Abstract

Zymomonas mobilis is an important microbial host that produces ethanol at high yields and titres, and has been used for industrial ethanol production. The synthesis of products other than ethanol at high titres in *Z. mobilis* requires redirection of flux away from ethanol; additionally, *Z. mobilis* is natively able to grow on hexose sugars but not pentose sugars. Here, we attempted to engineer *Z. mobilis* for the production of L-lactic acid and D-lactic acid; and utilization of xylose.

We identified highly active LDHs [L-LDH from *Bacillus subtilis* and D-LDH from *Lactobacillus delbrueckii subsp. Bulgaricus*] for LA production in *Z. mobilis*. We then targeted disruption of ADH by deletion of *adh1* and *adh2* genes that are known to be responsible for ethanologenic ADH activity. Expression of the identified *ldh* gene combined with deletion of *adh1* and *adh2* led to almost complete redirection of carbon flux from ethanol to LA. The resulting L-LA producing strain (Zm-BsLΔA1ΔA2) and D-LA producing strain (Zm-LdLΔA1ΔA2) formed LA at high yield (~0.4 g/g glucose) and had significantly reduced yields of ethanol (~0.03 g/g glucose).

However, these strains utilized low amounts of glucose and showed growth cessation that could not be overcome even after adaptively evolving the strains. We found that growth cessation was caused by a reduction in pH because of LA formation, and that pH-controlled fermentations could overcome it. Batch fermentations with pH control showed complete consumption of 40 g/l glucose with production of L-LA and D-LA at high yields (~0.85 g/g glucose) and titres (~32 g/l).

The best D-LA producing strain was evolved for growth at higher glucose concentrations under low oxygen availability to increase LA titres further. Batch fermentations of the evolved D-LA strain conducted in shake-flasks under limited oxygenation showed complete utilization of 100

g/l glucose and production of ~83 g/l D-LA. The evolved strain was also able to grow on minimal medium with starting glucose concentrations of 40 g/l glucose and produced ~33 g/l D-LA. Moreover, the strain could grow robustly in a bioreactor with low aeration requirements and showed efficient production of D-LA on rich and minimal media with titres and yields comparable to shake-flask experiments.

We also attempted molecular engineering strategies to alter transcription and translation efficiency of the *pdc* gene or completely disrupt it. However, there were no changes in ethanol production by use of these strategies.

For xylose utilization, we introduced four xylose pathway genes from *B. subtilis* via genome integration. The engineered strains were further evolved for growth on xylose using multiple ALE protocols. However, the evolved strains showed extremely weak growth on plates containing xylose and no growth in liquid medium containing xylose.

Overall, this work showed L-LA and D-LA production in an engineered *Z. mobilis* strain at yields and titres comparable to those of native LA producers. This demonstrates the use of metabolic engineering for redirecting flux away from ethanol in *Z. mobilis* and furthers its use for generating non-native products.