Genetic engineering of lactic acid bacteria *Lactococcus lactis* for growth on cellulose

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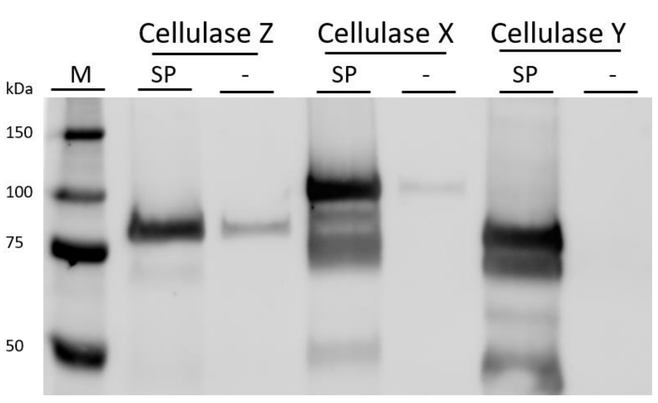
INTRODUCTION

Agriculture, the wood industry, the food industry, the paper industry, and households generate large amounts of lignocellulosic waste, which could be used as a cheaper and more sustainable alternative for the microbial fermentation of high-value chemicals such as lactic acid. The main component of lignocellulosic biomass is cellulose. In order to grow on cellulose, bacteria must be equipped with a set of enzymes that can degrade cellulose. These enzymes are cellulases and they can be anchored to the cell wall of the bacteria or secreted freely into extracellular environment. The products of cellulose degradation are cellodextrins of varying lengths. For their transport into the cell, the bacteria must have appropriate transporters. For the degradation of the imported cellodextrins, the bacteria must also be equipped with enzymes that break them down into individual glucose units, which in turn can be used in metabolism. Since lactic acid bacteria do not possess endogenous cellulases, genetic engineering for heterologous expression is possible.

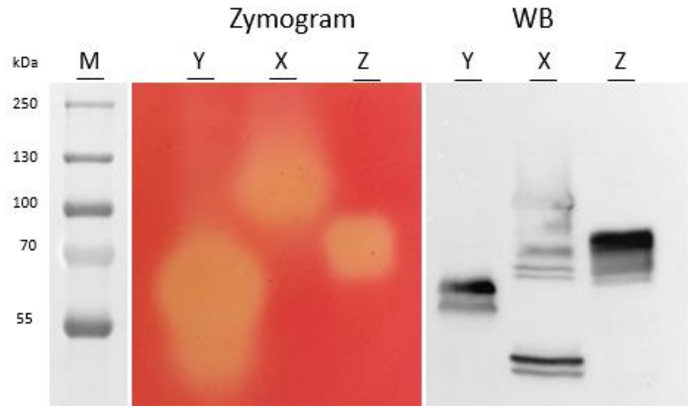
RESULTS

Secretion of heterologous cellulases in *L. lactis*



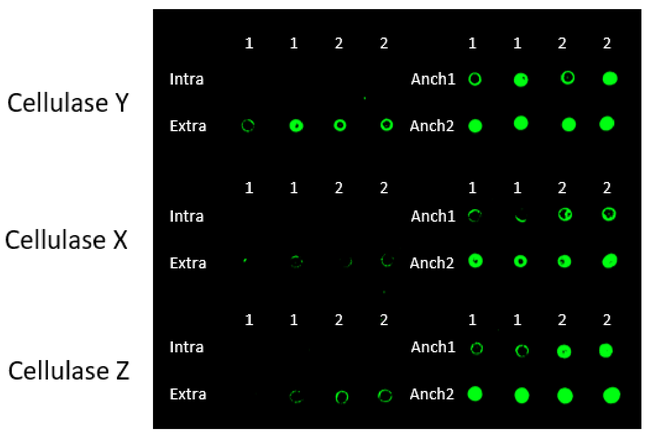
Western blot of concentrated conditioned medium from *L. lactis* cells expressing cellulases Y, Z, or X with (SP) or without (-) secretion signal sequence. The signal sequence SP enables *L. lactis* cells to secrete cellulases. Cellulases were detected with antibodies against the tag.

Zymography assay of secreted cellulases



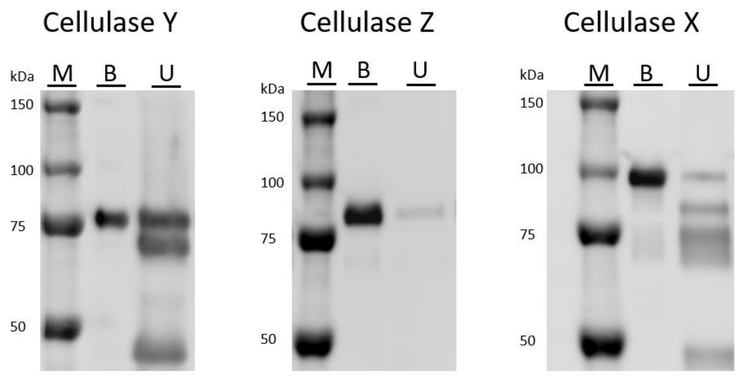
The activity of secreted cellulases (Y, X, and Z) on carboxymethylcellulose (CMC) was confirmed by zymography of concentrated conditioned culture media. The zymogram was incubated overnight at 30°C, and enzymatic activity was subsequently detected by Congo red staining.

Surface display of cellulases on *L. lactis* cells



Dot blot analysis of intact *L. lactis* cells in suspension expressing cellulases (Z, X and Y) intracellularly (Intra), secreting cellulases (Extra), or displaying cellulases on their surface (Anch1 and Anch2). Each cellulase was anchored to the cell wall by two different anchors (Anch1 or Anch2). The Anch2 anchor allows better surface display for all cellulases compared to the Anch1 anchor. The cellulases were detected with antibodies against the tag.

Binding of cellulases to crystalline cellulose



Western blot of fractions of cellulases (Y, Z or X) bound (B) and unbound (U) to crystalline cellulose. Conditioned medium containing secreted cellulase (Y, Z or X) was incubated for 1 h with crystalline cellulose. Cellulases Z and X can bind completely to crystalline cellulose, whereas cellulase Y can bind only partially to crystalline cellulose.

CONCLUSIONS

We have successfully developed *L. lactis* strains expressing three different heterologous cellulases that originate from different cellulolytic bacteria. So far, our obtained results show that functional cellulases can be expressed by lactic acid bacteria *L. lactis*.

- We have shown that signal peptide enables secretion of all three cellulases from *L. lactis* cells.
- We compared two different anchors for surface display of cellulases on *L. lactis*. The Anch2 anchor allows better surface display for all three cellulases on *L. lactis* cells.
- We confirmed the catalytic activity of all three cellulases on the soluble modified cellulose substrate CMC.
- We confirmed that all three cellulases can bind to crystalline cellulose.

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