

## Abstract

*Zymomonas mobilis* is well-known for its ability to produce ethanol at high yields and titres, and has been used for industrial-scale ethanol production. It has an extremely high glucose uptake rate, a low biomass to product ratio, and ferments glucose to almost theoretical yields of ethanol using the Entner-Doudoroff (ED) pathway. *Z. mobilis* lacks a complete TCA cycle and hence converts pyruvate to ethanol to regenerate cofactors – NAD and NADP – for maintaining redox balance.

Metabolic engineering studies done so far to assess the potential of *Z. mobilis* as a production host have focussed mainly on two aspects – engineering of biosynthetic pathways for synthesizing products beyond ethanol; and broadening substrate range for ethanol production.

For synthesizing products beyond ethanol, *Z. mobilis* has been explored for the production of 2,3-butanediol, isobutanol, polyhydroxybutyrate, and lactic acid (LA) by introducing respective pathway genes. Among these, LA is an important platform chemical with applications in food, cosmetics, and chemical industries; and as a monomer for the production of polylactic acid. Presently, the majority of LA (~90%) is produced through the microbial fermentation route, which offers high enantiomeric purity (specificity for L-LA or D-LA) and benign operating conditions. LA fermentation involves a lactate dehydrogenase (LDH) enzyme that couples the reduction of pyruvate to lactate with the oxidation of NADH to NAD. The type of enzyme – L-LDH or D-LDH – involved in the production of LA, determines the enantiomer (L-LA or D-LA) produced by a microorganism.

Native producers of LA – lactic acid bacteria – have complex nutritional requirements, which makes LA purification an expensive process. However, yeast and other microbial hosts with simpler nutritional requirements have been engineered for LA production by heterologous expression of LDH from different sources. This has been combined with reduction of fluxes

toward ethanol formation pathway in native ethanol producers (yeast and *Z. mobilis*) for increasing yields of LA. In *Z. mobilis*, D-LA production was accomplished by introducing a D-LDH encoding gene from *E. coli* and down-regulating PDC activity. However, the engineered strain showed an equimolar amount of ethanol formation alongside D-LA

The synthesis of products other than ethanol at high titres in *Z. mobilis* requires redirection of flux away from ethanol. Previous studies that attempted to block ethanol formation by knocking out PDC were unsuccessful, most likely due to PDC being an essential gene in *Z. mobilis*. Thus, exploration of other approaches for rediverting flux away from ethanol is essential.

For broadening substrate range, most studies in *Z. mobilis* have focussed on introducing pathways for utilization of pentose sugars. *Z. mobilis* can only metabolize hexose sugars natively; therefore, pathways for the uptake of pentose sugars like xylose and arabinose have been introduced to broaden this bacterium's substrate range. For xylose utilization in *Z. mobilis*, previous studies have used xylose assimilation genes from *E. coli* followed by adaptive lab evolution to improve xylose uptake rates.

In this work, we attempted to engineer *Z. mobilis* for the production of L-lactic acid and D-lactic acid; and utilization of xylose. We also attempted molecular engineering strategies to alter transcription and translation efficiency of the *pdc* gene or completely disrupt it.