

# Functional strain redundancy and phage resistance diversity in Swiss raw mixed cultures

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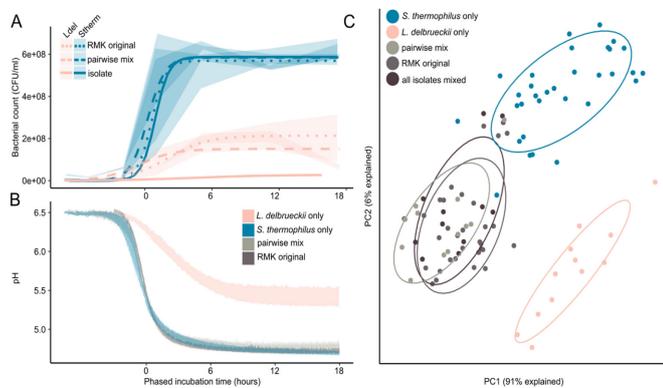
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## Introduction

Undefined starter cultures are so far poorly characterized bacterial communities from environmental origin used in cheese making. They are phenotypically stable and have evolved through domestication by repeated propagation in closed and highly controlled environments over centuries. This makes them interesting for understanding eco-evolutionary dynamics governing microbial communities. While cheese starter cultures are known to be dominated by a few bacterial species, little is known about the composition, functional relevance, and temporal dynamics of strain-level diversity. Here, we applied shotgun metagenomics to an important Swiss cheese starter culture used for hard cheeses

## Results: Functional strain redundancy

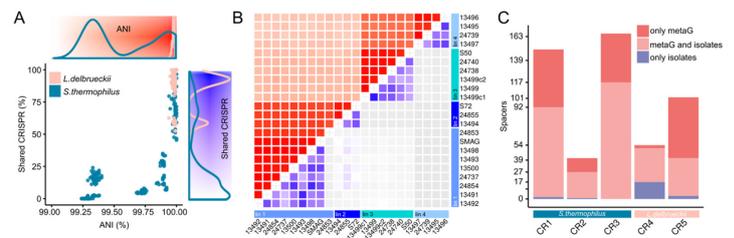
While the composition of the community is simple, the two species and the multiple coexisting strains may contribute important cheese-related functional properties (i.e., rapid acidification and flavor volatiles production) and express different ecological traits that facilitate their stable coexistence (i.e., growth or metabolic potential). We noticed that the strains, however, are highly redundant for the growth (Fig. 1A), acidification (Fig. 1B) and volatile production capacity (Fig. 1C). Moreover, they grew and acidified much better in co-culture than in isolation. The volatile profile is clearly different when the two predominant species co-occur, independent from complexity of strain diversity.



**Fig.1:** A) Cell count of both species individual, original mixed culture and artificial mixture. B) Acidification of the same samples as in A). C) Principal component analysis of the metabolic profiles of *S.thermophilus* and *L.delbrueckii* (and mix) after 24 h of growth at 37 °C. Different treatments are highlighted in colors and with the surrounding eclipse.

## Results: Phage resistance diversity

Ecological theory predicts that functional redundancy without niche specialization prevents stable coexistence, which is in contrast with our results. We hypothesize that phage predation may contribute to the maintenance of low levels of strain diversity in the analyzed cheese starter culture. Here, we observed that while the isolated lineages and isolate are genomically highly similar (large average nucleotide identity), they share only very few CRISPR spacers (Fig. 2A-B). While we have isolated the large majority of core-genome SNV diversity, many novel CRISPR spacers remain in the metagenome (Fig. 2C).



**Fig.2** CRISPR spacer diversity of *L. delbrueckii* and *S. thermophilus*. A) The correlation of fraction of shared CRISPR spacers and ANI. B) The heatmap of the genomic (ANI: top heatmap; from white to red) and CRISPR spacer diversities (bottom heatmap; from white to blue) of *S. thermophilus*. C) The amount of metagenomic (metaG) and genomic (isolates) CRISPR spacers according to the five arrays.

## Conclusion

Undefined starter cultures are bacterial communities which are highly stable and dominated by only a few coexisting strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *lactis*. Genome sequencing, metabolomics analysis, and co-culturing experiments of 43 isolates show that these strains are functionally redundant, but differ tremendously in their phage resistance potential. Moreover, we identified two highly abundant *Streptococcus* phages that seem to stably coexist in the community without any negative impact on bacterial growth or strain persistence, and despite the presence of a large and diverse repertoire of matching CRISPR spacers. Our findings show that functionally equivalent strains can coexist in domesticated microbial communities and highlight an important role of bacteria-phage interactions that are different from kill-the-winner dynamics.

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