303d A Novel Microbial Platform for the Anaerobic Synthesis of Oxidized Chemicals: Anaerobic Production of Acetic Acid in *E. Coli*

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Current aerobic methods used to produce oxidized chemicals via fermentation do not compete with petrochemical routes due to their high capital and operating costs. Fermentation is a leading expense, which can account for between 50 and 90% of total production costs of commodity chemicals. Production of acetate, for example, result in a loss of ~50% of the sugar as CO_2 and can not use sugars obtained from lignocellulosic biomass, which constitute a low-cost (~0.05 $/lb), sustainable alternative for producing bulk chemicals. We are developing a novel microbial platform for the anaerobic production of oxidized chemicals from plant biomass sugars. The proposed *E. coli*-based platform will use nitrate respiration to convert 5- and 6-carbon sugars into acetate. This process has lower capital and operating costs than existing aerobic processes. Furthermore, the reduction of nitrite obtained from nitrate respiration will generate ammonium and alkalinize the medium, thus reducing the use of base for pH control and ammonium salts in the fermentation and purification processes. Metabolic engineering is being used as a rational approach to obtain these microbial biocatalysts that will produce acetate via nitrate respiration. The work to be presented at the meeting includes the engineering of *E. coli* for the efficient use of nitrate as electron acceptor and nitrogen source under anaerobic conditions and the construction of strains for the anaerobic production of acetate acid as the main fermentation product.

Our strategy to maximize the conversion of nitrite into ammonia and avoid its accumulation in the culture medium is based on preventing the extrusion of nitrite produced by the more active membrane-associated nitrate reductases (NarG and NarZ) and avoiding the production of nitrite via the periplasmic nitrate reductase (NAP). Using this approach, we have disrupted, both singularly and in combinations, the genes involved in these processes: i.e. *napFDAGHBC*, encoding periplasmic nitrate reductase NAP; *narK*, encoding a nitrite/nitrate transporter; and *narX* encoding sensor NarX which is part of the homologous two-component regulatory system NarX/NarL (*in vitro* molecular studies have shown that NarX negatively regulates the NarL protein by acting as a NarL-phosphate phosphatase). To this end we have created all single, double, and triple mutants. Wild-type *E. coli* W3110 and all recombinant strains have been evaluated for cell growth, sugar consumption and their capacity for the reduction of nitrate and nitrite. The most robust growth and sugar consumption behavior (both volumetric and specific rates) were achieved in the triple mutant strain W3110[D*napFDAGHBC*, D*narK*, D*narX*], which also exhibited the lowest accumulation of nitrite in the medium due to the simultaneous reduction of nitrate to nitrite to ammonium. On the other hand, to achieve the synthesis of acetate at high yields we inactivated genes involved in the synthesis of competing fermentation products including lactate (gene *ldhA*, encoding lactate dehydrogenase) and succinate (genes *frdABCD* encoding fumarate reductase). Double mutant W3110[D*ldhA*, D*frdABCD*] produced acetate as the main fermentation product at a yield exceeding 90% of the theoretical maximum. Our current efforts focus in the integration of the aforementioned modifications into a single strain capable of simultaneously reducing nitrate to nitrite to ammonium and producing acetate at high yield and productivity.