# Cracking the Dairy Code: How Science is Creating Crystal Ball-like Models for Dairy Products

Predicting specific compounds in final dairy products using the information about microbial community structure, fermentation conditions and dairy substrate.

Cheese, the product of milk undergoing a fermentation process, presents a wide array of flavors, colors, textures, and forms. Beyond its deliciousness, cheese serves as a rich source of fats, minerals, vitamins, and protein – a nutritional goldmine. In our modern era, the demand for exceptional cheese with a sensory punch is skyrocketing.

Central to the captivating world of cheese are compounds like diacetyl, acetyl, acetic acid, acetaldehyde, and ethanol. These unsung heroes hold the key to cheese's quality and its remarkable flavor profile.

## Microbial community and cheese flavor

Microbes serve as a vital component in the fermentation of cheese, giving rise to delectable and distinctive flavors. Microorganisms in cheese are the primary generators of enzymes and have a significant influence on its flavor and quality. Conventionally fermented cheeses boast intricate microbial communities, often leading to inconsistent quality and notable flavor variations. Given that naturally occurring cultures, adopted through the back-slopping technique, tend to lack a specific composition, and can differ among dairy products, a more controlled fermentation path can be achieved through the introduction of carefully selected lactic acid bacteria.

## Long-read sequencing

Cheese production involves the use of complex and diverse starter cultures comprising numerous species, which can further divide into dozens of different strains. These bacterial strain level interactions significantly influence the ultimate quality of the product.

Thanks to recent breakthroughs in DNA sequencing, we've entered a new era of cheese exploration. Think of it as deciphering a secret code within the microbes. Long-read DNA sequencing technology is like a magnifying glass that reveals new details about microbial world that short-read technologies have missed.

It helps us piece together the complete genetic puzzle of these bacteria, without missing a beat.

## The project

In our project, two different undefined DL-starter cultures were obtained from Danish dairies, and approximately 400 isolates were purified from these cultures. Using cutting-edge long-read nanopore technology, the complete genomes of these isolates have been successfully reconstructed. As a result, we now possess not only knowledge of the bacterial strains present in the starter cultures, but also insights into their genetic potential and seek to decipher their role in flavor formation.

Our primary objective in this project is to develop a robust model capable of predicting specific compounds through detailed metagenomics. In order to achieve this goal, we will use individual strain to simulate cheese fermentation and monitor the volatile compounds produced by each strain, we will then gradually increase the complexity of the microbial communities, by combining two, three, four or more strains, and analyzing their genetical content and the compounds produced in cheese fermentation. Finally, based on multivariate data analysis, we hope to develop a model for the prediction of specific compounds based on bacterial strain-level characterization.

The tool developed to integrate microbial and flavor data from model fermentations will be applied to study real-life examples of dairy fermentations, specifically yellow cheese and buttermilk. Strains linked to given flavor com-

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## Project info:

*Title:* Cassandra: Quality modeling using detailed genomics
*Project manager:* Associate professor Lukasz Krych, Department of Food Science, University of Copenhagen
*Participants:* Arla Foods amba
*Project period:* March 2021-September 2025
*Objective:* To develop a robust and cost-effective system for predicting production of specific compounds during mixed mesophilic starter culture-based dairy fermentations using strain-level metagenomics.

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pounds will be mixed according to the model, the ultimate goal is to successfully replicate the desired flavor profiles as anticipated. Through a validation process, the prediction models will be tested to determine whether they accurately align with the formation of specific compounds in the final dairy products. As a minimum, this exciting research holds promise for enhancing our understanding of microbial contributions to flavor development in cheese fermentations.

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### Industry benefit from this research

The flavor of many dairy product is a crucial factor influencing consumer acceptance and choice. Typically, achieving the desired flavor profile often involves a complex fermenta-tion process. The intricate microbial community structure involved in this process can lead to unstable quality and significant flavor fluctuations in dairy products.

This project aims to develop a predictive model capable of swiftly and accurately forecasting desired or undesired compounds in the final fermented dairy products. This prediction will be based on bacterial strain-level characterization utilizing whole genome-based bioinformatics, and multivariate data analysis. The intention behind this approach is to replace the conventional, time-consuming, laborious, and costly "trial and error" methods.

Furthermore, this research will lay the foundation to extend to a wider array of dairy products by translational predicting of compound profiles in the end products. As dairy products tend to exhibit more stable and appealing flavors, this improvement in quality can lead to heightened consumer satisfaction, consequently fostering increased customer loyalty and recurring business.

In conclusion, the knowledge of this research will elevate the consistency of dairy product quality, optimize production processes, and better align with consumer preferences.

## Summary

Cheese holds immense market potential, with consumers seeking richer flavors. The intricate genetics of cheese microbes shape its distinct traits, especially flavor. Long-read DNA sequencing enhances our ability to pinpoint these microbes accurately, exploring their roles in sensory development.

Long-read sequencing has a potential to resolve complete genomes from mixed communities, while merging genomics and metabolomics via multi-omics analysis helps predict cheese's aromatic compounds. But this isn't just about genes; it's foreseeing the molecules that captivate our taste buds.

Beyond cheese, these predictive models will transform diverse dairy items.

By leveraging metagenomics and fermentation nuances, we reshape not only cheese but hopefully the entire dairy landscape.

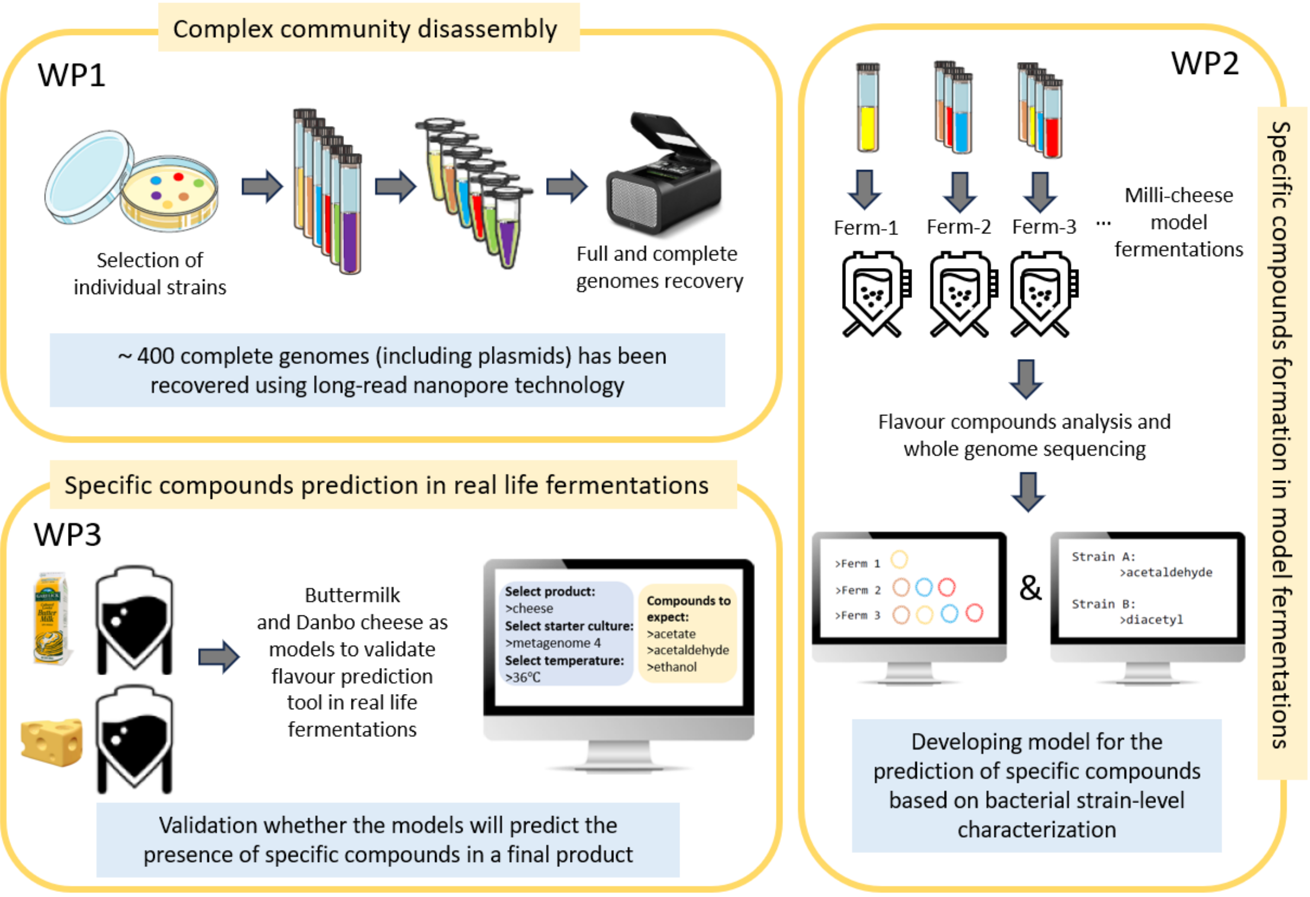

*Figure 1: Illustration of the project work packages*