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Metabolic control of *Clostridium thermocellum* via selective inhibition and compensatory product formation

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Clostridium thermocellum is a thermophilic, anaerobic bacterium that catabolizes recalcitrant plant fibers such as cellulose. Cellulose is depolymerized by an extracellular, membrane-associated enzyme system, and the sugars are then transported across the cell membrane for fermentation. C. *thermocellum* produces ethanol, but only as part of mixed-acid fermentation. Acetate is the major product under optimal growth conditions, and cellular reducing equivalents (e.g. NAD^+) are regenerated via hydrogenase enzymes, which produce H_2. However, reducing equivalents can also be recovered with dehydrogenase enzymes, such as lactate dehydrogenase and acetaldehyde/ethanol dehydrogenase. The ethanol yield was improved by as much as 350% when H_2 production was selectively inhibited with hydrogenase inhibitors, exogenous H_2, or elevated hyperbaric pressure. However, these gains were only realized when lactate production was controlled by limiting the rate of substrate (sugar) transport. Substrate limitation was achieved in a chemostat or by inhibition of sugar transport across the cell membrane. Fortuitously, the hydrogenase inhibitor methyl viologen (paraquat) also inhibited sugar transport by C. *thermocellum*, and stimulated ethanol production. The concept of driving compensatory product formation via selective inhibition of metabolic pathways is broadly applicable to microorganisms that make more than one product. In particular, the clostridia (including those that produce other solvents) are amenable to selective inhibition because of the sensitivity of their hydrogenases.

Biography

Michael Flythe received a Ph.D. in microbiology from Cornell University in 2006. He currently serves as a Research Microbiologist in the USDA-Agricultural Research Service, and on the faculty of the University of Kentucky. Flythe has authored more than 30 articles, proceedings, and book chapters on microbial physiology in the context of cellulosic biomass catalysis, silage production, and the gastrointestinal tracts of herbivorous animals.

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