Development of emerging model microorganisms: Megasphaera elsdenii for biomass and organic acid upgrading to fuels and chemicals.

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The native ability to condense acetyl-CoA groups to efficiently generate C4 to C8 compounds makes *M. elsdenii* a compelling platform for the production of fuels and chemicals from lactate and plant carbohydrates. Our overall objective is to develop *M. elsdenii* into a platform for the conversion of lignocellulosic biomass sugars and organic acids to hexanol and other valuable chemicals. *M. elsdenii* produces organic acids as fermentation products when growing on lactate and glucose, including butyric (four carbon), valeric (five carbon), hexanoic (six carbon), and in some cases octanoic (eight carbon) acids as major fermentation products, likely via a chain elongation pathway using acetyl-CoA. As the carbon chain length increases for the corresponding alcohols, fuel properties improve, making hexanol an appealing target as a next-generation gasoline blend stock beyond ethanol. We are engineering *M. elsdenii* to efficiently produce next-generation, drop-in lignocellulosic fuels such as hexanol at high yield and titer as a general bioengineering platform. While previous efforts to make products like alcohols in *E. coli* have been moderately successful, production of C6 and larger products remains low, suggesting that extending the chain elongation pathway beyond a single cycle remains a significant challenge in model organisms. Engineering *M. elsdenii* is an alternative approach with the potential for exceptionally high yields and titers of C6 products such as hexanol. We developed an initial method for transformation of DNA into two strains of *M. elsdenii* via methylome analysis, heterologous expression of DNA methyltransferases in *E. coli*, and electroporation. We further improved efficiency by creating an *E. coli* methylating strain that uses arabinose-inducible expression, resulting in an additional 10-fold increase in *M. elsdenii* transformation efficiency, to approximately 10,000 cfu/ug DNA. This has facilitated efficient and rapid strain construction. Expression of a bifunctional aldehyde/alcohol dehydrogenase (*adhE2)* gene using the native *M. elsdenii* ribosomal S4 protein promoter resulted in 5.3 mM butanol in *M. elsdenii* ATCC 25940 from lactic acid as the growth substrate. The deletion of a propionyl-CoA transferase in the *M. elsdenii* chromosome resulted in increased acetate co-consumption, loss of propionate production, reduced valerate production, and increased hexanoate and butyrate production from lactic acid as the growth substrate. *This is the first demonstration of metabolic engineering in Megasphaera and proof of concept that this approach may lead to the accomplishment of our longer-term goals.*