Poster Number 293: Engineering flocculent *Saccharomyces cerevisiae* strains for efficient lactose to ethanol fermentation.

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Lactose is a substrate available in large amounts due to the surplus of cheese whey (by-product of dairy industries) existing worldwide. Besides many other applications, lactose is an interesting feedstock for fermentation processes, namely for the production of bulk commodities such as bioethanol. Whey fermentation yields potable ethanol that can be used as fuel but also in food and beverage industries. There are many lactose-consuming microorganisms but most do not present physiological characteristics suitable for ethanol production bioprocesses. Therefore, there has been considerable research and development effort put in the construction of genetically engineered *Saccharomyces cerevisiae* strains able to ferment lactose. Our group has constructed a lactose-fermenting *S. cerevisiae* strain expressing the *LAC12* (permease) and *LAC4* (lactase) genes of *Kluyveromyces lactis*. The host was a highly flocculent strain, since we envisaged high cell density and continuous fermentation systems. The properties of the original recombinant were further improved using evolutionary engineering approaches, which yielded an evolved strain that fermented lactose faster with higher ethanol yield and with improved flocculation. In batch fermentations with concentrated whey containing 150 g/L initial lactose, the evolved strain produced 7% (v/v) ethanol in about 120 h. Supplementation of the whey with 10 g/L of corn steep liquor increased final ethanol to 9% obtained after 42 h of fermentation.