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 to efficiently generate C4 to C8 compounds makes *Megasphaera elsdenii* a compelling platform for the production of fuels and chemicals from lactate and plant carbohydrates. *M. elsdenii* produces organic acids as fermentation products from lactic acid and glucose, including butyric (4 carbon), valeric (5 cargon), hexanoic (6 carbon), and in some cases octanoic (8 carbon) acids as major fermentation products, likely via a chain elongation pathway using acetyl-CoA. As the carbon chain length increases, fuel properties improve. Energy density increases and hygroscopicity decreases. Virtually nothing is known about the metabolic pathways in *M. elsdenii* that result in organic acid formation beyond predictions based on genome annotation. We developed the first methods of DNA transformation of *Megasphaera elsdenii*, developed selectable markers and chromosomal deletion methods opening this organism to advanced physiological studies and bioengineering. We used these tools to identify genes involved in valeric acid production and to engineer butanol production from lactic acid. We generated a metabolic reconstruction of glucose and lactic acid conversion to hexanoic acid using the DOE KBase platform. We continue to develop genetic tools to enable rapid and complex construction of strains capable of producing long chain carbon molecules at high yield and high titer.

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