The response of *Pseudomonas putida* to a sorghum lignolysate

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Project Goals: The goal of the Joint BioEnergy Institute is to establish the scientific knowledge and new technologies in feedstock development, deconstruction and separation, and conversion needed to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

There is strong interest in lignin valorization to produce valuable products. Nevertheless, the bioprocessing of lignin into bioproducts is a major bottleneck because of the structural complexity of the biopolymer. Here, we employed cholinium-based ionic liquid pretreatment to obtain a soluble aromatic-rich fraction by base-catalyzed depolymerization (BCD) of sorghum that had been depleted of sugars by enzymatic hydrolysis. Growth of *Pseudomonas putida* on BCD liquor coincided with disappearance of aromatic peaks, consistent with the complete utilization of *p*-coumarate, ferulate, and the other aromatic monomers present in the depolymerized substrate. The growth of *P. putida* in the BCD liquor was higher than that observed on individual aromatic substrates, suggesting that the BCD liquor contained additional carbon sources beyond lignin-related aromatics. Aromatic-independent growth was confirmed in a *P. putida* mutant strain that was unable to grow on p-coumarate and ferulate. Metabolite analysis demonstrated that the sorghum BCD liquor was a mixture containing at least four distinct substrates for *P. putida* (aromatic monomers, amino acids, cholinium and fatty acids). Comparative proteomic analysis revealed the significant upregulation of aromatic, amino acid and cholinium catabolic pathways as well as genes for fatty acid β-oxidation and acetate assimilation. These results indicate that lignolysates obtained from plant biomass are complex substrates whose assimilation into microbial hosts requires multiple metabolic pathways responding in concert.

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