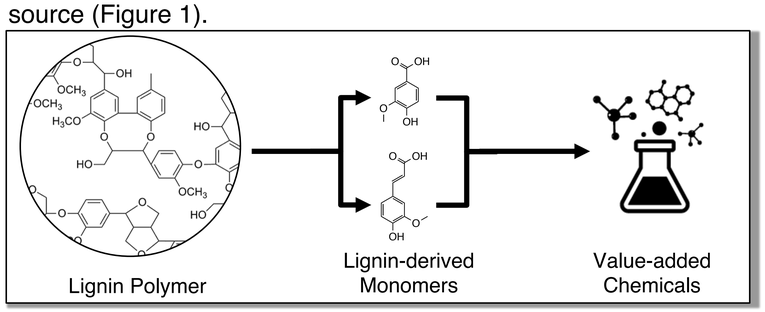

Figure 1: From lignin to value-added chemicals.

Modeling

To allow efficient manipulation of *P. putida*, an *in silico* genome-scale model of the organisms metabolic network was used to predict the effect of various changes (Figure 2).[4] The tool enables the identification and prediction of beneficial gene deletions as well as how additional genes would effect the growth of the bacteria and the ability to produce various chemicals.

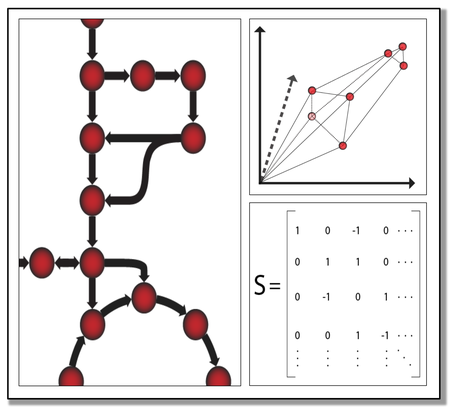

Figure 2: Symbolic and mathematic representations of a genome-scale model.

Pseudomonas putida Toolbox

To verify its effectiveness in *P. putida,* dCas9 was used to inhibit the expression of a green fluorescent protein-encoding gene that was inserted into the genome.[5] Two different regions were targeted: the coding region, to halt transcription, or the promoter region, to prevent binding and initiation (Figure 3). Both methods were effective, reducing the relative fluorescence by over 60% and 90% (Figure 4).

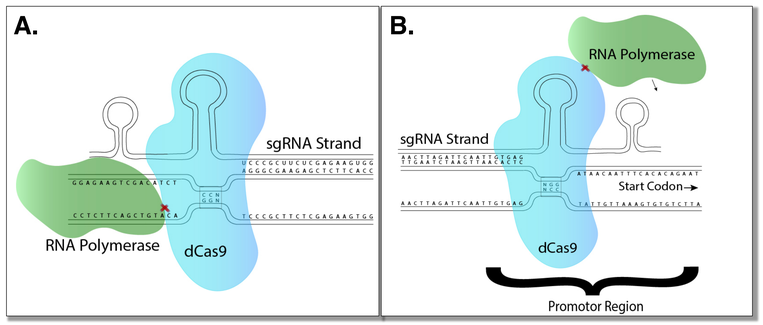

Figure 3: A. Termination of transcription by dCas9; B. dCas9 as a repressor to prevent binding and initiation.

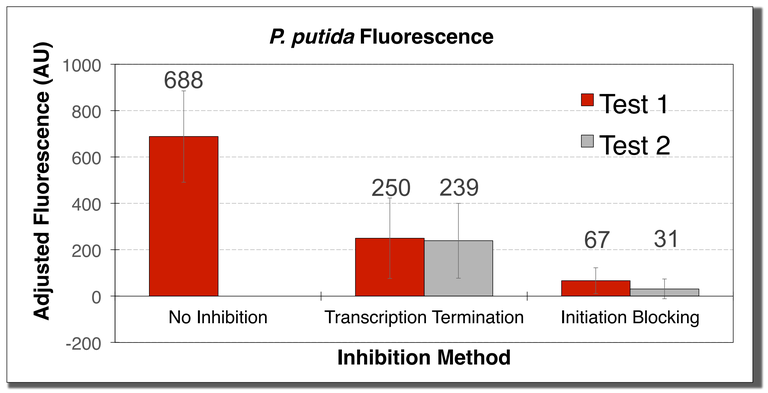

Figure 4: Measurement of fluorescence in the presence and absence of functioning dCas9 system.

For genetic manipulation and circuit construction, plasmid copy number of broad-host-range vectors and promoter strength was characterized. The standardized pSEVA collection containing the RSF1010 (pSEVA651) and RK2 (pSEVA621) replication origins as well as the pBBR1 origin were compared using the expression of fluorescent protein (Figure 5).[6] The relative copy number was normalized to the pBBR1 vector. Promoter strength was tested using the fluorescence of sfGFP in plasmids with pBBR1 replication origin (Figure 6).

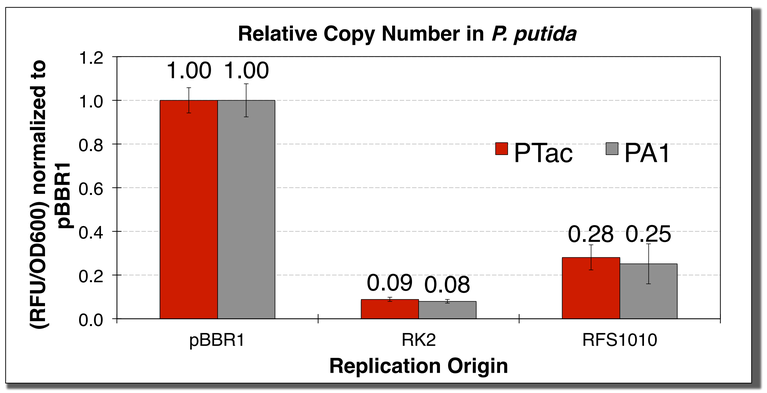

Figure 5: Three plasmids with different replication origins were tested using fluorescence of GFP to determine the relative copy number of the plasmids in *P. putida*. Results were verified using two different promoters: Ptac and PA1

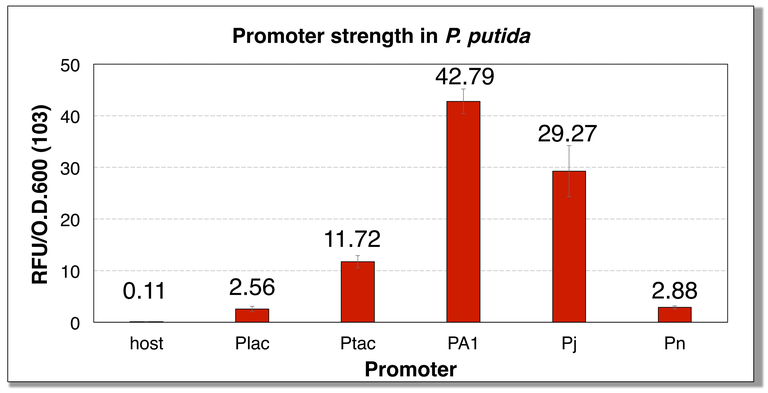

Figure 6: Promoter strength was determined for various constitutive promoters by measuring the fluorescence of GFP in *P. putida* following 24 hours of cultivation

Conclusions and Future Work

We have established a toolbox that enables the manipulation and tuning of the genetic contents in *P. putida*. Future efforts will focus on pathway and strain engineering to construct *P. putida* strains that can accumulate value-added chemicals from lignin-derived compounds. Molecules including vanillin, succinate, and pyrogallol have been produced by *P. putida* strains. We aim to further expand and improve the current product profile for applications ranging from plastics to pharmaceuticals to fuels.

Citations

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