***Pseudomonas putida* soil isolates that metabolize C5 sugars and lignin-derived aromatics**

Mee-Rye Park[1,2] (mrpark@lbl.gov), Rahul Gauttam[1,2], Bonnie Fong[1,2], Joseph Stevens[1,2], Blake Simmons[1,2], and Steven Singer[1,2]

[1]Joint BioEnergy Institute, Emeryville, CA, 94608; [2]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA, 94720

http://jbei.org

Project Goals: The Joint BioEnergy Institute (JBEI) performs fundamental research to improve the conversion of biomass to biofuels and bioproducts. A critical aspect of current research is to maximize the carbon in plant biomass that is converted by microbial hosts. To achieve this goal, we have isolated new potential hosts within members of genus *Pseudomonas* that are natively capable of utilizing C5 sugars and lignin-derived aromatics.

Pseudomonas putida is promising host for biomass conversion to fuels and chemicals because it metabolizes sugars and aromatics from plants. However, the best studied strain, *P. puitda* KT2440, lacks the native ability to metabolize C5 sugars (xylose and arabinose), the most abundant components of hemicellulose from grasses. To maximize the range of components which can be obtained from lignocellulosic hydrolysate, pentose (C5) sugars should be utilized as feedstock for value-added products. Here, we report *Pseudomonas putida* isolates obtained from soil that grow on C5 sugars as well as lignin-derived aromatics. These isolates were in two clusters closely related to *P. putida* KT2440 and were capable of growing on glucose and *p*-coumarate at rates comparable to KT2440. One set of isolates grew on xylose and the second set of isolates grew on xylose and arabinose. Genomic analysis of the isolates indicated that homologs of the Weimberg pathway for xylose oxidation (*xylD-XylX-XylA*) were present in both isolate genomes and an oxidative pathway for arabinose oxidation (*araD-araX-AraA*) was present in the one strain. Transformation protocols were established for the both strains and deletion of the periplasmic glucose dehydrogenase eliminated the ability of the strains to grow on C5 sugars, indicating both C6 and C5 sugars were oxidized by the same protein. A CRISPRi system for one strain has been established and this system of gene knockdowns, along with RNA-seq experiments will identify the genes important for xylose and arabinose metabolism. Preliminary experiments indicated that the strain can produce Type III polyketide synthases and non-ribosomal peptide synthases, suggesting it could serve as an alternative host for fuels and chemical production.

Funding statement. *This work was performed as part of the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy.*