Construction of a platform strain for anaerobic bioprocessing and its application to biofuel production

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Report

We constructed a *Lactobacillus plantarum* double-knockout mutant for the L- and D-lactate dehydrogenase genes. By overexpressing pyruvate decarboxylase and alcohol dehydrogenase in this double-knockout strain, we were able to produce 56.7 g/L of ethanol from 150 g/L of glucose (at 100 h of fermentation). Unlike conventional metabolic engineering hosts, such as *Saccharomyces cerevisiae* or *Escherichia coli*, our double-knockout strain can be used to produce various value-added products, without aeration or vigorous agitation. This anaerobic bioprocessing system should allow the development of economical bio-production processes.

Research aims

Metabolic engineering is a promising method for maximizing the production of specific metabolites. *S. cerevisiae* and *E. coli* are common hosts for metabolic engineering. However, these microorganisms require an aerobic growth phase (with aeration and vigorous agitation) before the fermentation production phase. As a consequence, bioproduction systems are often economically unsustainable, particularly for the bulk production of chemicals or biofuels. Moreover, because of the requirement for aeration during the growth phase, it is usually challenging to scale-up the fermentation process. This study aimed to construct a platform microorganism for anaerobic bioprocessing (ABP), which requires neither aeration nor vigorous agitation.

Methods

The bacterium *L. plantarum* maintains a similar growth rate under both aerobic and anaerobic conditions, which enables the growth-associated production of lactate without the need for aeration and vigorous agitation. Therefore, *L. plantarum* is a suitable host for ABP. However, the strain converts most of the fermented sugar into lactate.

To prevent the production of lactate, we disrupted both L- and D-lactate dehydrogenase genes (*ldhL1* and *ldhD*, respectively) in *L. plantarum* (Fig. 1). Moreover, to redirect the phosphoketolase pathway (which produces equimolar amounts of pyruvate and acetate from pentose sugars) to the pentose phosphate pathway (which only produces pyruvate from pentose sugars), we replaced the phosphoketolase gene (*xpk1*) with the transketolase gene (*tkt*) from *Lactococcus lactis*. Next, to promote ethanol fermentation, we introduced in the Δ*ldhL1* Δ*ldhD* strain pyruvate decarboxylase (*PDC*) and alcohol dehydrogenase (*ADH*) genes from various origins. Finally, for the strain showing the highest ethanol titer in our initial tests, ethanol fermentation was performed in a 1 L jar fermenter using a glucose concentration of 150 g/L.

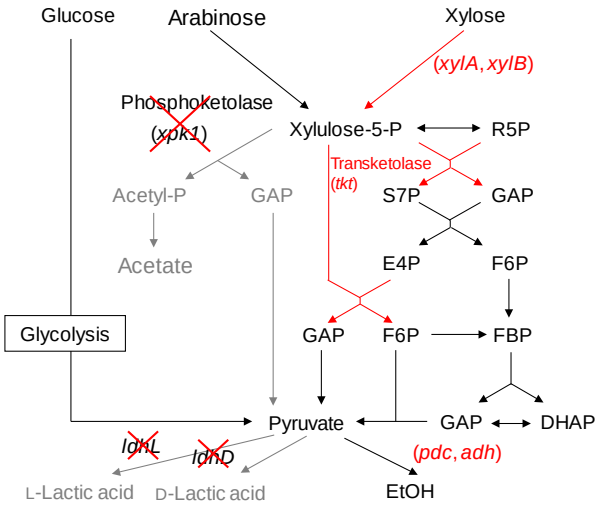

Fig. 1. Metabolic pathways in the ABP platform microorganism.

Results

We have previously reported the construction of a Δ*ldhD* strain of *L. plantarum*, which was confirmed to produce L-lactate with a high optical purity[1]. To construct the Δ*ldhL1* Δ*ldhD* strain, we disrupted the *ldhL1* gene in the Δ*ldhD* strain. As expected, the resulting Δ*ldhL1* Δ*ldhD* strain was unable to produce lactic acid. Remarkably, this strain could only grow under aerobic conditions, probably due to an inability to oxidize the NADH produced through glycolysis. Furthermore, the disruption of the *xpk1* gene in the Δ*ldhL1* Δ*ldhD* strain hindered cell growth on pentose. However, we were able to restore cell growth by introducing the *tkt* gene into the disrupted *xpk1* locus. Since *L. plantarum* can naturally use arabinose but not xylose, we are now trying to introduce in the Δ*ldhL1* Δ*ldhD* strain the *xylA* and *xylB* genes to allow the strain to use xylose as an alternative carbon source.

To evaluate the Δ*ldhL1* Δ*ldhD* strain as a platform microorganism for ABP, we expressed in this strain the *PDC* and *ADH* genes to promote ethanol production (Fig. 1). We tested the *PDC* genes from three different bacteria, *Acetobacter pasteurianus* (*ApPDC*), *Zymomonas mobilis* (*ZmPDC*), and *Lactobacillus florum* (*LfPDC*), and the *ADH* genes from *Oenococcus oeni* (*OoADH*) and *Z. mobilis* (*ZmADH*). We first performed ethanol fermentation using the different *PDC* and *ADH* expression strains, and a glucose concentration of 100 g/L (Fig. 2).

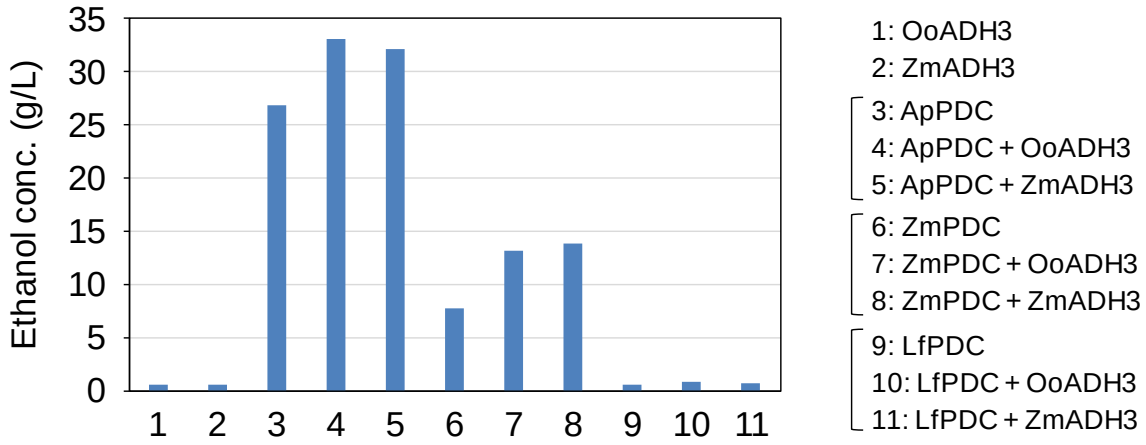

Fig. 2. Ethanol fermentation in Δ*ldhL1* Δ*ldhD* strains expressing the indicated PDC and ADH genes. All strains were grown for 48 h at 37°C in 100 mL flasks, using 30 mL of modified MRS medium supplemented with 100 g/L glucose and 15 g/L $CaCO_3$.

While the strains expressing *OoADH* or *ZmADH* alone did not produce ethanol, those expressing *ApPDC* or *ZmPDC* were able to produce ethanol. This result suggests that *L. plantarum* endogenous *ADH* can catalyze the reduction of acetaldehyde to ethanol. Moreover, the expression of *ApPDC* or *ZmPDC* restored the anaerobic growth of the Δ*ldhL1* Δ*ldhD* strain. Our data also show that the co-expression of *PDC* and *ADH* further increased the ethanol titer, with the *ApPDC*/*OoADH* strain producing the highest titer of ethanol of 33.0 g/L.

Encouraged by these results, we used the *ApPDC*/*OoADH* strain to perform a scaled-up ethanol fermentation, using a 1 L jar fermenter and a glucose concentration of 150 g/L. Under these conditions, the *ApPDC*/*OoADH* strain produced 56.7 g/L of ethanol in 100 h of fermentation (Fig. 3). The observed ethanol yield (g per g of consumed sugar) was 0.40, which was lower than the theoretical yield of 0.51. Since we found that lactate and acetate were still being produced, further improvement of the metabolic pathways in the Δ*ldhL1* Δ*ldhD* strain will be required to improve ethanol yield and purity.

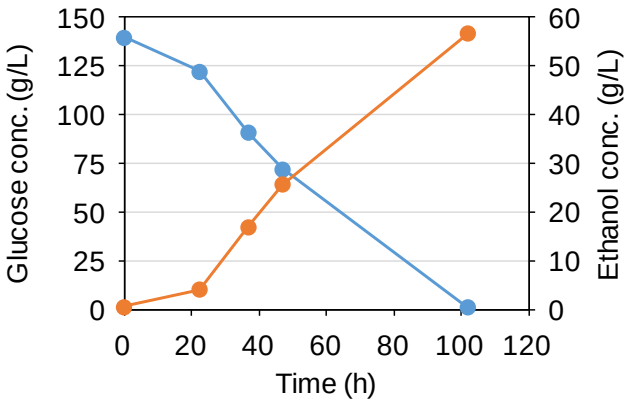

Fig. 3. Ethanol fermentation using the *ApPDC*/*OoADH* strain. Temperature and pH were maintained at 37°C and 6.0, respectively.

Conclusion

In this study, we successfully produced ethanol using an engineered *L. plantarum* strain, which was unable to produce lactate. Remarkably, the strain enabled the growth-associated production of ethanol under anaerobic conditions and without aeration or vigorous agitation. We believe that this ABP system using a platform microorganism can be further developed to allow the economical production of various chemicals and biofuels.

References

1) Okano, K., Uematsu, G., Hama, S., Tanaka, T., Noda, H., Kondo, A., and Honda, K. (2018) Metabolic engineering of *Lactobacillus plantarum* for direct L-lactic acid production from raw corn starch. *Biotechnol. J.* **13:** 1700517.