Development of *Lactococcus lactis* strains for potential growth and lactic acid production on cellulosic biomass

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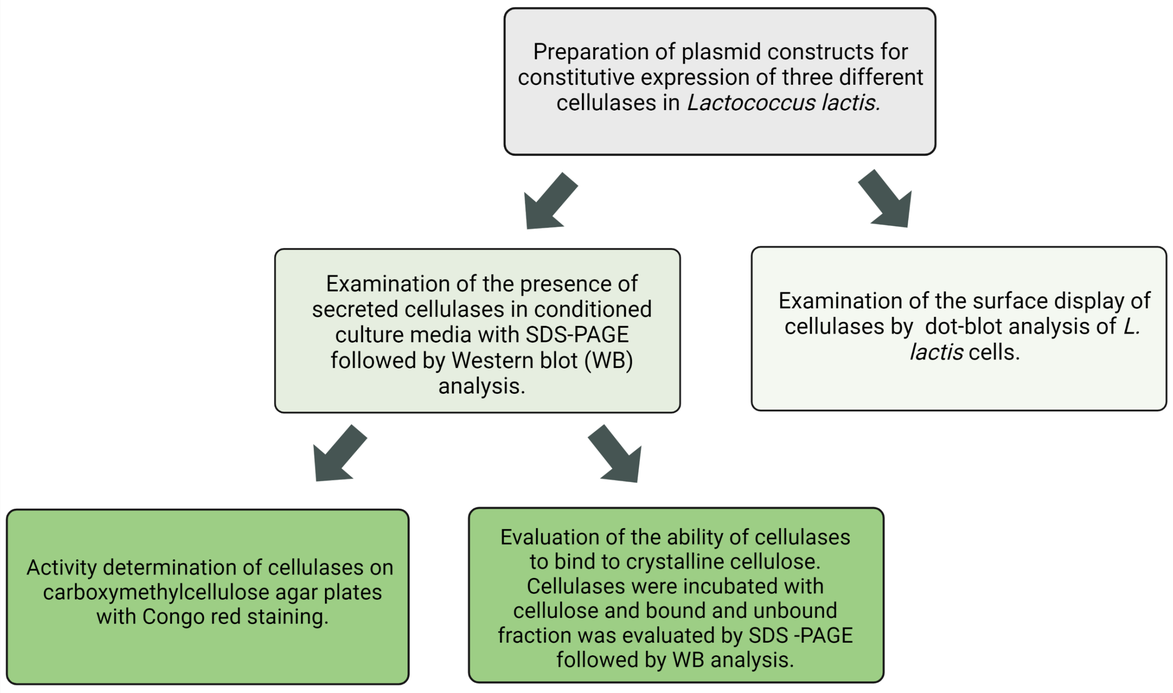
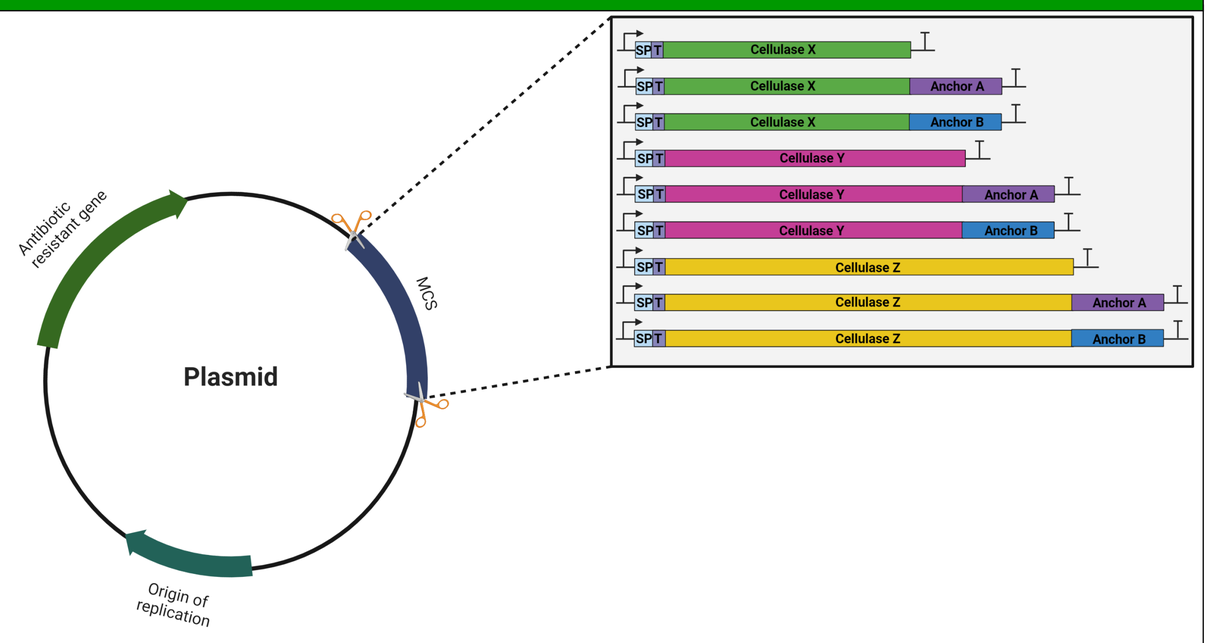
INTRODUCTION

The circular economy and the transition to more sustainable sources of raw materials, biochemicals and their production is a current and important topic being discussed in the framework of the European Green Deal. One of the main goals is to limit greenhouse gas emissions in order to reduce climate change and achieve climate neutrality. To achieve the aforementioned goal, it is crucial to reduce dependence on fossil fuels by developing more sustainable biochemicals that would be competitive in price with petrochemical alternatives. Polylactic acid (PLA) is a good example of a biodegradable polymer and a viable replacement for conventional petroleum-based plastics. PLA is made from lactic acid, which is produced through fermentation of sugars derived from crops that are also used in human and animal nutrition. This makes its production in large quantities and at an affordable price challenging. Plant waste would provide a significantly more sustainable and cost-effective option for the feedstock (substrate). Lactic acid bacteria (LAB) are the most common producers of lactic acid. To promote growth on cellulosic substrates, LAB must be equipped both with extracellular cellulases and transporter systems that enable them to decompose cellulose and uptake the products of the decomposition.

METHODS

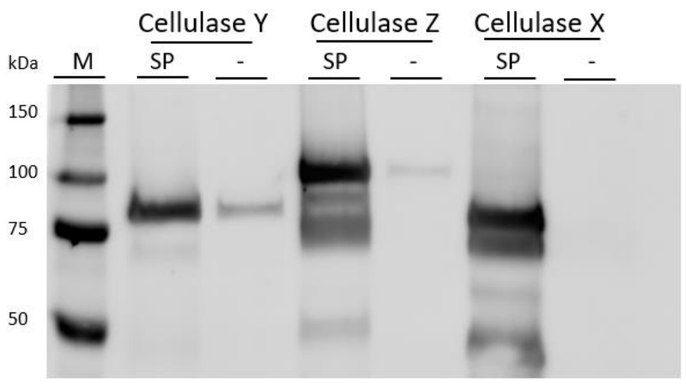




We prepared expression cassettes consisting of a constitutive promoter, a short tag sequence (T), a secretion signal sequence (SP), a cellulase coding sequence (X, Y and Z), an anchor sequence (A and B) and a transcrition terminator. The expression cassettes were inserted into plasmid for expression and replication in *L. lactis* cells

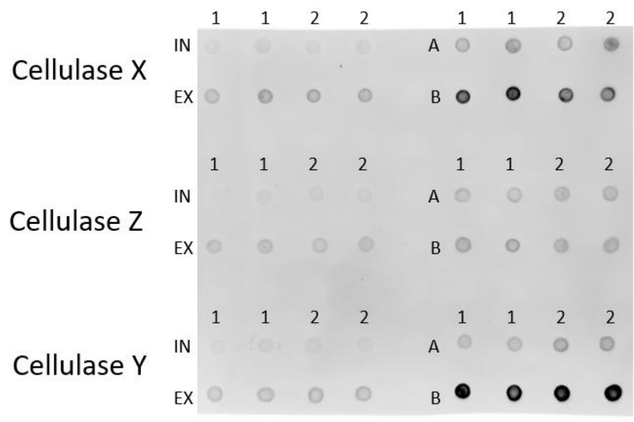
RESULTS

Secretion of heterologous cellulases in *L. lactis*



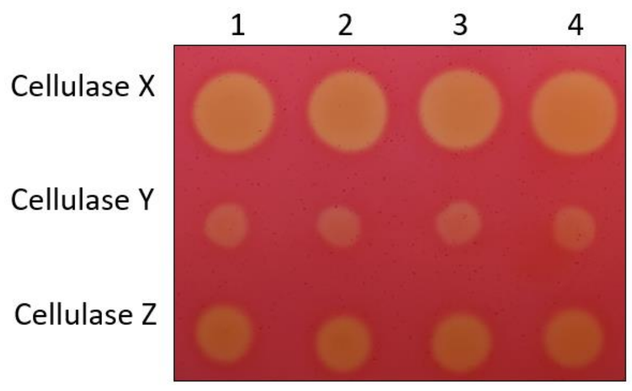
Western blot of concentrated conditioned medium from *L. lactis* expressing cellulases X, Y or Z with (SP) or without (-) secretion signal sequence. Cellulases were detected with antibodies against tag.

Surface display of heterologous cellulases in *L. lactis*



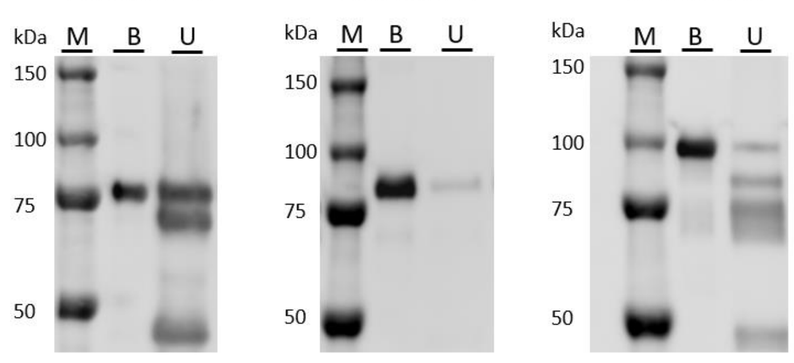
Dot blot of *L. lactis* cells expressing X, Y or Z cellulase inside the cells (IN), secreting cellulases into extracellular enviroment (EX) or expressing cellulases on their cell surface by two different anchors (A; anchor A or B; anchor B). 1 and 2 are biological repeats. Anchor B enables better surface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

Cellulase binding to crystalline celulose



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

CONCLUSION

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 outside *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.

ACKNOWLEDGMENTS

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