Engineering Bacterial CO_2 Fixation to Enhance Biofuel and Biochemical Yields

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The mass production of biofuels and chemicals through microbial fermentation is renewable solution to combat climate change through decreased greenhouse gas emissions. The primary factor affecting the economic viability of next generation biofuel fermentations is the cost of feedstock and feedstock pretreatment. One way to mitigate high feedstock costs is by maximizing feedstock conversion to the product of interest. The ability to achieve high mass yields from carbohydrate fermentations, however, is impeded by CO_2 loss during glycolysis through the decarboxylation of pyruvate when forming acetyl-CoA, whereby one third of hexose carbons cannot be recovered as useful products. One route to improving glycolytic yields is through the incorporation of CO_2 fixation pathways that can recapture CO_2 lost during fermentation.

Stoichiometric analysis of the six natural carbon fixation pathways shows that the best suited pathway for industrial fermentation is the Wood-Ljungdahl pathway (WLP), an anaerobic pathway that directly reduces CO_2 to form the biological building block, acetyl-CoA, using electrons derived from glycolysis or from a wide array of inorganic sources including molecular CO and H_2. Under mixotrophic fermentation, in which both gases and carbohydrates are consumed concurrently, 100% of carbohydrate carbons can be recovered in products, up from the 66% of carbohydrate carbon that can be recovered in traditional fermentation.

The viability of mixotrophic fermentation was tested in four bacteria that natively contain the WLP. All four acetogens demonstrated the capability to simultaneously consume sugars and gases by producing metabolites at yields higher than that which could be achieved through heterotrophic fermentation alone. Additionally, we showed through ^{13}C labeling that, in the case of *Clostridium ljungdahlii*, gas incorporation occurs concurrently with sugar consumption and that the relative rates of gas and sugar production allow for more than 40% of acetate to be derived from the carbon fixation rather than glycolysis thereby validating the concept of acetogenic fermentation.

Engineering the WLP in the established industrial organism *C. acetobutylicum* would allow for the conversion of carbohydrates into butanol, acetone, and ethanol at higher yields than is possible with traditional fermentation. In an attempt to achieve this goal, we focused on expressing 11 core genes coding for enzymes and accessory proteins, which required the development of a novel clostridial genetic system using two co-existing plasmids combined with a method to integrate a subset of the WLP genes into the *C. acetobutylicum* chromosome. While the engineered WLP in *C. acetobutylicum* showed functionality of the Eastern branch of the pathway due to the formation of labeled 5,10-methylenetetrahydrofolate from labeled formate and a functional Western branch in the formation of CO from CO_2, the connection of these two branches was not observed in any labeling of acetyl-CoA observed in the acetate and butyrate pools. Further efforts were undertaken to characterize the expression and activity of the vital CODH/ACS enzyme that catalyzes the reduction of CO_2 to CO and the condensation of CO with a methyl-group to form acetyl-CoA, and both activities were demonstrated through *in vivo* assays. Further engineering will be required to bring a functional heterologous WLP to fruition.