Spoilage of UHT milk by enzymes from microorganisms

Results from the MicroLink project explains strain variability in UHT milk spoilage potential between cold tolerant bacteria







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ABSTRACT

The project investigated underlying reasons for variability in spoilage potential among different strains of cold tolerant Pseudomonas bacteria in UHT milk. Main reason for variability in enzymatic spoilage potential among strains was related to differences in gene expression levels and was linked to genetic differences in the bacterial genomes. In contrast, the variability seemed to be less related to differences in protein cleavage specificity or variability in heat sensitivity of enzymes from different strains.

Approximately 1 out of 500 batches of Ultra High Temperature (UHT) milk end up showing quality problems after production. These quality problems can be gelation, sedimentation or formation of off flavours, and can occur during subsequent storage and shipping, at retail or at consumer level. Some of these quality problems can be traced back to the activity of quality-deteriorating enzymes originating from cold-tolerant (psychrotrophic) bacteria that can grow during refrigeration. The bacteria do not survive the heat treatment, but their enzymes do, which can subsequently reduce shelf life and ultimately lead to product waste. Stability of UHT milk is challenging, as it is often exported and transported outside the cool chain and has declared long shelf life. One of the most important enzymes in relation to this spoilage has been shown earlier to be AprX, a bacterial enzyme that degrades milk proteins.

To avoid these quality problems, it would be of great benefit to be able to predict tank milk batches suitable for UHT milk production. To address this, the MicroLink project, supported by the Danish Dairy Research Foundation, has been investigating relationships between type and level of psychrotrophic bacteria, spoilage potential and enzyme activity after heat treatment.

Variation between strains

When testing if milk is suitable for UHT products, it is a challenge that neither total bacterial counts nor the number of psychrotrophs themselves are sure predictors of UHT milk stability during subsequent ambient storage. Furthermore, the bacteriological test for psychrotroph counts takes 3 days, as they grow slowly, and this waiting time creates logistic problems relative to batch sorting.

The background for the lack of correlation between bacterial counts and spoilage potentials was initially investigated in a storage experiment

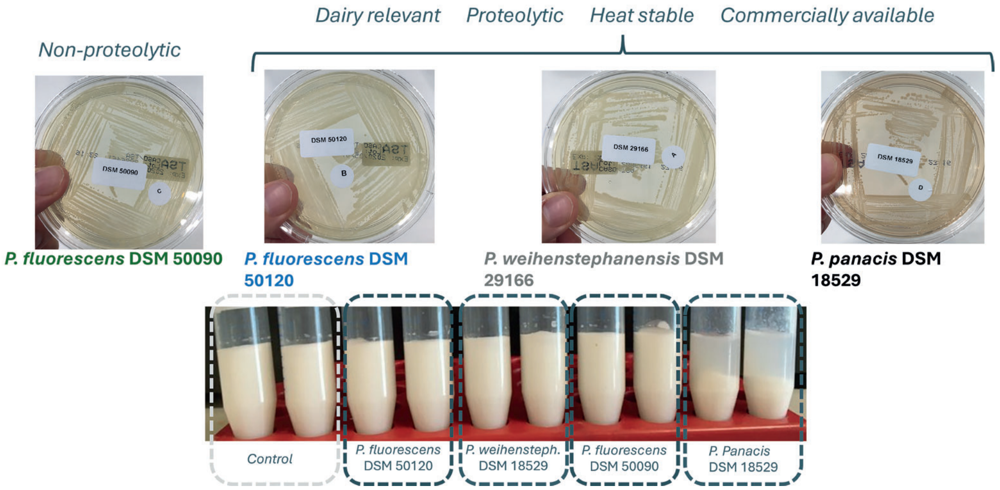

Figure 1. Overview of used commercial strains and their effect after inoculation into UHT milk and after storage for 45 d at room temperature. It is seen that P. panacis resulted in visible change in quality and phase separation/sedimentation. Control was without inoculation

(room temperature, 45 days) of UHT milk inoculated with four different Pseudomonas strains. The four strains varied a lot in their spoilage potentials (Figure 1), but importantly, a correlation between the expression level of the AprX gene and protein degradation in the UHT milk was found. There was little difference in enzyme specificity between strains, while strains differed markedly in the amount of peptides made and thereby extent of proteolysis. This indicated that spoilage is mainly linked to level of enzyme activity.

In the stored UHT milk, especially κ-casein was primarily hydrolysed at the beginning of the storage, but other milk proteins would eventually also be hydrolysed over time, especially αs1-casein. The extent of κ-casein cleavage is expected to contribute to instability and gelation of the milk.

Heat survival of enzyme activities

In the next set of experiments, enzyme activity after heat treatment was analyzed for 55 Pseudomonas strains from the strain library of spoilage bacteria at the Arla Innovation Centre. These strains were tested for protein degrading enzyme activity before and after UHT treatment (135°C, 10 s) carried out in a laboratory oil bath. The results confirmed large variation between strains' enzyme activities after cultivation, as more than 50 times difference from highest to lowest activity was observed among strains, when explored relative to degradation of caseins. On average, more than 90% of the enzyme activities survived the heat treatment across strains. Thereby it was shown that not only expression level of the AprX gene, but also the level of enzyme activities varies greatly among strains. Furthermore, these findings point to differences in spoilage potential among strains which were not due to differences in heat susceptibility of their enzymes, but merely an effect of the level of enzyme activity.

What can it be used for?

Genome sequencing of Pseudomonas with large variations in their level of enzyme activities showed that these could be grouped according to genetic differences. The project deciphered that variation in spoilage potential among strains is mainly determined by expression levels of spoilage enzymes. This knowledge is useful in relation to the development of methods for faster and targeted prediction of the suitability of individual UHT batches for the production of UHT milk, for example development of a PCR-based method to avoid food waste and quality deterioration of UHT milk. ●

PROJEKT FACTS

Title: MicroLink – Prediction via omics-technologies of influence of microbiological enzymes on quality deterioration in UHT milk

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Participants: Department of Food Science, Aarhus University; National Food Institute, DTU; Arla Foods Innovation Centre

Project Period: 2019-2023

Main Purpose: To use 'omics' techniques to better understand why some batches of UHT milk end up being spoiled and others not, despite low bacterial counts in tank milk used for the production.

PROJECTS RELATED TO THE DANISH DAIRY RESEARCH FOUNDATION