Heterologous expression of cellulases in *Lactococcus lactis* as a promising platform for the utilization of plant waste to develop high-value chemicals

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BACKGROUND

The circular economy and transition to more sustainable sources of raw materials, such as cellulosic biomass from plant waste and the sustainable production of chemicals is recognised as an important goal of the European Community. Lactic acid is a highly demanded biochemical due to its use in the food, pharmaceutical and cosmetic industries, as well as in polymer synthesis. It is mainly produced by microbial fermentation using lactic acid bacteria (LAB) [1]. Unfortunately, no cellulose-degrading enzymes have yet been identified in LAB [2]. The expression of heterologous cellulases that originate from cellulose-degrading organisms in the lactic acid bacterium *Lactococcus lactis* is an important step toward the development of *L. lactis* cells with cellulolytic capabilities.

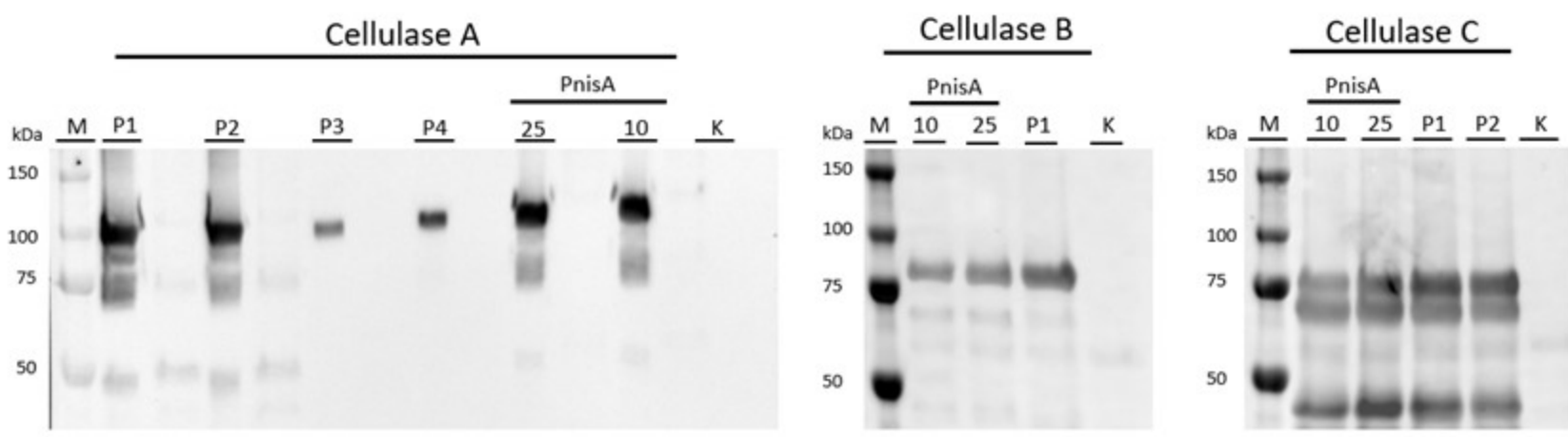
RESEARCH OVERVIEW

In this study, we have successfully developed several *L. lactis* strains that constitutively display three different cellulases on their cell surface or secrete them into the extracellular environment.

- We have confirmed the expression, secretion, and surface display of three different recombinant cellulases in *L. lactis*.
- To optimize the constitutive expression of cellulases, the expression from four different constitutive promoters was compared with the expression from the most commonly used inducible promoter (PnisA) to achieve efficient expression of heterologous proteins in *L. lactis*.
- We compared the two different anchors for surface display of cellulases.
- We confirmed the catalytic activity of recombinant cellulases on carboxymethylcellulose (CMC) and the ability to bind to crystalline cellulose.

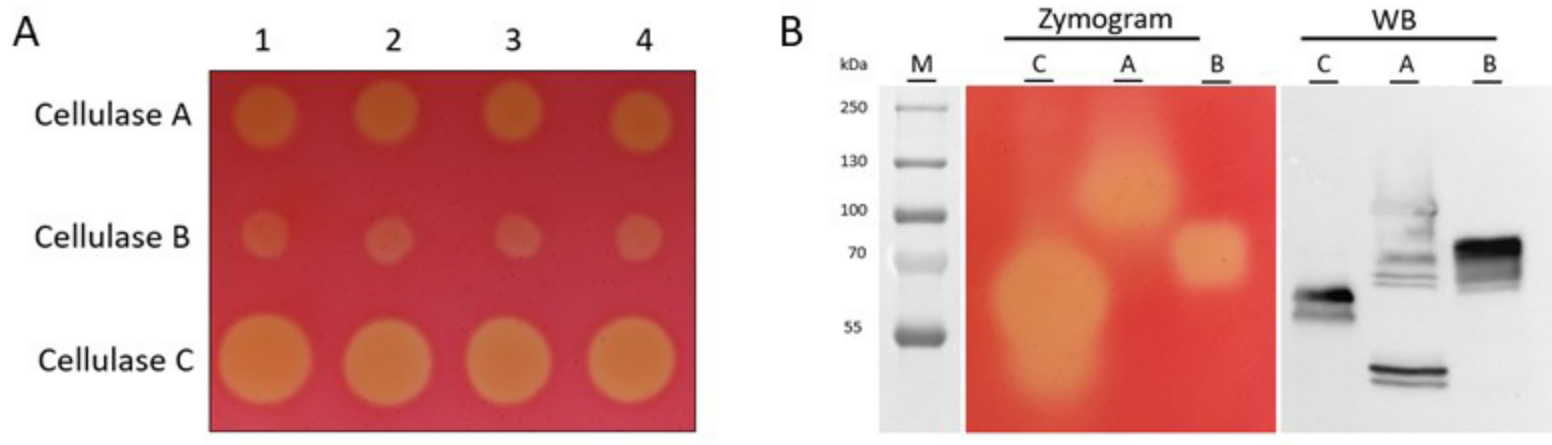
RESULTS

Inducible and constitutive expression and secretion of cellulases in *L. lactis*



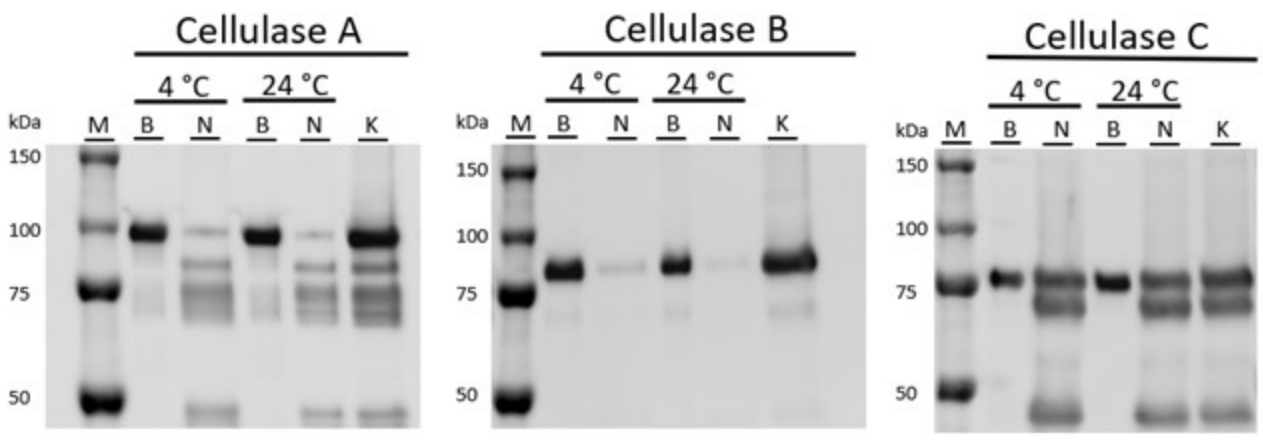
Western blot of concentrated conditioned media of *L. lactis* cells expressing cellulases A, B or C under different constitutive promoters (P1, P2, P3 or P4) or the inducible promoter PnisA induced with 10 ng/mL or 25 ng/mL of nisin. We have shown that expression of secreted cellulases by the constitutive promoters P1 and P2 was comparable to expression induced from inducible PnisA promoter. The PnisA promoter is the most commonly used promoter for efficient expression of recombinant proteins in *L. lactis*.

Activity of secreted cellulases on carboxymethylcellulose



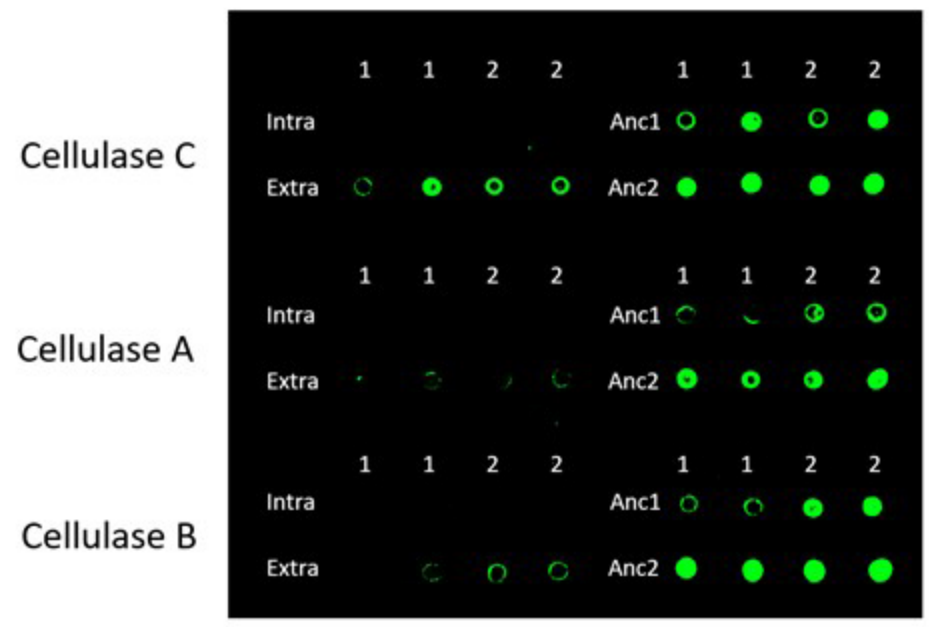
The activity of secreted cellulases on carboxymethylcellulose (CMC) was visualized by applying conditioned media on CMC-agar plates (A) or by zymography of the concentrated conditioned media containing secreted cellulases A, B or C (B). The plates and zymogram were incubated overnight at 30°C and enzymatic activity was subsequently detected with Congo red staining.

Cellulase binding to crystalline cellulose



Western blot of cellulases (A, B or C) secreted into the medium. Conditioned media were incubated with crystalline cellulose for 1 h at 4°C or 24°C. **B**; cellulase bound to cellulose; **N**; unbound fraction. **K**; TCA-concentrated medium without the addition of cellulose. Cellulase A and B can bind to crystalline cellulose completely, whereas cellulase C can bind only partially.

Surface display of cellulases in *L. lactis*



Dot blot of *L. lactis* cells expressing A, B or C cellulase intracellularly (Intra), secreting cellulase (Extra) or displaying cellulase on their surface by two different anchors (Anc1; anchor 1 or Anc2; anchor 2). 1 and 2 are biological repeats. Anchor 2 allows better surface display of cellulases than anchor 2.

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REFERENCES

[1] Kim J., Kim Y. M., Lebaka V. R., Wee Y. J. 2022. Lactic Acid for Green Chemical Industry: Recent Advances in and Future Prospects for Production Technology, Recovery, and Applications. Fermentation, 8, 11: 609

[2] Tarraran L., Mazzoli R. 2018. Alternative strategies for lignocellulose fermentation through lactic acid bacteria: the state of the art and perspectives. FEMS Microbiology Letters, 365, 15: 126.