Extensive diversity and rapid turnover of phage defense repertoires in cheese-associated bacterial communities

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Introduction

Phages are key drivers of genomic diversity in bacterial populations as they impose strong selective pressure on the evolution of bacterial defense mechanisms across closely related strains. The pan-immunity model suggests that such diversity is maintained because the effective immune system of a bacterial species is the one distributed across all strains present in the community. However, only few studies have analyzed the distribution of bacterial defense systems at the community-level, mostly focusing on CRISPR and comparing samples from complex environments. Here, we studied 27 cheese-associated species encompassing 2'778 bacterial genomes and 158 metagenomes, which are dominated by a few bacterial taxa and occur in relatively stable environments.

Results 1: Rapid turnover of CRISPR spacer diversity

We find that nearly identical strains of cheese-associated bacteria contain diverse and highly variable arsenals of innate and adaptive (CRISPR-Cas) immunity (Fig.1 A). Based on this information we inferred a a rapid turnover of CRISPR spacers with genomic distance (Fig.1 B).

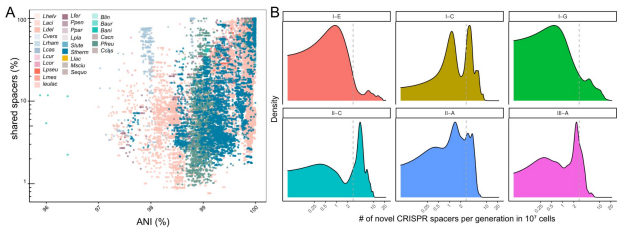

Fig.1: Genomic CRISPR divergence. A) CRISPR similarity vs. ANI of all pairwise strain combinations. B) turnover rate of different CRISPR-Cas subtypes calculated from A).

Results 2: Large diversity of CRISPR spacers in community

We find many more CRISPR spacers in the metagenomes of cheese samples (Fig. 2A). Moreover within every metagenomic sample novel CRISPR spacers are discovered (Fig. 2B). Overall, this collaborates the rapid turnover of defense mechanisms in these communities.

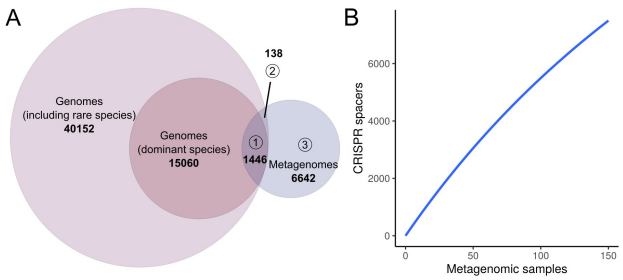

Fig.2: High diversity of CRISPR spacers in cheese communities. A) Venn diagram of genome or metagenome specific spacers. B) Rarefaction curve of CRISPR spacers over the different metagenomic samples.

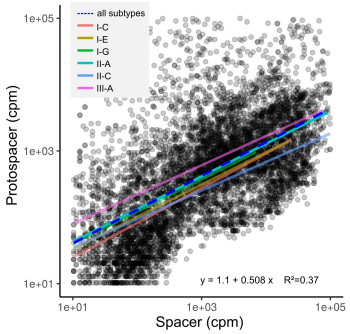

Fig.3: CRISPR spacer and protospacer abundance indicated in counts per million (cpm).

Results 2: Selection of phage-targeting CRISPR

CRISPR spacer abundance correlated with the abundance of matching target sequences across the metagenomes providing evidence that the identified defense repertoires are functional and under selection (Fig. 3).

Results 3: Incomplete CRISPR immunity

While the previously described characteristics align with the pan-immunity model, the detected CRISPR spacers only targeted a subset of the phages (vOTUS) previously identified in cheese, suggesting that CRISPR does not provide complete immunity against all phages, and that the innate immune mechanisms may have complementary roles (Fig. 4).

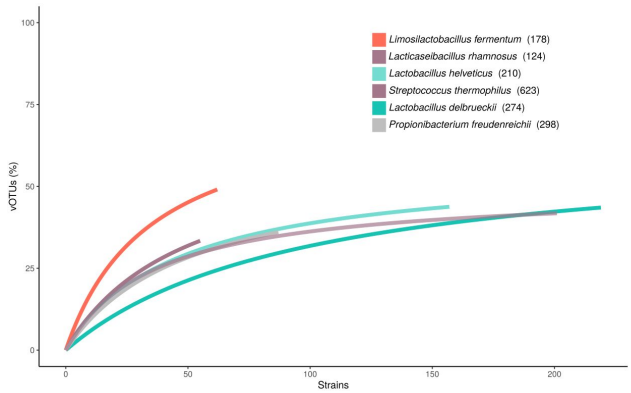

Fig.4: Protospacer diversity. The rarefaction curves of the metagenomic samples for all species with more than 50 genomes and more than 85 described vOTUs.

Conclusion

Our findings show that the evolution of bacterial defense mechanisms is a highly dynamic process and highlight that experimentally tractable, low complexity communities such as those found in cheese, can help to understand ecological and molecular processes underlying phage-defense system relationships.

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