Transcriptome profiling of *Lactococcus lactis* subsp. *cremoris* under conditions simulating Cheddar cheese manufacture

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## Introduction

During the manufacturing and ripening process, cheese undergoes many modifications due to glycolysis, proteolysis and lipolysis that contribute to the development of typical Cheddar flavor. These modifications occur through the catalytic action of the microbiota, including the primary starter culture containing *Lactococcus lactis*. These activities can vary from one lactococcal strain to another and can be at the origin of differences in starter performance. The availability of genomic sequences and the emergence of DNA microchip technology adds a new dimension to the study of the total lactococcal gene expression during cheese production.

The aim of this study was to develop a DNA microarray to study gene expression of four strains of *Lactococcus lactis* subsp. *cremoris* under simulated conditions of Cheddar cheese manufacture.

## Methods

### Growth conditions
- Two pre-cultures in modified Elliker medium at 22°C (18 h and 7 h).
- Pre-culture (12 h) in microfiltered milk 3.35% M.F. at 22°C.
- Incubation 5% in microfiltered milk 3.35% M.F., 1 h at 25°C (10^6 cfu/ml).
- Pease activity test 1.

### cDNA synthesis, labelling and hybridization
- Synthesis of cDNA from 30 µg of RNA extracted at stages T1, T2, T5.
- Labelling and purification of cDNA.
- Hybridization of 700 ng of labelled cDNA on the oligonucleotide chip.
- Washing and rinsing of slides (Chipmaker).

### Data analysis
- Average of probes and replicates was calculated using BRB ArrayTools2.
- Hybridization signals were normalized on the sample average for all genes with AMADA.
- For principal component analysis (PCA), the Euclidean distance was calculated between samples using the normalized data for all genes.
- SAM analysis (Significance of Microarray) was used to extract statistically significant changes in gene expression among samples. These statistical analyses were carried out using TIGR MeV.

## Results

### Development of a DNA chip

60-mer oligonucleotide probe design from a draft genome sequence of *L. lactis* subsp. *cremoris* SK11 (Genbank accession numbers CP000425 to CP000430)
- 1030 genes represented by 1 to 3 oligos
- 2537 specific oligonucleotides for SK11
- 27 oligonucleotide controls

\[ \text{X 4 replicates} = 10807 \text{ spots} \]

Figure 2: Growth and acidification of *L. cremoris* during the Pearce activity test

![Image](image2.png)

Figure 3: Principal components analysis of the expression profiles (Euclidean Distance)

![Image](image3.png)

- The profiles of SK11 and HP are similar and E8 and Wg2 were grouped together in the three conditions tested. The profiles of E8 and Wg2 are more distant from SK11 than that of HP.
- The profiles after salting are very different from the profiles obtained with temperature conditions 32°C and 38°C (Fig. 3).

### Conclusions

- This work constitutes the first study of lactic starters gene expression under conditions reflecting the processes applied in industry.
- These results improve our comprehension of diversity among the strains and their response to environmental variations.
- Strains SK11 and HP seem to be the most affected by the temperature variation and salting. Lytic cellular systems were induced by salting in these two strains.
- The genes identified may be responsible for differences in technological potential and will be used to develop specific markers to distinguish between the starter strains.
- Quantitative real time RT-PCR will be used for the validation of the results.

## References

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