153b Role of Pyruvate Dehydrogenase in the Anaerobic Fermentation of Sugars by *Escherichia Coli*

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Pyruvate is a key metabolite in central metabolism since it is the end product of glycolysis and most of the fermentative products are derived from it. Thus, metabolism at the pyruvate node is important from metabolic engineering point of view as variation in the way pyruvate is dissimilated would have significant impact on overall cell metabolism and the product distribution. We are focused in elucidating the activity and role of enzymes involved in dissimilation of pyruvate during fermentative growth of *E. coli* using metabolic flux analysis (MFA).

PDH (Pyruvate Dehydrogenase), the major enzyme responsible for dissimilation of pyruvate under aerobic conditions, was believed to be inactive under anaerobic conditions but recently it was found to be otherwise. However, PDH is not able to support fermentative growth of *E. coli* in minimal media in Pyrute Formate Lyase null background and its role under these conditions is unknown. For our study, we have created an *E. coli* strain devoid of the genes encoding PDH (*E. coli* W3110Δ*aceEF*; PDH-) and evaluated wild-type W3110 and otherwise isogenic PDH- during the anaerobic fermentation of glucose. We found that the PDH- strain grows at a much lower rate and exhibits a longer lag than W3110 indicating that PDH plays an important role on cellular metabolism during the anaerobic fermentation of glucose. Upon performing MFA, we found that there is significant flux redistribution in PDH- strain, and the most significant differences were found in the fluxes in pentose phosphate pathway (PPP). The flux through oxidative branch was very low in wild type as compared to PDH- mutant (about 100 folds lower) and the cross fluxes between PPP and EMP (Embden Myerhoff Parnas) were in opposite directions in the two strains. There was a positive flux from PPP towards fructose-6-phosphate and glyceraldehyde-3-phosphate in PDH- strain whereas these metabolites fed the PPP in W3110.

Based on our MFA results that there is an increase of flux through oxidative branch of PPP in PDH- mutant as compared to W3110, we postulated a hypothesis that the role of PDH under fermentative condition is to provide CO_2 in a carbon and energy efficient manner. Further experiments where we supplemented the growth media with CO_2 supported our hypothesis since the growth rate of both the strains equalized in CO_2 supplemented experiments indicating that the availability of CO_2 was the basis of difference between the two strains.

To further support our findings, we are currently focused on performing labeling experiments which are powerful tools in investigating cellular metabolism at a high degree of resolution. In these experiments, labeled substrate is fed to the system and analysis of the labeling pattern of intermediate metabolites provides information about the metabolic network topology and internal fluxes. Such an analysis for wild type strain W3110 supported our finding that the flux is indeed very low through oxidative branch of PPP. We plan to perform similar analysis for PDH- derivative of the wild type and in the meeting we will present our final results regarding the role of PDH under fermentative conditions.