Use of the emerging bacterial system *Lactococcus lactis* for functional expression of the protein histone deacetylase 14 (HDA14) from the green algae *Klebsormidium nitens* and of the plant model *Arabidopsis thaliana*

Recombinant Protein Production of Plant HDA14 in *L. lactis*: Toward Biochemical and Functional Characterizations

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Histone deacetylases (HDACs) regulate gene expression by deacetylating histones and other proteins. In plants, **HDA14** is a **Zn^{2+}-dependent chloroplast HDAC** involved in plant responses to environmental signals. But, HDA14 protein is difficult to produce in *Escherichia coli* (formation of inclusion bodies) and yield of soluble and functional protein are often low.

➢ **Our approach :** Use *Lactococcus lactis* as an alternative heterologous production system, already used for functional expression and characterizations of plant chloroplast / mitochondrial membrane proteins and exploit the **NICE (Nisin-Controlled Expression)** induction system for protein production.

Why *Lactococcus lactis* ?

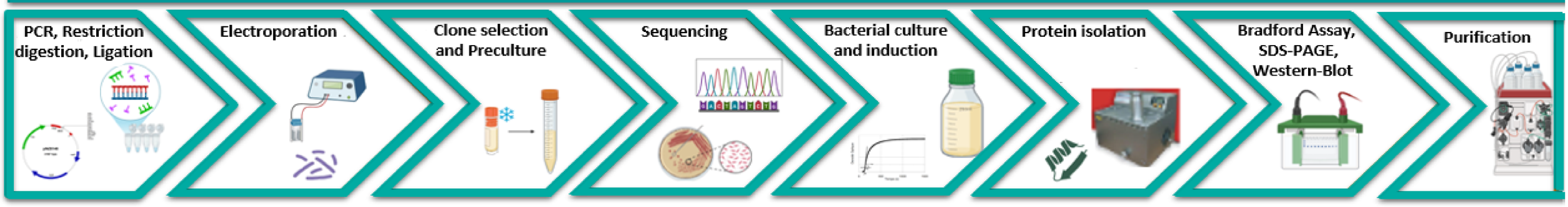
- ❑ GRAS microorganism (Generally Recognized As Safe)
- ❑ Low protease activity, no endotoxins
- ❑ Efficient soluble protein production



Objectives

- ❑ Develop a **HDA14 expression system** in *L. lactis*.
- ❑ Produce and purify recombinant HDA14 proteins from *A. thaliana* and *K. nitens*.
- ❑ Obtain functional soluble proteins for **biochemical and structural characterizations**.

Recombinant Construct Design — Construct Validation — Recombinant HDA14 Production — Biochemical Characterization



NICE system (Nisin Induced Controlled Gene Expression)

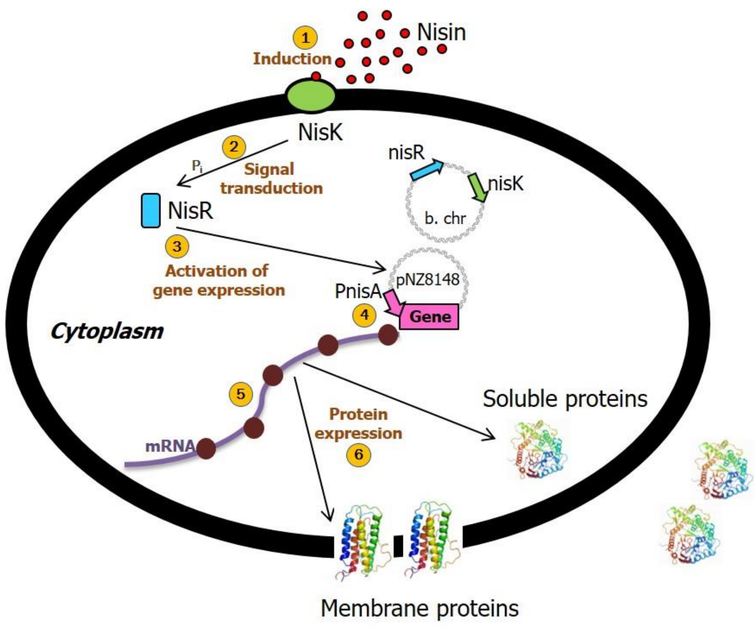


Figure 1. Overview of the NICE (*Nisin-Induced Controlled Expression*) system used in *L. lactis*. Nisin binds the sensor kinase NisK, leading to phosphorylation of the response regulator NisR, which activates the **PnisA** promoter and induces transcription of the recombinant gene.

NICE system → **tight and tunable gene expression, minimal background expression**, and **high recombinant protein yields**.

Detection of recombinant HDA14 by Western blot

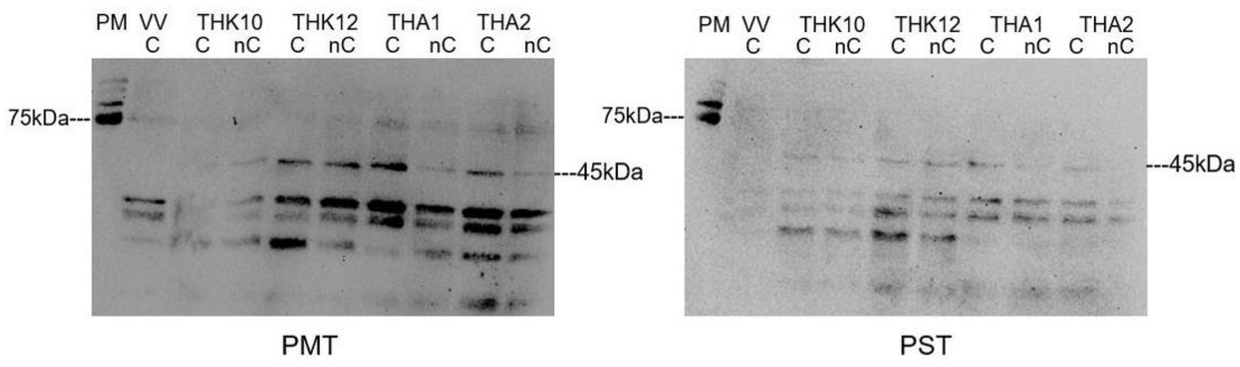


Figure 2. Western blot analysis of total membrane proteins (PMT) and total soluble proteins (PST) fractions from recombinant *L. lactis* expressing KnHDA14Δ and AtHDA14Δ (proteins lacking their predicted targeting sequences), using an antibody directed against the 6×His tag. C, heated samples; nC, non-heated samples; VV, empty vector; THK, His-tagged KnHDA14Δ; THA, His-tagged AtHDA14Δ

→ Specific immunoreactive signal at ~45 kDa detected for all HDA14 constructs, confirmes recombinant protein expressions of both AtHDA14Δ and KnHDA14Δ .
→ Selection of **His-AtHDA14Δ1** construct for subsequent purification steps.

Purification of His-AtHDA14 by affinity chromatography

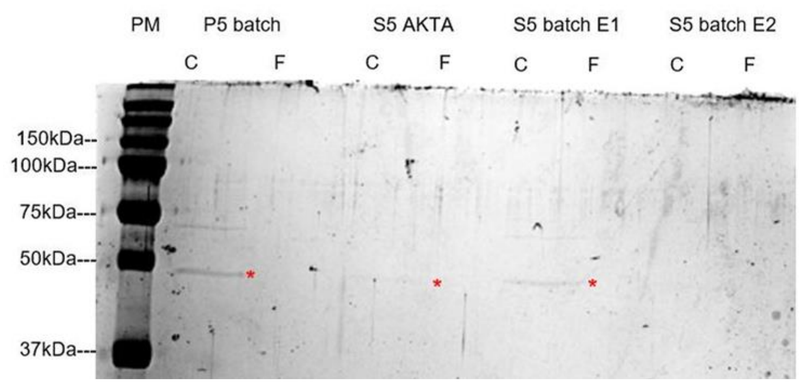


Figure 3. SDS-PAGE Coomassie blue staining of purified His-AtHDA14Δ obtained after affinity chromatography using either batch purification or an ÄKTA chromatography system (Cytiva) , followed by desalting and concentration. P5 and S5, protein production batches; E1 and E2, elution fractions 1 and 2; C, concentrated sample; F, filtrate after concentration

→ One signal at the expected molecular weight (~45 kDa*) observed in concentrated (C) tubes, confirming the presence of the recombinant protein.
❖ Need to optimize yield and purity to increase the quantity of protein recovered

Optimization of His-AtHDA14Δ1 production

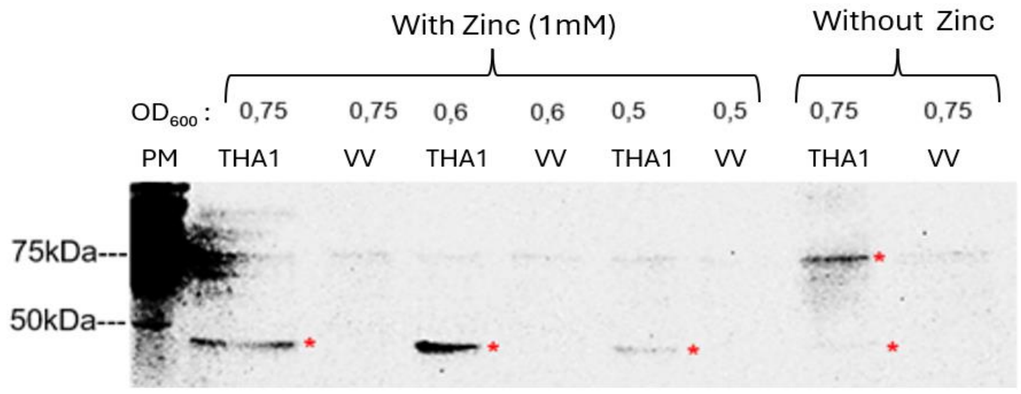


Figure 4. Western blot analysis of the different conditions of optimization of His-AtHDA14Δ1 production using an antibody against the 6×His-tag. THA, His-tagged AtHDA14Δ; VV, empty vector; OD_{600}, optical density at 600 nm; Δ, protein lacking its N-terminal targeting sequence.

→ Highest production obtained with induction at DO=0.6 in the presence of $ZnCl_2$.
→ In the absence of zinc : reduction of the 45 kDa signal and appearance of an additional band at ~75–80 kDa, suggesting protein dimerization resistant to denaturization

Conclusion

✓ Development of a *Lactococcus lactis*-based expression platform for HDA14 production
✓ Expression of recombinant HDA14 proteins from *A. thaliana* and *K. nitens*
✓ Purification by affinity chromatography of recombinant proteins

Perspectives

❖ Further optimization of recombinant protein production and purification.
❖ Improvement of protein yield and purity.
❖ Biochemical characterization of HDA14, including its deacetylase activity.

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