Transforming Lactococcus lactis into a microbial cell factory - DTU Orbit (23/04/2019)

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Biological conversion of lignocellulosic biomass to biofuels and -chemicals is a promising technology to reduce dependency on fossil fuels. This is important considering the environmental problems associated with consumption of the fossil fuels together with the fact that the reserves are limited and will be depleted if the increasing demand continues. However, one of the main challenges in the biological conversion is the identification of suitable platform organisms that can convert all the sugars present in the lignocellulosic biomass, including xylose. The aim of this PhD project was to investigate the potential of Lactococcus lactis as a platform organism for production of biofuels and -chemicals with a focus on characterization and optimization of the xylose metabolism. The plant isolate L. lactis KF147 was selected as the potential platform organism due to its natural ability to utilize both the pentose sugars xylose and arabinose. One of the desirable traits of a good platform organism is that it is easy to manipulate genetically. Since genetic manipulation usually involves introducing exogenous DNA, it is important that suitable methods are available. For this reason a standard protocol for preparing competent L. lactis KF147 cells was optimized resulting in a 100-fold increase in the transformation efficiency. Tools for introducing genes are likewise important. To expand the repertoire of genetic engineering tools available for L. lactis a novel tool named Repetitive Marker-Free Site-Specific Integration was developed. This tool facilitates repetitive rounds of site-specific integration of genes into the chromosome of L. lactis without leaving behind undesired vector elements. The site-specific integration is based on elements from the temperate lactococcal phage TP901-1, whereas excision of undesirable elements relies on a modified Cre-loxP system and 5-fluoroorotate mediated counter-selection. The plasmid used for the site-specific integration was termed pKv6 and when it is used for integrating genes, a new attachment site, attBmin, is likewise introduced, which can subsequently be used for the next round of integration. The xylose metabolism in L. lactis KF147 was characterized in a defined medium supplemented with 0.2%, 1% or 3% (w/v) xylose. The defined medium contains free arginine, and it was found that L. lactis KF147 co-metabolizes the arginine through the arginine deiminase pathway. To simplify further analysis arcA encoding the arginine deiminase was deleted, thus eliminating the arginine catabolism. We found that in L. lactis KF147 xylose is metabolized through two pathways namely the phosphoketolase pathway and the non-oxidative part of the pentose phosphate pathway. The only products formed were lactate, formate, acetate and ethanol, and the composition of the products depended on the xylose concentration. As xylose concentration increased, the proportion of xylose metabolized through the pentose phosphate pathway also increased. The effect from deleting and over-expressing the phosphoketolase pathway was also studied. The latter was achieved by introducing an additional copy of the ptk gene encoding the phosphoketolase. When the phosphoketolase pathway was deleted the product composition was similar to a typical mixed-acid pattern where formate, acetate and ethanol are formed in the ratio 2:1:1 in addition to lactate. For the strain with an inactive phosphoketolase pathway as well as for its parent, lactate production increased with xylose concentration, but in the phosphoketolase deficient strain up to almost three times more lactate was formed. In contrast when ptk was overexpressed the flux through the phosphoketolase pathway was 1.5 times higher compared to the parent strain when grown with 3% xylose. In addition, a clear shift towards a more mixed-acid fermentation profile was observed. The effect of adding additional arginine to the medium was likewise investigated in regards to growth of L. lactis KF147 and product composition. At low xylose concentration (0.2%) additional arginine greatly stimulated the growth of L. lactis KF147. In contrast, no effect of growth was observed at the higher xylose concentrations (1%, 3%). Irrespective of xylose concentration, arginine did affect the xylose and product fluxes, which all decreased as the arginine concentration increased. The final product yields were, however, not affected. L. lactis KF147 was also grown in rich M17 medium with xylose which showed that product composition strongly depends on the growth medium as the yield of lactate per xylose increase significantly. The pentose phosphate pathway present in L. lactis KF147 and other genome sequenced L. lactis strains is, however, a modified version of the known pentose phosphate pathway as no transaldolase gene is present in any of the strains. A codon-optimized version of the transaldolase gene ywJh from B. subtilis 168 was introduced in the phosphoketolase deficient strain. The effect of the introduced transaldolase gene was investigated in defined medium with 0.2% and 3% (w/v) xylose; however, no significant effects on either the growth rates or product formation were observed; even though expression of the introduced transaldolase was confirmed. The xyIT gene, predicted to encode a D-xylitol/linked symporter, was deleted and the effect on growth evaluated in defined medium with 0.2% and 1% (w/v) xylose. Only at the low xylose concentration was a reduced growth rate observed for the ΔxyIT strain compared to the parent strain. Based on this it was deduced that more than one transport system for xylose is present in L. lactis KF147. An adaptive evolution experiment was carried out with the goal of isolating mutants with improved growth on xylose. For this purpose the phosphoketolase deficient strain was applied. Of the 44 evolved strains screened for improved growth on xylose, three were selected for further detailed studies where specific growth rate and product formation were determined. These three strains underwent genome sequencing as well. The most interesting evolved strain was AD29 that, as the only of the investigated strains, exhibited both a pronounced accelerated growth on xylose (62.5% faster), and a changed fermentation profile with a clear increase in lactate production and corresponding drop in the production of formate, acetate, and ethanol. Three adaptive mutations were identified in AD29. Two is by all accounts involved in regulatory mechanisms either to stress (yhfB) or more globally (ytfF), and the last facilitate improved uptake of xylose (ptnC). Based on the above findings we conclude that L. lactis KF147 possesses many of the features a platform organism need, however whether the industry will find it attractive remains to be seen.

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