Plant waste as an alternative feedstock for more economical and sustainable lactic acid production

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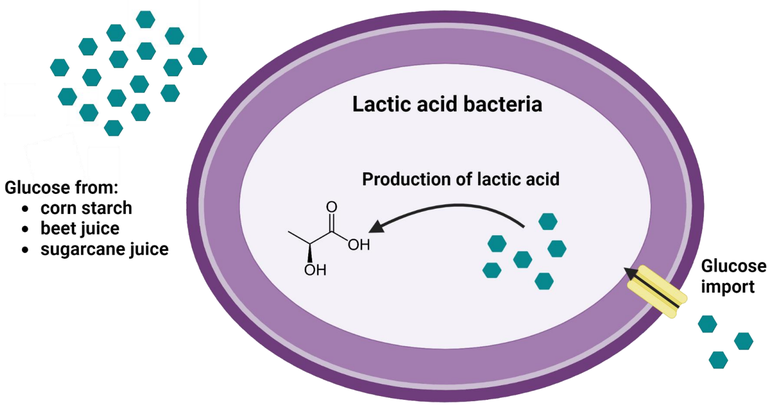
INTRODUCTION

PROBLEM: Expensive and unsustainable production of lactic acid

Energy crops intended for human and animal nutrition (corn, sugarcane, etc.)



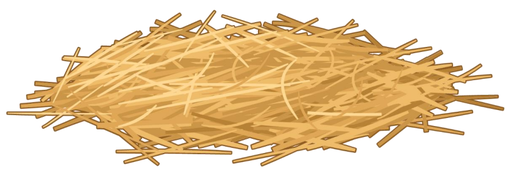
Most lactic acid is produced by microbial fermentation using crops that are also used for human and animal nutrition, so it is difficult to provide it in sufficient quantity and at a low price.

Consequences:

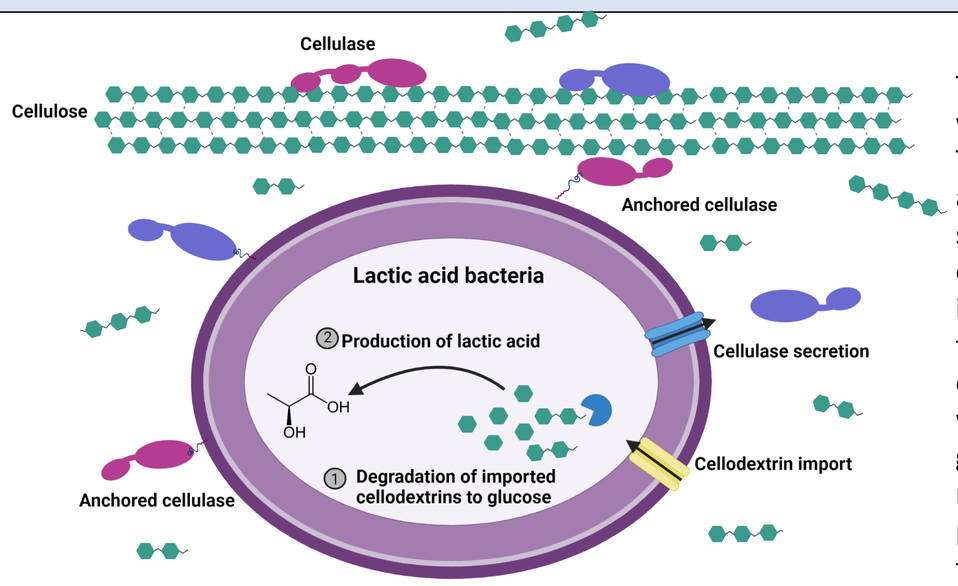
- High prices of environmentally friendly biochemicals that are produced from lactic lactic (PLA-based bioplastics, ethyl lactate….).
- Use of chemicals from unsustainable sources (oil or palm oil).

SOLUTION: Development of lactic acid bacteria for growth on cellulose

LIGNOCELLULOSIC PLANT WASTE



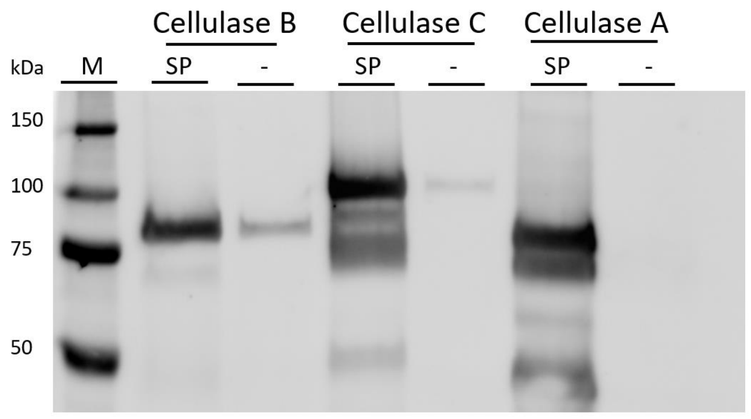
Many industries such as the wood industry, the paper industry, the food industry, agriculture and also households produce large amounts of lignocellulosic waste that could be used for the microbal fermentation of high value chemicals.



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 bacteria or freely secreted. The products of cellulose degradation are cellodextrins of varying lengths. For their transport into the cell, bacteria must have appropriate transporters. For the degradation of the imported cellodextrins, the bacteria must also be equipped with enzymes for their degradation into single glucose units, which can in turn be used in metabolism. Since lactic acid bacteria do not possess endogenous cellulases, genetic engineering for heterologous expression is possible.

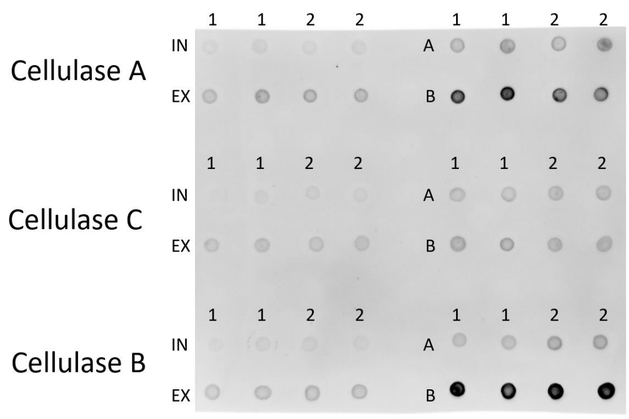
RESULTS: Heterologous expression of cellulases in *Lactococcus lactis*

Secretion of heterologous cellulases in *L. lactis*



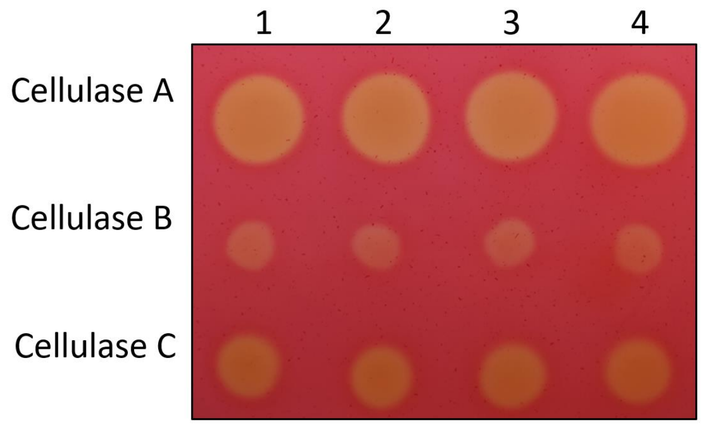
Western blot of concentrated conditioned medium from *L. lactis* expressing cellulases A, B or C with (SP) or without (-) secretion signal sequence. Cellulases were detected with antibodies against tag.

Surface display of heterologous cellulases in *L. lactis*



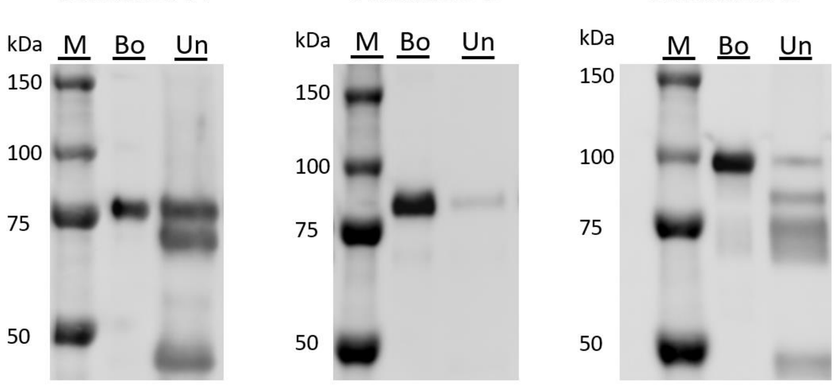
Dot blot of *L. lactis* cells expressing A, B or C cellulases inside the cells (IN), secreting cellulases into extracellular enviroment (EX) or displaying cellulases on their cell surface by two different anchors (A; anchor A or B; anchor B). 1 and 2 are biological repeats. Anchor B enables better surface display of cellulases in *L. lactis* than anchor A.

Activity assay of cellulases



The activity of secreted cellulases on carboxymethylcellulose (CMC) was evaluated by applying conditioned medium on CMC agar. The plates were incubated overnight at 30°C and enzymatic activity was subsequently detected by staining with Congo red solution. All three cellulases are active on CMC.

Binding of cellulases to crystalline celulose



Western blot of fractions of cellulases (A, B or C) bound (Bo) and unbound (Un) to crystalline cellulose. Conditioned medium with secreted cellulase (A, B or C) was incubated with crystalline cellulose for 1 h. Cellulases B and C can bind to crystalline cellulose completely, whereas cellulase A can bind to crystalline cellulose only partially.

CONCLUSIONS

We successfully developed *L. lactis* strains expressing all three heterologous cellulases that originate from different cellulolytic bacteria.

- We confirmed that signal peptide enables secretion of all three cellulases from *L. lactis* cells.
- We confirmed surface display of all three cellulases on *L. lactis* by two different anchors.
- We confirmed the activity of all three cellulases on the soluble modified cellulose substrate CMC.
- We confirmed that all three cellulases can bind to crystalline cellulose.

So far, our results show that functional cellulases can be expressed by the LAB *L. lactis*, which paves the way for the development of bioprocesses to produce lactic acid from plant waste.

ACKNOWLEDGMENTS

arrs SLOVENIAN RESEARCH AGENCY
ERA CoBioTech
REPUBLIKA SLOVENIJA
MINISTRSTVO ZA VISOKO ŠOLSTVO, ZNANOST IN INOVACIJE
CellA Chem