

Report from Cheeses sent to Safecheese project

All partners in the Safecheese project have sent cheeses. NGO, ParmReg, Faraday, Kaanlar, Moxlakis, Northumberland, CoProLat, Coquard and Muto have collected cheese samples and information of the process used to produce the various cheeses according to directives prepared by Matforsk. Samples from 170 cheeses have been collected.

A database containing information about the cheeses involved in the project, and their processing technology have been made.

(the cheese samples retrieved indicate an organization in 3 cheese types, namely Hard cheese (like Parmesan) Semihard cheese (like Port Salut) and Soft (like Brie). Challenge experiments and sensory evaluation of cheeses produced with selected protective strains will be performed with soft cheese late in WP2.

Challenge experiments with *Listeria monocytogens*, *Stapylococcus aureus* and *Escherichia coli* O157 in collected cheeses

180 cheese samples were collected from 65 producers throughout Europe, Turkey and Africa.

Cheeses were made into 50% slurries. *Listeria monocytogens*, *Stapylococcus aureus* and *Escherichia coli* O157 were added and their development were followed for 60 days.

Of these 180 samples, 53 slurries were found to exhibit some sort of growth inhibition against the added pathogens. The following figures show examples of how different cheese slurries influence on the development of the added pathogens

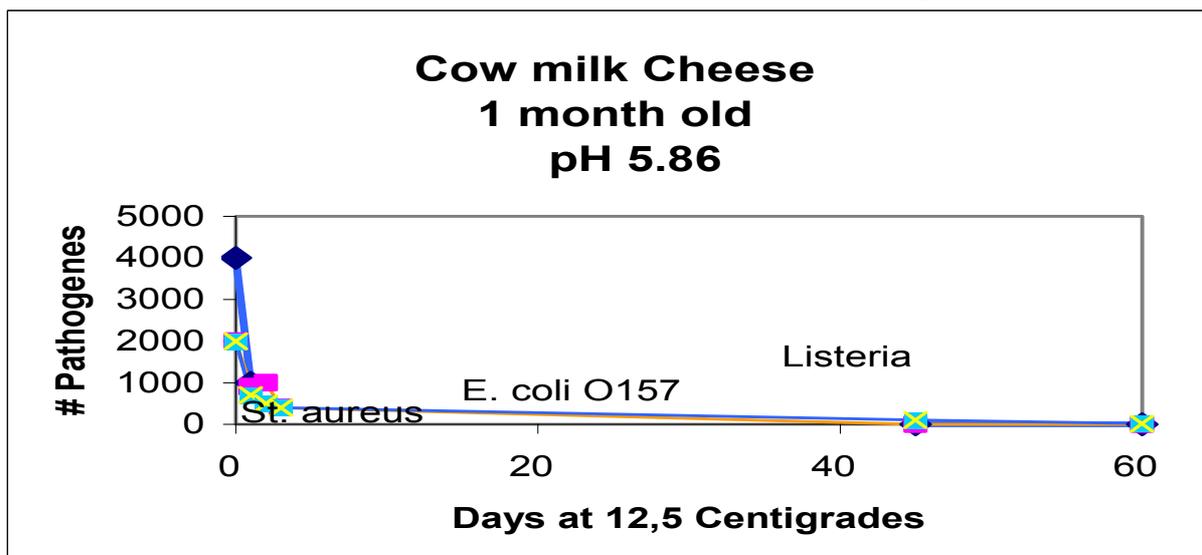
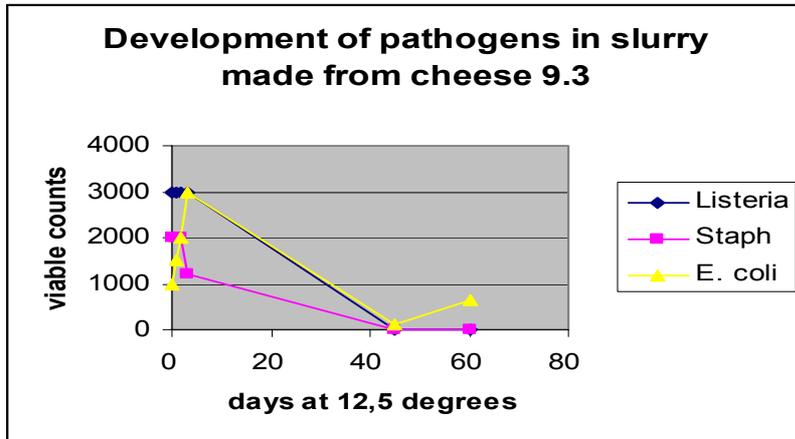
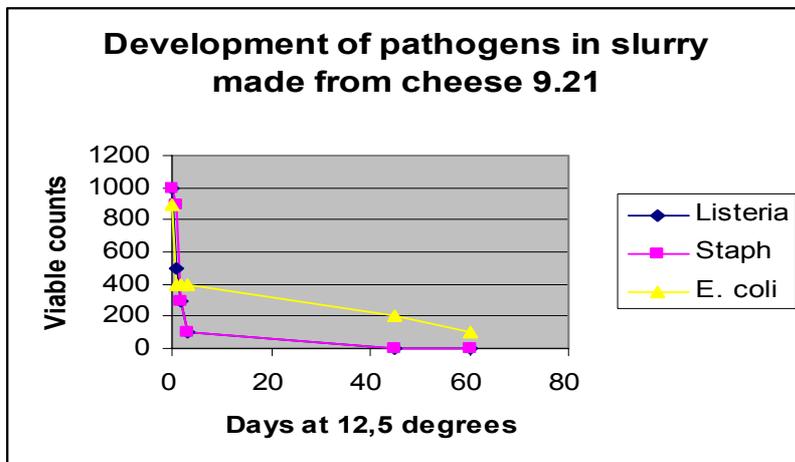


Fig 1. All pathogens are inhibited in their growth at an early stage.

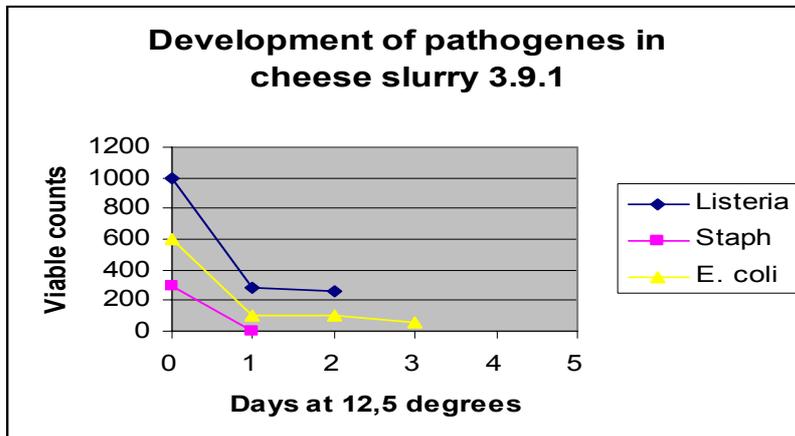
We have selected some representative cheeses for presentation as graphs.



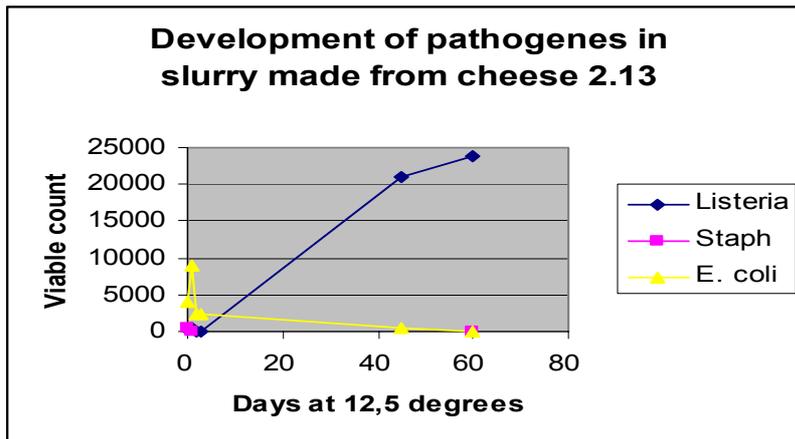
Cheese 9.3 pH 4.71
Soft Goat milk cheese from Madagascar collected by Couqard. Protective against Listeria. Some activity against E.coli. Staphylococci may survive and produce toxin the first days



Cheese 9.21 pH 5.36
Hard Goat milk cheese from France, collected by Couqard. Protective against Listeria and Staphylococci,



Cheese 3.9.1 pH 6.87
Curd from U.K. with unknown origin collected by Faraday. Protects well against all three pathogens. Graph show development first 3 days.



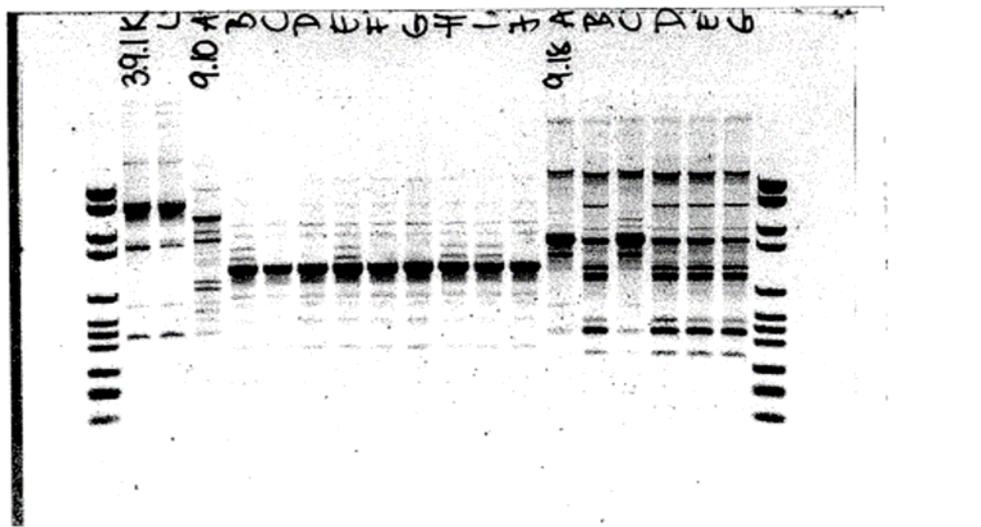
Cheese 2.13 pH 5.41
 Hard Cow milk cheese from Italy collected by Parma Reg.
 Show good effect against Staph and E.coli, while Listeria is increasing with time

Figure 1 shows the growth pattern obtained from one of these cheeses. This cheese slurry does not support growth or survival of any of our pathogens and represents one of our potential protective cheese samples.

From cheese slurries to individual bacterial isolates

Of the 53 growth inhibiting slurries we have selected 44 potential inhibiting cheese slurries with which we had to repeat the challenge experiments to verify their protective capability. After two verification experiments we have 21 cheese samples which have a protective potential, and will be subjected to further studies.

From each of the 21 cheese samples the most dominating bacteria is isolated and (RAPD) examined to decide their individuality. So far we have 69 promising isolates.



Picture 1 show the patterns of different bacterial isolates from cheeses selected for their protective quality. The RAPD method give a DNA profile. Equal profiles results from isolates of the same bacterium. Consequently; 3.9.1 K & L are identical isolates, 9.10 A is unique among the 9.10 isolates B, D, E, H, I are one and same strain, and 9.10 C, D, F, G & J are the same strain. The 9.18 isolates are indeed two; A = C and B = D = E = G. Thus this gel shows 6 different isolated bacterial strains.

Validation

Robust LAB protective bacteria are supposed to work in ordinary milk as well.

Consequently, the 69 isolates will be subjected to incubation in fresh milk seeded with the pathogens and followed for 14 days. Step one was to standardize the number of bacteria in our growth media, so that the same number of protective bacteria would be added to the tubes containing pathogens. Step two was to incubate with the pathogens and step three was to show that they belong to the group of Lactic Acid Bacteria (LAB).

We found none of the single isolates to give the wanted protection against all three pathogens. We thus decided to change strategy.



New strategy

We decided to score the effect of mixed cultures in fresh milk seeded with the pathogens.

We made up several different mixes, included the commercial available Listeria protective strains HOLDBAC from Danisco and BACTOFERM from Chr. Hansen.



Selection of strains for use in cheese making experiment

From this experiment we were able to perform a qualified selection of strains to use in the forthcoming cheese making experiment

The strains were our 9.12 and 5.3.4

The content of three protective cultures selected

HOLDBAC from Danisco

9.12 alone

5.3.4 mixed with Bactoferm from Chr. Hansen

How do the pathogens behave in brie/camembert made with protective culture

Next step in WP2: Cheeses made with and without pathogens; control and 3 protective cultures in two parallels, . ie 8 full cheese making procedures.

Development of pathogens will be followed for 45 days at 12,5 degrees.