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High-throughput Quantification of Cheese Bacteria

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Raw milk cheeses are considered richer in flavor than cheeses made from pasteurized milk due to the beneficial impact of the microbial community in raw milk. However, the impact of starter and non-starter lactic acid bacteria (NSLAB) on cheese quality in terms of flavor, texture and ripening stability is still incompletely understood in Swiss cheese varieties. So far, mainly culture-dependent methods with a limited set of selective media were used to study microbial communities of Swiss raw milk cheeses. Quantitative real-time PCR (qPCR) is a well-established method for detecting and quantifying bacteria. High-throughput qPCR (HT-qPCR) using microfluidics brings further advantages by providing fast results and by decreasing the cost per sample.

Recently, we validated our new HT-qPCR system targeting 24 bacterial species relevant for cheese quality in collaboration with the Genetic Diversity Centre (GDC, ETH Zurich). The developed qPCR assays were highly specific for the target species under identical amplification conditions. The HT-qPCR system offers a fast, accurate, and cost-efficient monitoring of desired and undesired microorganisms in cheese. As for example, with a 192.24 Dynamic array integrated fluidic circuit (IFC, Fluidigm Corporation) chip, a simultaneous screening of the 24 species in 56 cheese DNA samples in technical triplicates in a single run is possible.

Microfluidics brings many advantages, allowing thousands of reactions to be performed in parallel in very small volumes (nanoliter-scale) and thus consuming massively less material and reagents in comparison to standard qPCR. However, there is also a trade-off in terms of significantly higher detection limits. This disadvantage can be partially compensated by a multiplex-PCR step with a low number of cycles to selectively enrich the target DNA sequences. This preamplification step can be used to qualitatively detect bacterial species with low abundance (< 8×10^4 genome equivalents/g cheese).

The use of the new HT-qPCR approach will provide more comprehensive data on the growth and succession of microbial species during cheese ripening, thus providing a better understanding of the influence of individual species on cheese quality. Moreover, we see a great potential for new diagnostic possibilities regarding the identification and monitoring of microbially induced cheese quality defects.

In conclusion, HT-qPCR is a fast, reliable and economic approach to quantify quality-relevant bacterial species in cheese and has promising applications, such as monitoring the composition of the bacterial microbiome of raw milk cheeses to ensure consistent product quality.

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Fig. 1. Raclette du Valais AOP, a smear-ripened, full-fat semi-hard cheese made from raw milk. The cheese microbiome and thus also the quality of raw milk cheeses is influenced by environmental factors as well as manufacturing and ripening conditions.

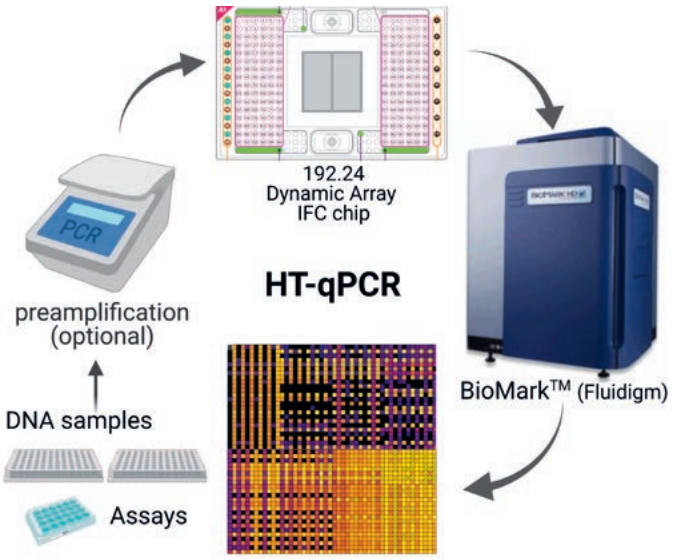

Fig. 2. Overview of the HT-qPCR workflow (created with BioRender.com). DNA samples and qPCR assays were loaded on the Dynamic Array integrated fluidic circuit (IFC, Fluidigm Corporation) chip. The Biomark instrument (Fluidigm Corporation) performed qPCR for the 4608 singleplex-qPCR reactions on the chip in parallel and recorded the fluorescence signal with a high sensitivity camera. Quantification cycle values were compared to qPCR standard dilution series and the number of copies in the DNA samples was calculated.

Reference

M. Dreier, H. Berthoud, N. Shani, D. Wechsler, P. Junier, *Front. Microbiol.* **2021**, *11*, 619166. https://doi.org/10.3389/fmicb.2020.619166.

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