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Synthetic co-cultivation of A. woodii and drakei for production of medium-chain organic acids from H2 and CO2

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Gas fermentation could play a critical role in transitioning towards a renewable circular industry and achieving CO2-emission goals. The acetogen *Acetobacterium woodii* is known for its fast utilization of CO2 and H2, and native acetate production. While recombinant strains were able to produce acetone, isobutanol, and lactate, a broadening of the product spectrum is essential for future application prospects. A further option is the co-cultivation of the CO2 assimilator *A. woodii* with natural chain-elongators such as *Clostridium drakei*. Lactate was chosen as an intermediate metabolite to combine the capabilities of both strains in a synthetic co-culture. An *A. woodii* strain was engineered by deleting its native lactate dehydrogenase and introducing a fluorescence-tagged D-lactate dehydrogenase (LDHD) from *Leuconostoc mesenteroides,* which enables lactate production. The fluorescence-activating and absorption-shifting tag protein (FAST) was N-terminally fused to the LDHD to confirm gene expression. Autotrophic bottle-scale co-cultivations of the engineered strain with *C. drakei* resulted in the production of 4 mM caproate and 18.5 mM butyrate from H2 and CO2. The FAST-mediated fluorescence was similar in intensity to monocultures of the engineered *A. woodii* strain, while lactate was only measured in traces, indicating lactate consumption by *C. drakei*. Furthermore, the synthetic co-culture was tested in a stirred-tank bioreactor with *in-situ* H2 generation, representing the first proof-of-concept for this method of caproate production.