153g Improving the Efficiency of Xylose Utilization and Xylitol Production in E. Coli

Patrick C. Cirino, Reza Khankal, and Jonathan W. Chin

Efficient microbial conversion of biomass into value-added products requires that pentose and hexose sugars can both be assimilated by the organism. E. coli has proven to be a suitable biocatalyst for the overproduction of native metabolites and non-native compounds using both types of sugars. Xylose is the second most abundant sugar constituent derived from lingocellulosic materials. In E. coli, xylose uptake occurs primarily through a high-affinity, ATP-binding cassette (ABC) transporter (XylFGH), even in the presence of high xylose concentrations. A second, low-affinity proton symporter (XylE) is also present but does not appear to play a significant role in xylose transport. The efficiency of xylose utilization in this organism is therefore suboptimal due to energetic requirements for nutrient uptake.

The research described here is aimed at improving the efficiency of xylose uptake in general, and more specifically at maximizing production of xylitol via xylose reduction in E. coli. As expected, stoichiometric modeling of E. coli metabolism shows that the ATP requirement for xylose transport is a key limitation to xylitol production, and engineering a less energy-intensive uptake mechanism is a prerequisite for further metabolic optimization. We have characterized the effects of deletions in the native xylose transport systems and overexpression of a variety of known or potential xylose transporters on xylose uptake, growth on xylose and xylitol production. These gene knockouts and additions were studied in wild-type E. coli (strain W3110), strains expressing a cAMP-insensitive catabolite activator protein mutant (CRP*) in which diauxie is eliminated and xylitol-producing strains expressing xylose reductase. The implications of our results in improving cofactor supply for whole-cell biocatalysis will be discussed.