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

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The native ability of the Gram-positive bacterium, *Megasphaera elsdenii*, to efficiently generate C4 to C8 compounds via the condensation of acetyl-CoA makes it a compelling platform for the production of fuels and chemicals from lactate and plant carbohydrates. Our overall objective is to develop *M. elsdenii* for the conversion of lignocellulosic biomass sugars and organic acids into hexanol and other valuable chemicals. It produces organic acids including butyric (four carbon), hexanoic (six carbon), and in some cases octanoic (eight carbon) acids as major fermentation products when grown on lactate or glucose, likely via a chain elongation pathway using acetyl-CoA units. As carbon chain length increases, fuel properties improve, with the energy density increasing and hygroscopicity decreasing. We used DOE KBase to generate a metabolic reconstruction of the conversion of glucose and lactate to hexanoic acid in *M. elsdenii* and designed a strategy for engineering the strain. We developed the first methods for DNA transformation of two strains and have continued to develop genetic tools to enable more rapid and complex strain construction. Using a combination of heterologous genes for aldehyde dehydrogenase and butanol dehydrogenase in *M. elsdenii*, we have demonstrated production of butanol. We continue to engineer the strain to produce long chain carbon molecules at high yield and high titer and will present a progress report on those efforts.

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