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► To cite this version:

Leïla Mekmene, Magali Genay, Daniel Mollé, Julien Jardin, Christian Corre, et al.. prtH2 not prtH is the ubiquitous cell wall proteinase gene in [*i*]Lactobacillus helveticus[/i]. 16^{ème} Colloque du Club Des Bacteries Lactiques, May 2009, Toulouse, France. 2009. hal-01190820

HAL Id: hal-01190820

<https://hal.science/hal-01190820>

Submitted on 1 Sep 2015

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prth2, not *prth*, is the ubiquitous cell wall proteinase gene in *Lactobacillus helveticus*

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Introduction

Cell envelope proteinases (CEPs) of lactic acid bacteria are responsible of the degradation of casein for their nutritional requirements. Thus, they produce peptides which affect textural properties and biological activities. In *Lactobacillus helveticus*, two CEPs have been described, **Prth** and **Prth2**, with low percentage of amino acid identity (22%). However, the distribution of the genes encoding these proteinases in this species remains unclear as well as the mechanisms of formation of functional peptides.

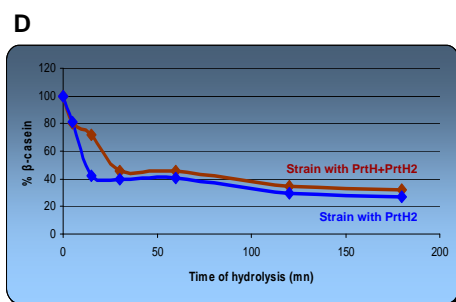
Aim : The objective of this study was to exploit the biodiversity of strains of *L. helveticus*, isolated from various biotopes, both in terms of presence or absence of these genes and in terms of nucleotide sequence, and studied their transcription in dairy matrices and the proteolytic activity of these enzymes.

Distribution of *prth* and *prth2* genes^a

We studied the distribution of the genes by using PCR and Southern blotting experiments. The *prth2* gene was ubiquitous in 29 strains of *L. helveticus* studied, whereas only 18 of them also exhibited the *prth* gene. Sequencing of 350-bp internal fragment of these genes revealed the existence of intraspecific diversity.

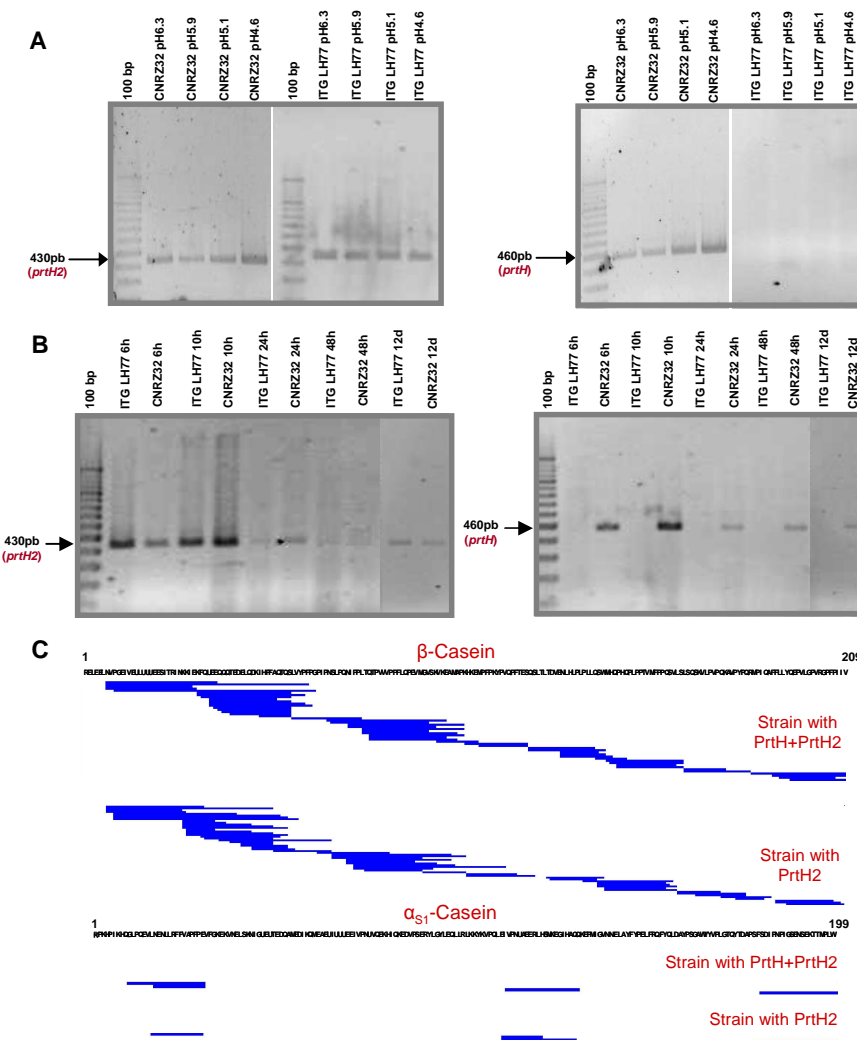
CEPs activity in milk and towards purified caseins

In order to clarify the relationship between genomic CEPs content and phenotypic characteristics of *L. helveticus* strains, we studied their proteolytic activity on milk caseins and towards purified caseins (β et α_{S1} -CN). There is no marked differences between the proteolytic activity of strains possess 1 or 2 CEPs, both on milk caseins (C) and purified caseins (D).



Detection of *prth* and *prth2* transcripts in dairy matrices^a

Expression of the 2 CEP-encoding genes was followed during the growth in dairy matrices of 2 strains, ITG LH77 and CNRZ32, which possess 1 and 2 CEP-encoding genes, respectively. Both genes were shown to be expressed by *L. helveticus* at each stage of growth in milk (A) and at different stages of mini-Swiss-type cheese making and ripening (B).



Conclusion

The difference at the genetic level was less marked when the proteolytic activity of enzyme was measured on milk and purified caseins. This difference in the number of CEPs present in strains did not necessarily influenced the resulting proteolytic activity.

^a M. Genay, L. Sadat, V. Gagnaire and S. Lortal. 2009. *prth2*, not *prth*, is the ubiquitous cell wall proteinase gene in *Lactobacillus helveticus*. Applied and Environmental Microbiology., Vol. 75, No. 10, p. 3238-3249.