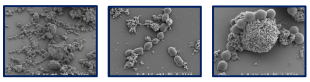
Streptococcus thermophilus in soya milk: growth and metabolic activity

Mylene Boulay, Maher Al Haddad, Françoise Rul



Streptococcus thermophilus in soya milk: growth and metabolic activity

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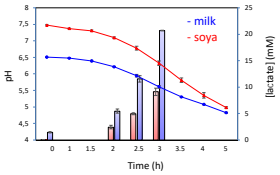
Context and objectives

Fermented plant-based food is booming in our diet and soya products fermented by lactic acid bacteria (LAB) are appealing because of their potential health and nutritional benefits.

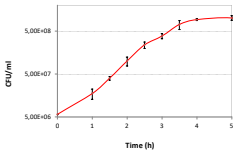
Whereas the physiology of one of the main starters, *Streptococcus thermophiles*, is well characterized in dairy matrices, its behaviour, metabolic activities and techno-functional properties are less documented in soya. Here, we characterized the growth of *S. thermophilus* in soya milk, identified the carbon source it uses and explore its global metabolism, with a focus on nitrogen metabolism.

1- *S. thermophilus* LMD-9 rapidely acidifies soya milk, by lactate production



- High and rapid acidification
  - pH_{5h} ➔ 4.98 (milk: 4.83)
  - V_{max} ➔ x 1,5 soya/milk
- Correlation between pH drop and lactate production
- Higher production of lactate in milk / soya:
  ⇨ related to higher bacterial population in milk

2- *S. thermophilus* LMD-9 grows in soya milk, via sucrose consumption

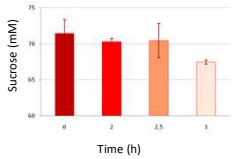


- *S. thermophilus* growth in soya:

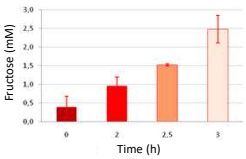
Final population 1,1 10^9 CFU/ml

μ_{max} 1,8h^{-1}

- Sugar dosages in soya culture supernatants

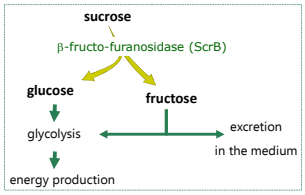


➔ 4 mM decrease of sucrose (⇔ 1.36 g/L)



➔ 2,1 mM increase of fructose (⇔ 0,38g/L)

- consumption of sucrose
- production of fructose
- no detection of glucose



Reference : Boulay M, Al Haddad M, Rul F. *Streptococcus thermophilus* growth in soya milk: Sucrose consumption, nitrogen metabolism, soya protein hydrolysis and role of the cell-wall protease PrtS. Int J Food Microbiol. 2020 Dec 16;335:108903. doi: 10.1016/j.ijfoodmicro.2020.10890

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Experimental design

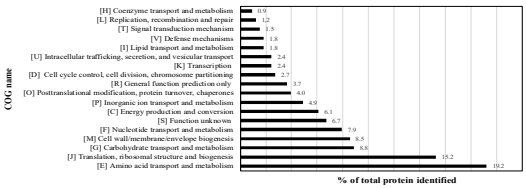
***S. thermophilus* LMD-9** wt and Δ*prtS* (constructed by gene interruption with a Ery K7)

10^6 CFU/ml in 37°C

- milk (powder, 10% reconstituted, Sigma)
- soya milk (SojaSun, Triballat-Noyal)

- acidification: pH and lactic acid measurements (HPLC)
- growth: enumerations on M17 lactose (1g/L)
- sugars: glucose, fructose, lactose, sucrose measurements (HPLC, Aminex HPX-87H column)
- proteolytic profile: SDS-PAGE (4–12%)
- proteomic analysis (coll. PAPPSO Plateform INRAE; http://pappso.inra.fr/):
  - cell-envelope and cytosolic protein fractions of exponential bacterial cultures
  - separation by LC-MS/MS (UltiMateTM 3000 RSLCnano system coupled to a LTQ-Orbitrap DiscoveryTM mass spectrometer in CID mode
  - protein identification: X! Tandem software – comparison with protein databases of *S. thermophilus* LMD-9 (GenBank v. 2013, 1710 entries), soya (Uniprot v. 16-11-14; 150,681 entries), and potential contaminants - Filtration: X! Tandem Pipeline – a peptide E-value of 0.01, a protein E-value of -4, and the presence of 3 peptides per protein minimum.

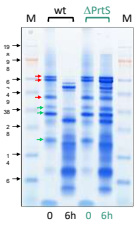
3- Proteins related to nitrogen metabolism predominates in soya cultures of *S. thermophilus*



- Proteomic analysis of LMD-9 soya culture
  - identification of 328 proteins
  - 19% of proteins are related to amino acid (AA) transport and metabolism
    ➔ including the cell-wall protease PrtS, AA and peptide transporters for potential use of of soya proteins by their hydrolysis and by transport of peptides
  - 9% of proteins are related to carbohydrate metabolism
    ➔ including proteins for potential transport and metabolism of sucrose, the main sugar of soya milk (PstI, PstH, ScrA, ScrB)

4- The cell-wall PrtS contributes to *S. thermophilus* LMD-9 growth in soya milk and to soya protein hydrolysis

- Δ*prtS* mutant: final population decreased by 2,8 fold compared to wt strain
  ➔ PrtS is involved in *S. thermophilus* growth in soya milk
- Proteolytic profiles of soya culture supernatants (SDS-PAGE)
  ➔ Marked proteolysis of the main soya proteins (➝ ; globulines 7S and 11S probably) for the wt strain after 6h of growth
  ➔ More restricted proteolysis in absence of PrtS protease
  ➔ Probable involvement of other bacterial proteases in soya protein hydrolysis



Conclusions – Perspectives

- *S. thermophilus* LMD-9 grows rapidly in soya milk, consumes sucrose and produces lactate, a molecule of interest for matrix acidification and for gut physiology. The first proteomic map in soya of *S. thermophilus* presented here underlines the prevalence of nitrogen metabolism.
- As in milk, the cell-wall protease PrtS is involved in growth of *S. thermophilus*. Its abitiy to hydrolysis soya proteins could help to improve digestibility of soya based food and provide health benefits, such as reducing the allergenicity of soya proteins or modulating the production of bioactive peptides.

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