DIRECT CELL-TO-CELL EXCHANGE OF MATTER IN SYNTHETIC *CLOSTRIDIUM* SYNTROPHIES ENABLING CO_2 FIXATION AND AN EXPANDED METABOLIC SPACE

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In microbial fermentations to produce metabolites, at least 33% of the sugar-substrate carbon is lost as CO_2 during pyruvate decarboxylation to acetyl-CoA. Previous attempts to reduce this carbon loss focused on engineering a single organism. In nature, microorganisms live in complex communities where syntrophic interactions result in superior resource utilization. Microbial communities are ubiquitous in nature and have a wide range of applications, including production of biofuels and chemicals. Syntrophic and other microbial co-cultures/consortia carry out efficient bio-transformations that are the result of multiple complementary metabolic systems working together. It is now well appreciated that the capabilities of multi-microorganism systems cannot be predicted by the sum of their parts. Rather, synergistic interactions at different levels often result in better overall performance of these systems. Importantly, integration of diverse metabolic systems through syntrophic dependencies make co-culture systems robust to environmental fluctuations.
Clostridium organisms are of major importance for developing new technologies to produce biofuels and chemicals. Three major types of *Clostridium* organisms have been the focus of studies for the sustainable production of fuels and chemicals. Solventogenic clostridia utilize a large variety of biomass-derived carbohydrates (hexoses, pentoses, disaccharides, and hemicellulose), and can produce C2-C4 chemicals. Acetogenic clostridia can fix inorganic H_2, CO_2, and CO to generate C2 acids and alcohols. Other specialized clostridia possess diverse biosynthetic capabilities for production of a wide variety of metabolites including C4 – C8 carboxylic acids and alcohols, which could serve as commodity chemicals, biofuels, or biofuel precursors. Here, we first examined a synthetic syntrophy consisting of the solventogen *Clostridium acetobutylicum*, which converts simple and complex carbohydrates into a variety of chemicals, and the acetogen *C. ljungdahlii*, which fixes CO_2. This synthetic co-culture achieved carbon recoveries into C2-C4 alcohols almost to the limit of substrate-electron availability, with minimal H_2 and CO_2 release. The syntrophic co-culture produced robust metabolic outcomes over a broad range of starting population ratios of the two organisms. Significantly, the co-culture exhibited unique direct cell-to-cell interactions and material exchange among the two microbes, which enabled unforeseen rearrangements in the metabolism of the individual species that resulted in the production of non-native metabolites, namely isopropanol and 2,3-butanediol. Next, we expanded this co-culture system to include *C. kluyveri*, which can metabolite ethanol and acetate to produce C6 and C8 carboxylic acids. Both *C. acetobutylicum* and *C. ljungdahlii* produce ethanol and acetate, which makes *C. kluyveri* and ideal partner for a triple synthetic co-culture system capable to converting biomass-derived carbohydrates to C6 and C8 chemicals.

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