Title: Design, Detect, Evolve: Engineering Syringol Degradation in ADP1

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Project Goals: Our long-term objective is to exploit bacterial aromatic compound metabolism for biotechnology. Applications range from lignin valorization to the degradation of environmental pollutants. A critical first step is to expand natural pathways using foreign and modified genes to create synthetic pathways with high conversion efficiency. A soil bacterium, *Acinetobacter baylyi* ADP1, is being used in this project to create a novel route for the catabolism of syringol, an aromatic component derived from lignin pyrolysis. To enable syringol consumption, for which there is no known metabolic route, we are combining parts of characterized pathways. Biosensors are being developed to facilitate the modification of enzyme substrate specificity, and laboratory evolution is being used for growth-based selection. The genetic malleability and catabolic versatility of this strain make it an ideal host for synthetic pathways. New catabolic functions can be used in ADP1 and/or ported for use in other organisms.

Abstract Text: Lignin is a vastly underutilized renewable resource. Initial processing yields a heterogenous mixture of compounds, including many aromatics. One approach to produce commercially valuable compounds from a lignin-derived mixture is to funnel as much carbon as possible through bacterial central metabolism and then into a desired product. To increase the consumption of lignin-derived mixtures by *A. baylyi* ADP1, our goal is to expand its repertoire for using lignin-derived aromatics. There is no known route for biological degradation of syringol. The development of a novel route for syringol degradation would contribute to lignin valorization and to a generalizable method to create catabolic modules. A three-part pathway was designed with candidate enzymes (indicated for each step, Fig. 1).

Multiple strains were constructed with foreign/synthetic DNA integrated in the ADP1 chromosome. To obtain a functional pathway, we are using two approaches: (1) a method for growth-based selection and evolution and (2) a method for improvement based on the ability of biosensors to detect pathway intermediates independently from growth.

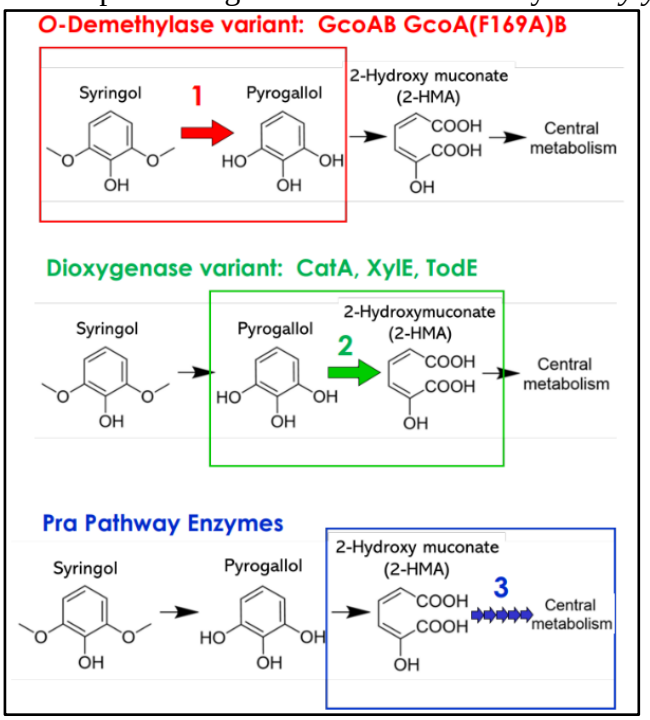

Fig. 1. Synthetic pathway for syringol catabolism

The first approach involves selection for growth on syringol during adaptive evolution with a method called EASy (Evolution by Amplification and Synthetic Biology) [1]. This method specifically amplifies a chromosomal region of ADP1, leading to a tandem array of that region. Multiple gene copies increase enzyme expression, thereby enhancing catalytic activity of a non-optimal enzyme. In addition, gene duplication provides a larger segment of DNA for the occurrence of advantageous mutations. In short, EASy is a way to offer an advantage to engineered strains prior to selective growth. Unlike in previous applications, I sought to amplify two different chromosomal regions of the same strain. The goal of this novel use of the method is to increase the number of copies of genes encoding separate parts of the pathway independently to prevent the accumulation of toxic intermediates. I successfully accomplished the simultaneous and independent EASy amplification of two chromosomal regions. Numerous amplified strains were constructed, and qPCR confirmed a wide range of the number of genes and different ratios of gene dosage in the two regions. These strains provide the starting cultures for laboratory evolution.

Since growth is an imperfect metric for enzyme activity, a parallel approach is being used to create this pathway: biosensor-based detection of pathway intermediates. Strains were constructed to express an O-demethylase variant GcoA(F169)B that demethylates syringol [2] (Fig. 1). To optimize this activity in our pathway, a biosensor is being developed to detect the reaction product, pyrogallol. For this purpose, we successfully respecified a biosensor that detects a similar compound [3] to detect pyrogallol in *E. coli*. An approximately two-fold response to pyrogallol was observed with no induction by its native inducer. Current work will improve the range of fluorescent response to pyrogallol, and this biosensor will be introduced into ADP1.

Initial successes with both approaches provide a foundation for continuation. Expansion of the "traditional" EASy method allows us to select the optimum ratio of initial gene expression in a pathway. Strains with various gene dosages are being used in experiments to select for growth on syringol. To complement this approach, we will improve biosensors for the detection of pyrogallol, allowing us to perform directed evolution of strains based on pathway flux rather than growth.

References/Publications

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