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Aim

To replicate sensory qualities of traditional items while enhancing nutritional value and supporting environmental, ethical, and safety goals. This study compares the microbiota of plant-based fermented cheese analogues with conventional cheeses using both culture-dependent and molecular approaches, providing insights into their microbial ecology.

Method

Cheeses of both animal (Mozzarella, Gouda, semi-hard cheese) and plant (Mozza, Vera) origin were purchased from the market and analyzed via both classical microbiological and metabarcoding analyses. DNA was extracted using the Macherey-Nagel Bioanalysis kit, followed by the amplification of V3-V4 region of 16S rRNA gene and ITS 1-2 loci for bacterial and fungal diversity, respectively.

Results

- **Yeasts and Lactic Acid Bacteria (LAB)**

co-existed in most samples, showing significantly higher yeast counts than LAB and Enterococci in **plant-based products** (Fig 1).

- **Fungal metabarcoding** revealed substantial diversity e.g., *Malassezia*, *Debaryomyces*, *Penicillium* between dairy and plant matrices - *Saccharomyces* was exclusively detected in plant-based products (Fig 2).

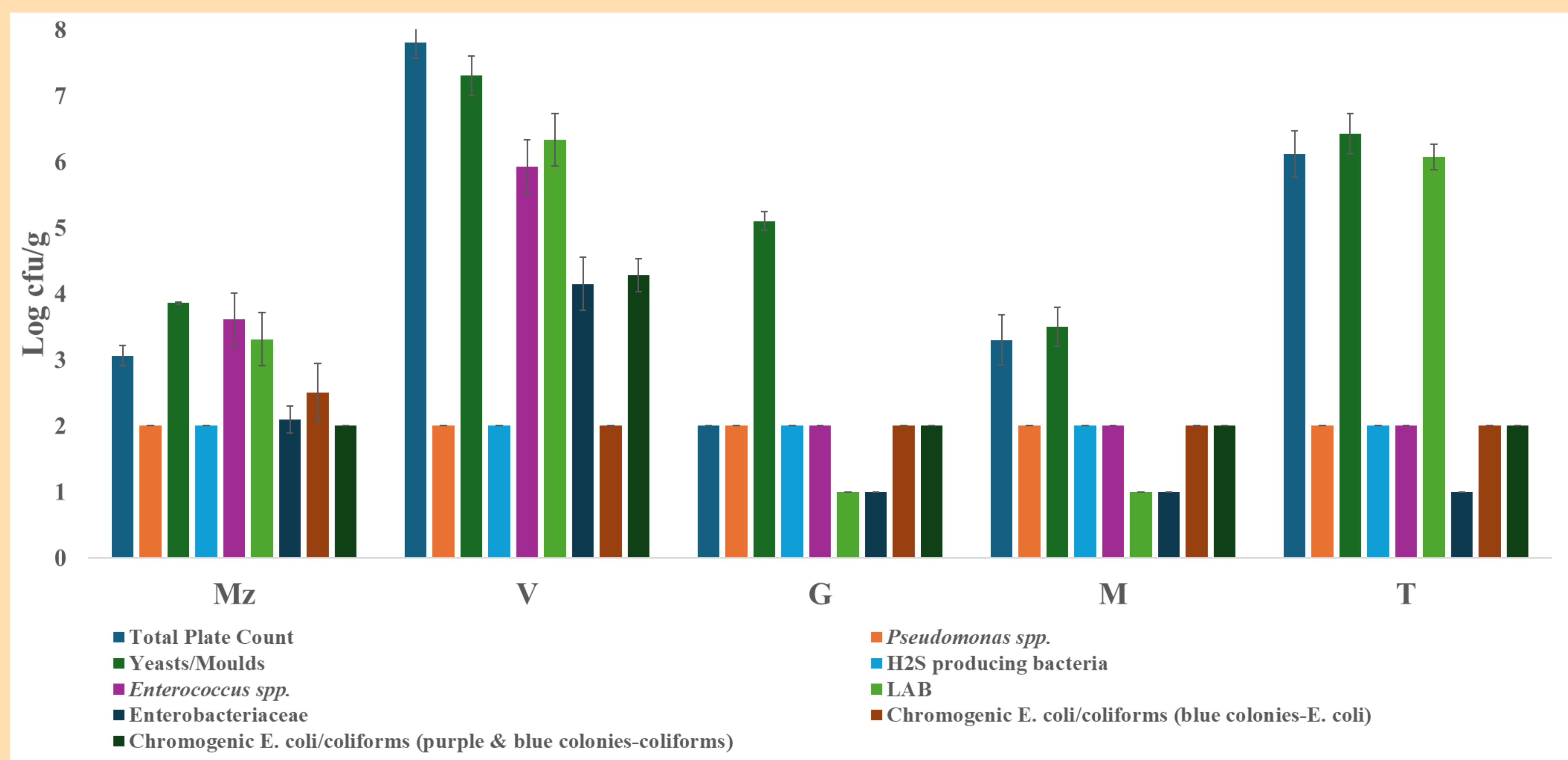


Fig. 1: Microbial population changes of dairy and plant-based dairy products (Mz: Plant based Mozza ,V: Plant-based Vera, G: Dairy Gouda, M: Dairy Mozzarella, T: 3-month cured dairy cheese.). Total Viable Counts (TVC), *Pseudomonas* spp., H₂S producing bacteria, LAB, yeasts, Enterobacteriaceae, *E. coli*/Coliforms. Each point is the mean of three replicates. Error bars represent the standard error.

- **16S metabarcoding analysis** showed distinct bacterial communities between matrices: **Plant-based:** dominated by *Lactocaseibacillus* and *Leuconostoc* while **Dairy:** dominated by *Streptococcus*, *Lactococcus*, and *Acinetobacter* (Fig. 3).

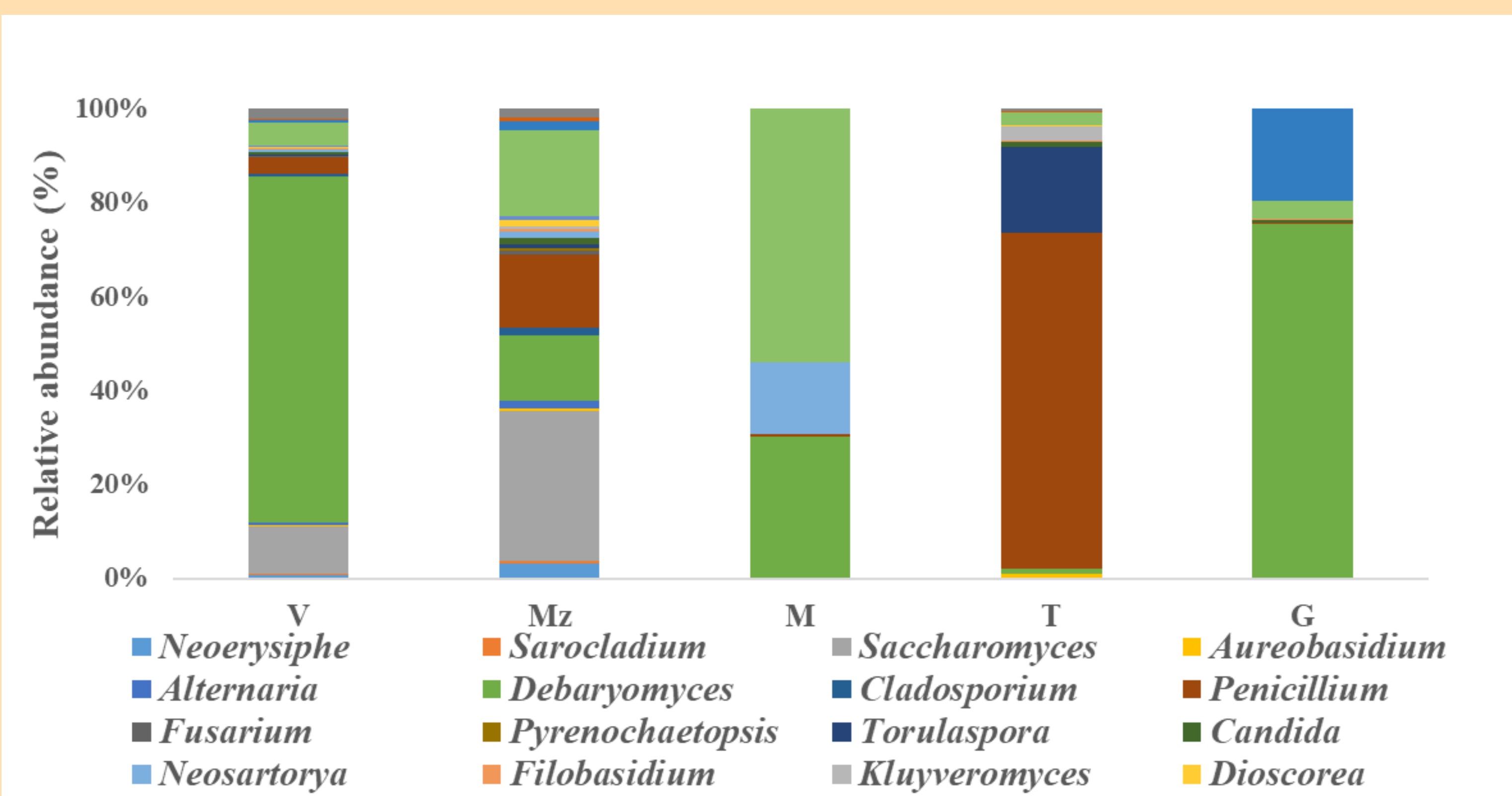


Fig. 2: Relative abundance (%) of fungal genera of dairy and plant-based dairy products as revealed by ITS metabarcoding analysis (Mz: Plant based Mozza ,V: Plant-based Vera, G: Dairy Gouda, M: Dairy Mozzarella, T: 3-month cured dairy cheese.)

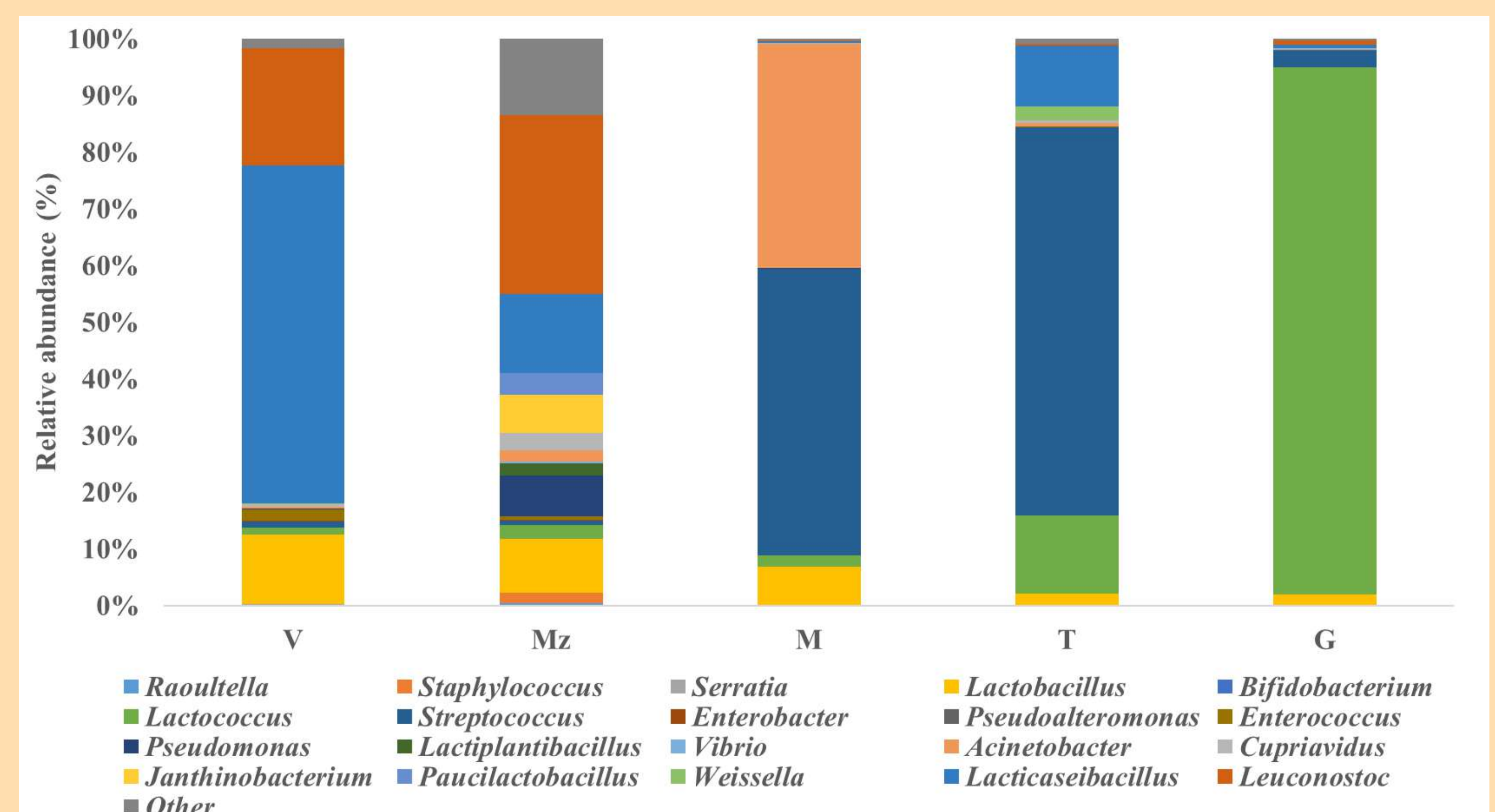


Fig. 3: Relative abundance (%) of bacterial genera of dairy and plant-based dairy products as revealed by 16S rRNA metabarcoding analysis. (Mz: Plant based Mozza , V: Plant-based Vera, G: Dairy Gouda, M: Dairy Mozzarella, T: 3-month cured dairy cheese.)

Conclusion

This work provides a holistic snapshot of the microbiota presented in plant-based cheese analogues, revealing noteworthy differences compare to dairy products. These findings contribute valuable insights for the optimization of fermentation processes and the development of consistent, high-quality plant-based cheeses in response to growing consumer demand.

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