

New tool could help maintain quality during cheese production

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Dutch type cheeses, notably edam and gouda, are made using complex starter cultures, that have been employed for centuries. Due to changes in strain composition within a culture, the quality frequently fluctuates. A team of Norwegian investigators has developed a tool that could be used to monitor the strains within a culture with high resolution, in order to maintain cheese quality. The research is published in *Applied and Environmental Microbiology*, a journal of the American Society for Microbiology.

Quality typically falls when [bacterial strains](#) in starter cultures become infected by viruses called bacteriophage. This is particularly problematic in industrial [cheese](#) production, which uses "frozen batch inoculum," according to the report. Unlike traditional "back-slopping" methods, in which samples from previous cheeses are used as starter cultures, frozen batch inoculum ensures that the bacteria, and thus the cheese product, will not vary.

However, while preventing bacterial evolution, frozen batch inoculum fails to preclude phage evolution. Thus, phage frequently gain the upper hand on the invariant bacteria.

Monitoring could offer prompt detection of [quality](#) problems, said corresponding author Helge Holo, PhD, Professor of Microbiology, Norwegian University of Life Sciences, Aas, Norway. Then, countermeasures could quickly mitigate the problems.

For example, the tool could identify strains that are most important to cheese quality. Then, those strains could be engineered to resist phage, or other strains having similar influence on taste and cheese quality, with less phage susceptibility, could substitute for those that are less resistant to phage, said Dr. Holo.

The tool the researchers developed is the use of next generation sequencing to sequence a gene called *epsD*. This is a protein that manufactures exopolysaccharide, an important compound made largely of sugars, that sits on the outer surfaces of cells. It is thought to be involved, among other things, in resisting phage.

In the study, the investigators isolated more than 200 strains of bacteria from three commercial starter cultures. From these, they sequenced the genomes of 95 strains. They then searched for the most variable gene that was present in all strains. The purpose was partly because such a gene could be used to determine how diverse the strains are—important information about starter cultures.

The relevant gene was *epsD*, and it was present in 93 of the 95 strains. Each strain's *epsD* is slightly different from all of the others, and thus, the strain can be identified from the *epsD* sequence.

Additionally, the tool can quantify the number of *epsD* sequences from a given strain, which enables determining the number of bacteria of that strain that are present. That is important because "The loss of abundance, or disappearance of an *epsD* sequence indicates that something has gone wrong," said Dr. Holo. "That could be a phage attack."

Mixed starter cultures used in the production of Dutch type cheeses are composed of undefined mixtures of certain subspecies of *Lactococcus lactis*, and certain species of the genus, *Leuconostoc*, according to the report.

From a scientific point of view, "The high degree of sequence variation of epsD represents evolutionary diversification, indicating a history of selection pressure." Much of that selection pressure likely occurs when bacteriophage infect one or a few of the strains, said Dr. Holo.

(Bacteriophage are very specific, in that a certain bacteriophage will only infect a limited number of bacterial [strains](#).) Bacteriophage are likely the ultimate cause of much of the fluctuation in quality that occurs in cheese cultures, she said.

Dr. Holo suggested that the "The presence of phages would be a driving force for keeping and not losing the [epsD] genes. The evolutionary diversification of epsD might reflect a long history of exposure to phages with different specificities."

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