Temperature drives substantial metabolic changes in the gas fermenting *Clostridium autoethanogenum* as revealed by multi-omics characterization

Payal Chirania[1,2*] (pchirani@vols.utk.edu), Richard J. Giannone,[1] Nancy L. Engle,[1] David T. Reeves,[1,5] Hunter Zeleznik,[3] Vicki Liu,[3] Loan Tran,[3] Steven D. Brown,[3] Michael Köpke,[3] Timothy J. Tschaplinski,[1] Robert L. Hettich,[1] and **Michael C. Jewett**[4]

[1]Oak Ridge National Laboratory, Oak Ridge, TN; [2]UT-ORNL Graduate School of Genome Science and Technology, University of Tennessee, Knoxville, TN; [3]LanzaTech Inc, Skokie, IL; [4]Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL; [5]The Bredesen Center, University of Tennessee, Knoxville, TN.

https://www.energy.gov/science/ber/articles/cell-free-technology-accelerates-industrial-biotechnology

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.
Non-model organisms have unique traits and offer significant advantages and benefits for biomanufacturing. One example is gas fermenting acetogens capable of converting low-cost waste feedstocks to fuels and chemicals, deployed today at commercial scale for conversion of steel mill emissions to ethanol. Yet, engineering these non-model organisms is challenging due to lower transformation and recombination efficiencies, longer cycle times and a more limited set of genetic tools compared to model organisms *E. coli* or yeast. Cell-free systems can guide and accelerate non-model organism strain development. This interdisciplinary venture, cBioFAB, combines advancements in cell-free and *Clostridium* engineering metabolic engineering to develop industrial-robust production strains for conversion of lignocellulosic biomass to next-generation biofuels and bioproducts.

Abstract Text: The fermentation of waste gases or syngas (a mixture of H_2/CO/CO_2) is a sustainable alternative for producing commodity chemicals and biofuels[1]. A small subset of acetogens, such as *Clostridium autoethanogenum*, can directly convert syngas into non-petroleum-based fuels, including ethanol, butanol, and other industrially-relevant chemicals. However, multiple factors such as pH, temperature, media composition, etc., affect end-product titers and process stability, and thus must be understood to better control product output. Studies on other acetogens have reported increases in ethanol production at temperatures lower than the organism's optimum[2]. However, few studies have examined the response of *C. autoethanogenum* outside its optimal growth temperature. Here, *C. autoethanogenum* cultures were grown at either 30°C or 40°C and characterized at a molecular level using a multi-omics approach (metabolomics, proteomics, and lipidomics). Notably, the product profiles varied between the two temperatures; at 40°C, the proportion of ethanol in liquid product was reduced to ~56% compared to ~73% at

30°C, with more carbon diverted towards the production of acetate (~27% compared to ~9% at 30°C) at the higher temperature. Initial omics analyses revealed that enzymes from both ethanol producing pathways (i.e., from acetate or acetyl-CoA) exhibited significantly reduced abundances at 40°C. In contrast, enzyme abundances for other products (acetate, lactate, and 2,3-butanediol) increased at the higher temperature. High temperature caused a dramatic metabolic shift in the microbe, whereby ~63% of the quantified proteins showed a significant difference in abundance. Among these, proteins from predicted bacterial microcompartment (BMC) gene clusters exhibited the highest fold changes with temperature. At 40°C, multiple proteins from the relatively smaller BMC cluster CAETHG_3273-3290 (18 genes) decreased, whereas those from the larger BMC cluster CAETHG_1810-1841 (32 genes) increased in abundance. Whereas both correspond to glycyl radical enzyme microcompartments (GRMs), limited information is available about the role of these temperature responsive BMCs. Interestingly, the larger cluster is a GRM1-type BMC which is predicted to metabolize choline into acetyl-phosphate or ethanol, which could further explain the increased acetate levels observed at 40°C. Although not grown on choline, the upregulation of these proteins could affect lipid metabolism and may explain the altered carbon partitioning leading to the large and diverse accumulation of alkyl-glycerol conjugates, including 1-myristoyl-glycerol, 1-palmitoleoyl-glycerol, and 1-myristoleoyl-glycerol, at 40°C as revealed by metabolomics. Overall, the acetogen undergoes significant metabolic changes to adapt to the temperature change, all of which provide key insights into its metabolism. 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, offer opportunity to direct the generation of a range of products.

References

1. Fackler, N.; Heijstra, B. D.; Rasor, B. J.; Brown, H.; Martin, J.; Ni, Z.; Shebek, K. M.; Rosin, R. R.; Simpson, S. D.; Tyo, K. E.; Giannone, R. J.; Hettich, R. L.; Tschaplinski, T. J.; Leang, C.; Brown, S. D.; Jewett, M. C.; Kopke, M., Stepping on the Gas to a Circular Economy: Accelerating Development of Carbon-Negative Chemical Production from Gas Fermentation. *Annu Rev Chem Biomol Eng* **2021,** *12*, 439-470.
2. Kundiyana, D. K.; Wilkins, M. R.; Maddipati, P.; Huhnke, R. L., Effect of temperature, pH and buffer presence on ethanol production from synthesis gas by "*Clostridium ragsdalei*". *Bioresource Technol* **2011,** *102* (10), 5794-5799.

This work is sponsored by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research. Award number DE-SC0018249.