**Multi-omics analyses reveal temperature-induced metabolic changes in the industrially relevant microbe *Clostridium autoethanogenum* affecting its product profile**

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**Project Goals: The interdisciplinary clostridia Foundry for Biosystems Design (cBioFAB) project addresses the complex challenge of designing, building, and optimizing biosynthetic pathways in biological systems by combining efforts from university, government, and industry partners. The goal of the project is to accelerate engineering efforts in non-model organisms through in vitro and in vivo metabolic pathway prototyping, computational modeling, and integrated omics analysis. Through these diverse approaches, the project seeks to provide the tools to enable high-level synthesis of next-generation biofuels and bioproducts from lignocellulosic biomass and expand the breadth of platform organisms that meet DOE bioenergy goals.**

Even with the advent of next-generation sequencing, there are limited options for many key metabolic genes considered for next-generation biofuel and bioproduct synthesis, and most genes found in public repositories are derived from type strain or environmental sequences with unproven performance. To expand the pool of available sequences that are likely to result in high performance, we have sequenced and mined the largest collection of industrially deployed Clostridium strains, evolved over several decades of intense development. To rapidly prototype the performance of identified genes and to develop improved, industrial-robust production strains for conversion of lignocellulosic biomass to next generation biofuels and bioproducts, we are establishing a new interdisciplinary venture, the cBioFAB that combines advancements in cell-free and Clostridium engineering metabolic engineering.

The utilization of industrial waste gases or syngas (a mixture of $H_2/CO/CO_2$) is a sustainable alternative for the production of commodity chemicals and biofuels[1]. Syngas is readily fermented to valuable solvents via the Wood-Ljungdahl pathway inherent in the microbial catalyst, *Clostridium autoethanogenum*; an organism that has been deployed commercially for ethanol production. However, for achieving high ethanol productivity and process stability, factors affecting fermentation, such as pH, temperature, media composition, etc., must be understood and controlled. Studies on other *Clostridium* species have reported that both substrate consumption and solvent profiles are significantly impacted by changes in temperature. For example, increases in ethanol production are observed at temperatures lower than an organism's optimum[2]. While studies have determined the optimal growth temperature for *C. autoethanogenum*, few have explored its response outside its optimal range. Hence, studies investigating the effect of temperature on the growth, metabolism, and membrane fluidity of this industrially-relevant microbe are needed to inform future optimization efforts. To that end, *C. autoethanogenum* cultures grown at varying temperatures (30°C and 40°C) were characterized at a molecular level

using a multi-omics approach (metabolomics, proteomics, and lipidomics). Initial results show that while CO uptake was similar at the two temperatures, significant differences in the product profiles were evident. At 40°C, a smaller proportion of the product was ethanol (56% vs. ~73% at 30°C), as more carbon (C) flux was diverted towards acetate (27%). Proteomics analyses revealed that the increased ethanol production could be driven by enzymes considered critical for diverting C flux from acetate to ethanol by reduction of acetate to acetaldehyde (Aldehyde ferredoxin oxidoreductases)[3]. These enzymes were ~4X more abundant in the 30°C cultures. Metabolomics analyses revealed the accumulation of multiple glycerol-conjugated fatty acids at 40°C, suggesting a shift in C flux to fatty acid biosynthesis and glycerolipid metabolism pathways via acetate at higher temperatures. Additionally, differences in the constituent membrane phospholipid species were also observed at the two temperatures, which along with metabolomics results, suggest that adaptive alterations to the membrane fluidity could be occurring. Interestingly, glycerolipids accumulating at 40°C such as 1-myristoyl-glycerol, can be used as food emulsifiers and are commercially relevant. Beyond these, several other proteins (621 of the 1,831 proteins quantified) and metabolites (including some newly identified in this study) were differentially abundant between the two conditions, indicating that temperature substantially impacts the metabolism of *C. autoethanogenum*. Since this chassis organism can thrive at a range of temperatures (20°C - 44°C), the adjustment of fermentation operating parameters, combined with omics-guided metabolic engineering efforts, enable the generation of a variety of valuable products.

## References

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