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High-level ethanol production by metabolically engineered *Lactococcus lactis* using economically renewable feedstocks

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Lactococcus lactis, one of the best characterized lactic acid bacteria (LAB), is well-established within the dairy industry and has been demonstrated to have potential as a cell factory for a broad range of useful compounds. Arguments for choosing *L. lactis* as production platform include its high glycolytic flux, well-characterized metabolic network, well-developed toolbox and general robustness. Here we provide another good example of how *L. lactis* can serve as a cell factory, by engineering a derivative that can convert waste stream material from the dairy industry into ethanol. The engineering efforts include substantial rewiring of metabolism, where we 1) inactivate competitive pathways by knocking out lactate dehydrogenase (LDH), phosphotransacetylase (PTA) and native alcohol dehydrogenase (ADHE), 2) introduce the heterologous enzymes-pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADHB) sourced from *Zymomonas mobilis*, and finally 3) incorporate lactose metabolism (substrate engineering). High yield and titer ethanol production is achieved using a low-cost medium containing a cheap nitrogen source derived from corn steep liquor. The results obtained demonstrate great potential for commercial production of ethanol.