Influence of smear microbiota on the survival of *Escherichia coli* in raw milk cheeses

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Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are a food safety issue for raw milk dairy products. Optimal development of starter cultures generally controls the growth of generic *E. coli*, thereby preventing spoilage by early blowing. However, the infectious dose of highly pathogenic STEC is low, making their presence in food problematic even at minimal levels. The growth and survival of *E. coli* in the core of raw milk cheeses has been described, but their behaviour in cheese smears remains to be investigated.

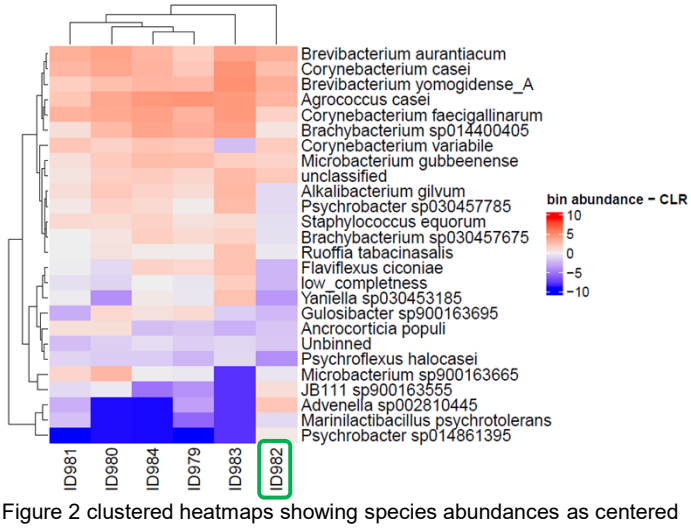

Figure 2 clustered heatmaps showing species abundances as centered log-ratios (CLR). Classsification of bins according to gtdb-tk.

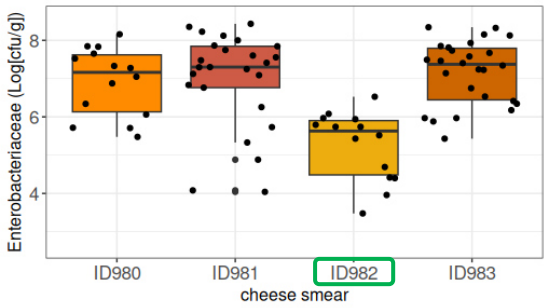

Figure 3. Enterobacteriaceae cell count in the cheese rind

Results

Raw milk spiked with generic *E. coli* was processed into semi-hard smear cheese. Survival in the core and in the smear was monitored during ripening. The smear allowed better survival of *E. coli* than the cheese core, with <2.5 log and >4 log decrease after 105 days, respectively (Fig1). Complex smear microbial consortia isolated from four ripening facilities were used to inoculate smear brines, mimicking the old-young smearing process. *E. coli* survival was dependent on the consortia used, with reductions ranging from 1 to 2.5 log (Fig1). Shotgun metagenomics of the original consortia revealed the presence at dominant level of the genus *Advenella* (order Burkholderiales) in the most antagonistic smear **ID982** (Fig2). The antagonistic effect of smear ID982 encompassed all Enterobacteriaceae (Fig3).

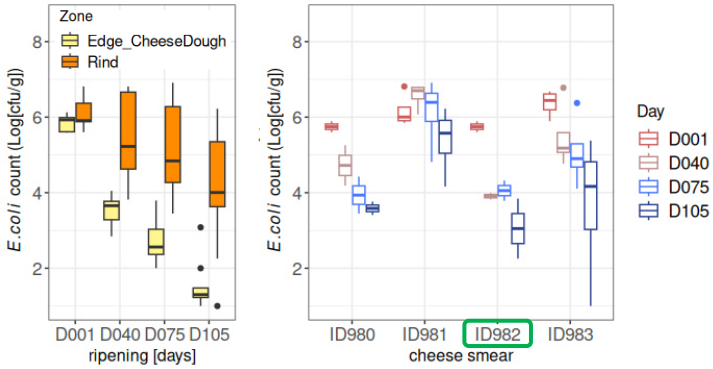

Figure 1. *E. coli* survival in the cheese matrix

Materials & methods

Pilot-plant

- Spiking of raw milk with 1'000 *E.coli* / mL
- Ripening cellar at 14°C and 92% relative humidity
- Smearing with brine at 3% sodium chloride twice a week

Wet lab

- Incubation on CHROMagar™ *E.coli*: 24h at 37°C and VRBG Agar for Enterobacteriaceae: 48h at 30°C

Dry lab

- TruSeq DNA PCR-free libraries, sequencing on Illumina Novaseq 6000
- Metagenomes processed with nf-core/mag v3.1.1 pipeline: assembly using SPAdes; binning using MetaBAT2, MaxBin2, and CONCOCT, followed by refinement with DAS Tool based on co-abundance. Only refined bins retained for downstream analysis

Summary
The study demonstrates that *Escherichia coli* survival in raw milk semi-hard cheeses is influenced by the smear microbial consortia. As plant-associated Burkholderiales strains have been shown to inhibit gram-negative bacteria in a contact-dependent manner, future work will explore the potential of *Advenella* strains as natural antagonists to enhance food safety in cheese production.

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