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Autotrophic lactate production from H_2 + CO_2 using recombinant and fluorescent FAST-tagged *Acetobacterium woodii* strains

Frank R. Bengelsdorf * [1], Alexander Mook [1], Jan Herzog [2], Lotta Guhl [2], Miriam Bäumler [3], Jonathan P. Baker [4], Nigel P. Minton [4], Peter Dürre [1], Dirk Weuster-Botz [3], An-Ping Zeng [2]

[1] Ulm University – Germany
[2] Hamburg University of Technology – Germany
[3] Technical University of Munich – Germany
[4] University of Nottingham – United Kingdom

Autotrophic lactate production was achieved by metabolic engineering of *Acetobacterium woodii*. Therefore, the genes (*lctBCD*) of the native Lct dehydrogenase complex, which is mediating consumption of lactate, were knocked out. Next, *A. woodii* was engineered to express a gene coding for a D-lactate dehydrogenase (LDHD) that originated from *Leuconostoc mesenteroides*. Additionally, this LDHD was N-terminally fused to the oxygen-independent fluorescence-activating and absorption-shifting tag (FAST). In autotrophic batch experiments using H2 + CO2, cells expressing the LDHD fusion protein produced up to 18.8 mM of lactate (Mook et al., 2022). The same strain produced 8.1 g L-1 of lactate (91 mM) with a rate of 0.27 g L-1 h-1 in a batch operated stirred-tank bioreactor with continuous gassing (Herzog et al. 2022). The N-terminal FAST caused a clear and brilliant fluorescence during exponential growth and in the stationary phase. For cells expressing the FAST-tagged LDHD fusion protein, flow cytometry at the single-cell level revealed phenotypic heterogeneities within the cell-culture (Mook et al., 2022). Thus, FAST provides a useful reporter tool to analysis gene expression during growth of *A. woodii* and enables quicker and more targeted strain optimization.