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UNRAVELING ACETOGEN GAS FERMENTATION USING QUANTITATIVE SYSTEMS BIOLOGY

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The world faces an increasing need for sustainable production of fuels and chemicals. Recently, acetogens have gained significance as cell factories for converting inexpensive and abundant waste feedstocks (e.g. syngas [CO, H2, CO2], industrial waste gases) into high-value products using gas fermentation. Furthermore, acetogens are ancient microorganisms as they arguably use the first carbon fixation pathway on Earth to fix CO2. Although biochemistry of the Wood-Ljungdahl pathway is well understood, better understanding of acetogen metabolism is needed for their rational metabolic engineering. We have developed a systems biology platform for advancing the understanding and engineering of acetogen metabolism through unravelling regulatory features and building the first quantitative links between carbon, energy, and redox metabolism. We used steady-state autotrophic chemostat cultivation coupled to quantitative gas, transcriptomics, proteomics, metabolomics analyses to reconstruct acetogen metabolism in silico using genome-scale metabolic models and thermodynamic metabolic flux analysis. This systems-level approach led us to propose a novel regulatory mechanism for carbon distribution and factors limiting metabolic robustness in acetogens. Metabolic modelling could accurately predict growth phenotypes and absolute proteomics data quantified allocation of protein resources in the model-acetogen Clostridium autoethanogenum. Coupling flux and proteomics data identified the prevalence of post-translational regulation of metabolic fluxes by adjustment of apparent in vivo catalytic rates of enzymes. We further used differential RNA-sequencing to decipher transcriptional architecture and to identify a key novel promoter motif together with a transcriptional regulator associated with essential genes for autotrophic growth of acetogens. Our work both serves as a reference dataset and advances fundamental understanding of regulation in the ancient metabolism of acetogens. It also suggests strategies for improving the performance of gas-fermenting acetogens through rational metabolic engineering.