Synthetic & Syntrophic Clostridium Co-culture Enables a Superior Metabolism, Cell Fusion & Material Exchange, and the Formation of Hybrid Bacteria

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ABSTRACT

Our demand for energy is steadily growing, and despite the current abundance and low cost of fossil fuels, the reserves of petroleum, coal, and natural gas are quickly being depleted. As a result, new technologies capable of utilizing low value feedstocks, like biomass, and waste gasses (H₂, CO, CO₂) must be developed to meet our need for fuels and commodity chemicals. A new and interesting solution is to utilize the capabilities of organisms found in nature. One example is the solventogenic Clostridium acetobutylicum, which can utilize a wide variety of sugar substrates, most notably all 6-C and 5-C monosaccharides, as well as complex polysaccharides, like starch and hemicellulose (a major component of biomass) to produce solvents, such as acetone, butanol, and ethanol, in the process known as the Acetone-Butanol-Ethanol (ABE) fermentation. Unfortunately, in microbial fermentations at least 33% of the sugar-substrate carbon is lost as CO₂ during pyruvate decarboxylation to acetyl-CoA, with the corresponding electrons lost in the form of H₂ gas. Previous attempts to reduce this carbon and electron loss focused on engineering of single organisms. In nature, microbial syntrophy is universal in nature, profoundly affecting the composition and function of microbiomes. Therefore, presented here is 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 captures the CO₂ and H₂ waste. The co-culture achieved carbon recoveries into C2-C4 alcohols almost to the limit of substrate-electron availability, with minimal H₂ and CO₂ release, and produced robust metabolic outcomes. Thus, syntrophic cultures offer a flexible platform for metabolite production, with
superior carbon recovery that can be applied to electron-enhanced fermentations enabling even higher carbon recoveries.

Beyond superior fermentation yields, this syntrophic co-culture exhibited direct cell-to-cell fusion events and the direct material exchange among the two microbes. This 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. Furthermore, transmission electron microscopy demonstrated cell-wall and cell-membrane fusions between the two organisms, where *C. ljungdahlii* appeared to invade *C. acetobutylicum* through pole-to-pole fusion. Correlative fluorescence-transmission electron microscopy and flow cytometry demonstrated large-scale exchange of proteins and RNA between the two organisms. Dividing hybrid cells were identified containing stained proteins from both organisms, thus demonstrating the persistence of cells with exchanged cellular components, here defined as *hybrid* bacterial cells. The formation of hybrid bacterial cells facilitated the unique co-culture phenotype, and the production of non-native compounds. Moreover, the exchange of plasmid, and possibly genomic, DNA was observed in the co-culture, indicating the cell-to-cell fusion may be a new mechanism for the horizontal gene transfer in bacteria. Although unanticipated and never previously reported, these phenomena are likely widely distributed in nature, have profound implications for species evolution and the function of microbial communities, and could find utility in biotechnology. They may shed a new light onto little-understood phenomena such as antibiotic heteroresistance of pathogens, pathogen invasion of human tissues, and the evolutionary trajectory and persistence of unculturable bacteria.