Three distinct proteases are responsible for the overall cell surface proteolysis in *Streptococcus thermophilus*

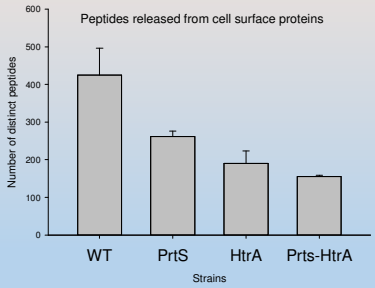
Mylène Boulay, Coralie Metton, Christine Mézange, Lydie Oliveira Correia, Thierry Meylheuc, Véronique Monnet, Rozenn Gardan, Vincent Juillard
Université Paris-Saclay, INRAE, AgroParisTech, MICALIS Institute, 78350 Jouy-en-Josas, France

Context and objectives

Streptococcus thermophilus is a LAB widely used as a starter in dairy industry. In this species, only two extracellular proteases have been characterized: (i) the cell-wall anchored protease PrtS that is not present in all strains and is essential for optimal growth in milk and (ii) the membrane-anchored protease HtrA that is present in all strains. This bacterium is considered as poorly proteolytic, and is therefore also regarded as potential bacterial chassis for heterologous protein production. In this context, we did develop a peptidomic approach to re-analyze the cell surface proteolysis of the *S. thermophilus* LMD9 strain and identify a new functional surface-located protease.

1. PrtS and HtrA both contribute to recycle cell surface proteins

Identification by LC-MS/MS of peptides released in Chemically Defined Medium (CDM) during growth of LMD9 and protease mutant strains.



An important residual proteolytic activity in the double mutant
→ **Working hypothesis: existence of another cell surface protease**
Similar situation in L. lactis

2. An *in silico* approach identifies a new surface protease candidate

59 putative proteolytic enzymes encoded in LMD9 (genome mining)

↓

11 putative proteases predicted at the cell surface (Locate P)

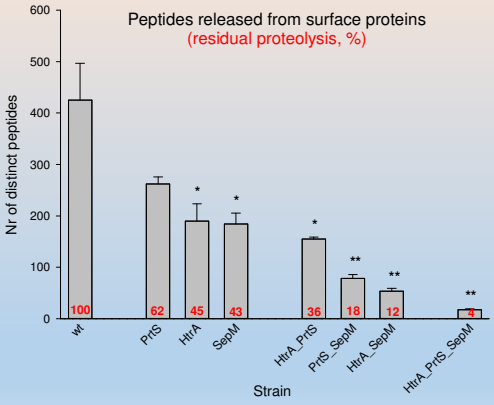
↓

7 putative cell-surface located putative proteases expressed during growth (peptidomics data)

5 already known: PrtS, HtrA, DacA, DacB, LepB
2 unknown proteases in S. thermophilus:
- ✓ STER_RS05675, without counterpart in *L. lactis*
- ✓ STER_RS07910, homologous to YwdF in *L. lactis*
  60% identity with the characterized SepM protease of *S. mutans*

→ ***In silico* prediction: SepM, a promising membrane-anchored candidate**
→ **Construction of a SepM mutant in LMD9 to ascertain the prediction**

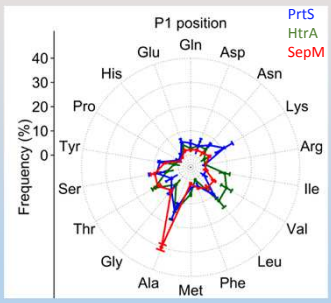
3. SepM is a functional surface protease in *S. thermophilus*



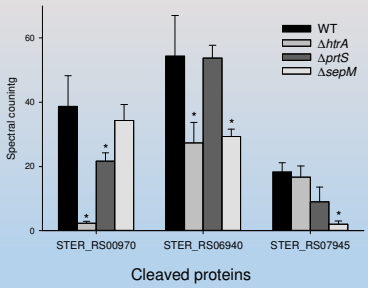
→ **PrtS, HtrA and SepM account for most of the cell surface proteolysis**

4. PrtS, HtrA and SepM have different cleavage preferences

Cleavage specificity



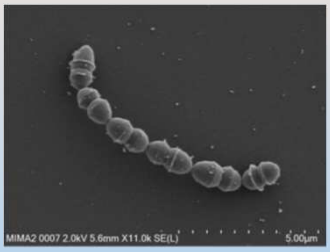
Substrate specificity



SepM has a strong preference for Ala in P1 position
PrtS, HtrA and SepM have different targets

5. SepM plays a role in cell morphology

Scanning Electron Microscopy of *S. thermophilus* (MIMA2, MICALIS) (exponential growth phase in CDM)

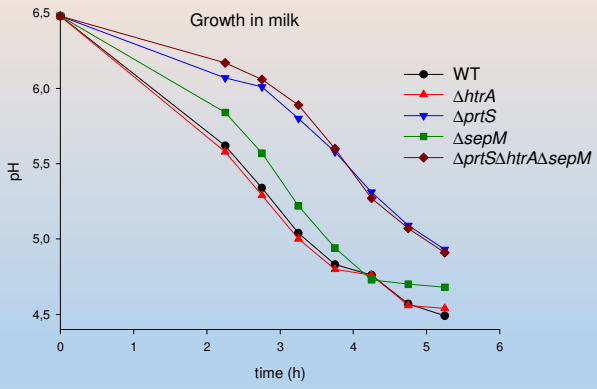


Wild-type strain

SepM mutant strain

→ **Inactivation of *sepM* affects cell division and presumably induces extracellular cell vesicles release**

6. Only PrtS is required for rapid growth in milk



→ **Inactivation of *sepM* does not significantly affect growth in milk**

Conclusion and perspectives

Three proteases are present at the cell surface of *S. thermophilus*. SepM, the newly identified protease, is anchored to the membrane and faces the external medium. The *sepM* encoding gene is present in all *S. thermophilus* strains. The three proteases participate in the cell surface protein turnover, acting synergistically on different substrates. Their deletion almost completely abolish the cell surface proteolysis, indicating that no other proteases acts significantly at the surface of *S. thermophilus*. Obtention of a mutant strain fully deprived of surface proteolysis opens the way to biotechnological applications.