

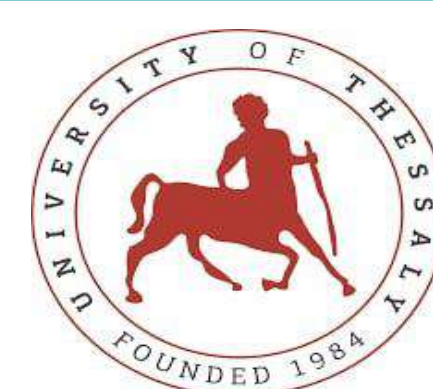
Microbial Profiles of Dairy- and Plant-based Brie: Substrate-Driven Differences Revealed by Culture-dependent methods and Metagenomics

Natalia Chatzimpyrou^{a, b}, Dimitrios A. Anagnostopoulos^a, Evangelia A. Karamani^a, Athanasios Tsiartsafis^a, Stavroula Letsiou^a, Ioannis S. Boziaris^a, Myrsini Kakagianni^b and Foteini F. Parlapani^{a*}

^a Laboratory of Marketing and Technology of Aquatic Products and Foods, Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Fytokou Street, 38446 Volos, Greece, , e-mail: dianagnost@uth.gr, ekaramani@uth.gr, atsiartsafis@uth.gr, sletsiou@uth.gr, boziaris@uth.gr, fwparlap@uth.gr

^b Department of Food Science and Nutrition, School of Agriculture Sciences, University of Thessaly, 43100, Karditsa, Greece, e-mail: chnatalia@uth.gr, mkakagianni@uth.gr

(*Corresponding author: Foteini F. Parlapani, e-mail: fwparlap@uth.gr)



Introduction

- **Fermented cheese analogues** are rapidly expanding, yet how the underlying **substrate** (dairy vs plant) shapes microbial assembly, succession, and quality/safety remains under-characterized
- We present a **comparison** of a traditional **dairy Brie** and a **plant-based Brie** using a combined **culture-dependent** and **next-generation sequencing (NGS)** workflow
- By integrating standard **plate counts** for key microbial groups with **16S rRNA (V3–V4)** and **ITS1–2** metabarcoding, alongside **pH** and **water activity** profiling, we delineate **substrate-driven differences** relevant to product **stability** and **safety**

AIM

To quantify **substrate-driven** differences (dairy vs plant) in Brie microbiomes

Objectives

- Compare **culture-dependent counts**: TVC, LAB, yeasts/moulds, *Enterobacteriaceae*, *Pseudomonas* spp., H₂S-producing *Enterobacteria*, *Enterococcus*, chromogenic *E. coli*/coliforms
- Profile **bacterial** (16S V3–V4) and **fungal** (ITS1–2) communities via NGS
- Relate microbial metrics to **physicochemical parameters** (pH, a_w)

Materials & Methods

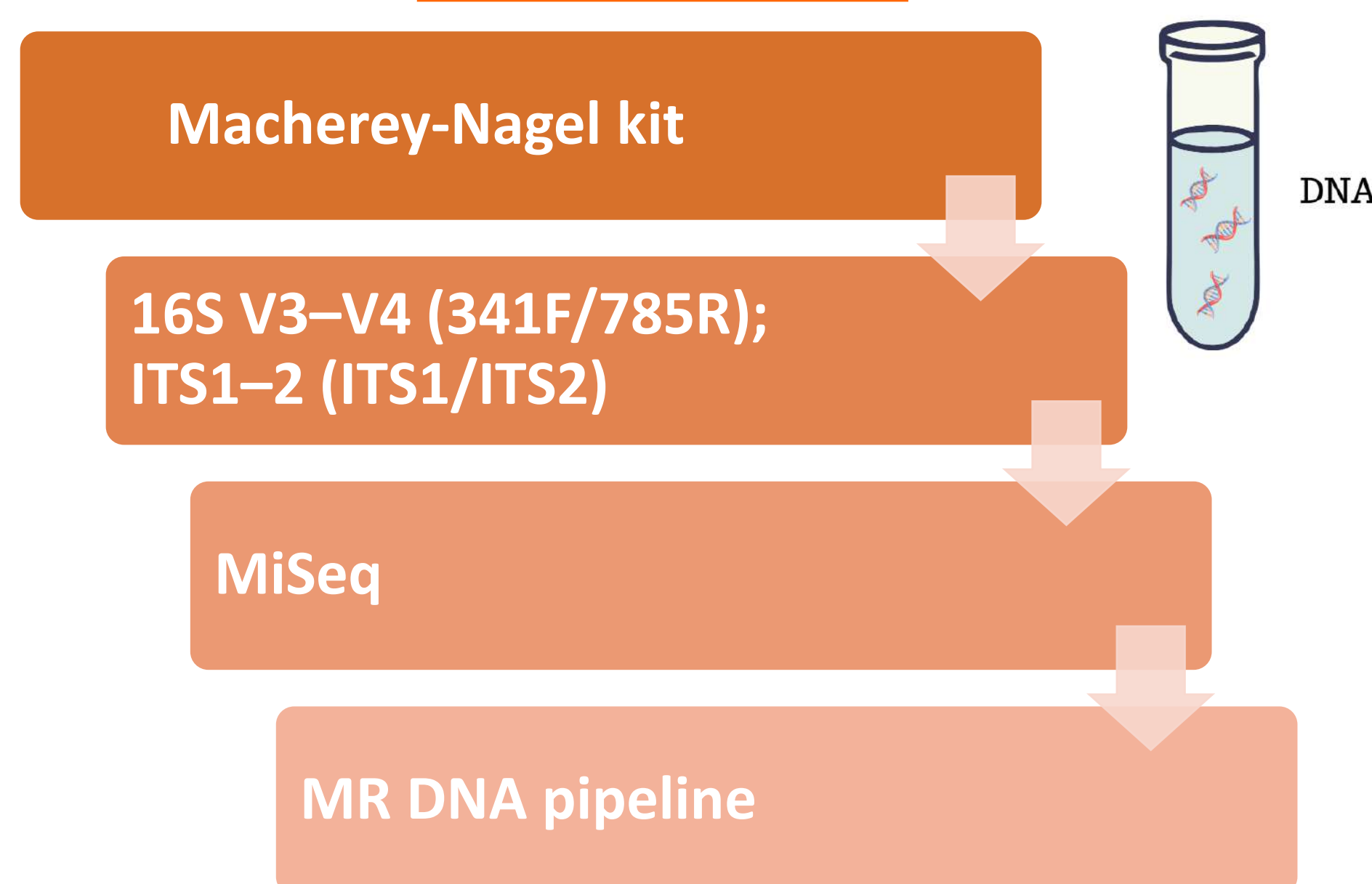
Enumeration of microbial populations

- **TVC** (TSA, 25 °C, 48–72 h)
- **LAB** (MRS, 25 °C, 72 h)
- **Yeasts/moulds** (YEPD, 25 °C, 3–5 d)
- ***Enterobacteriaceae*** (VRBGA, 37 °C, 24 h)
- ***Pseudomonas*** (CFC, 25 °C, 48 h)
- **H₂S-producing *Enterobacteria*** (Iron Agar, 25 °C, 48 h)
- ***Enterococcus*** (Slanetz, 37 °C, 48 h)
- **Chromogenic *E. coli*/coliforms** (HARL, 37 °C, 24 h)



Results in log CFU/g (mean ± SD, n=3)

DNA Extraction



denoising → zOTUs → BLASTn vs curated NCBI; α/β -diversity in QIIME2)

pH & a_w measurements

pHmeter
 a_w analyzer
Triplicate measures

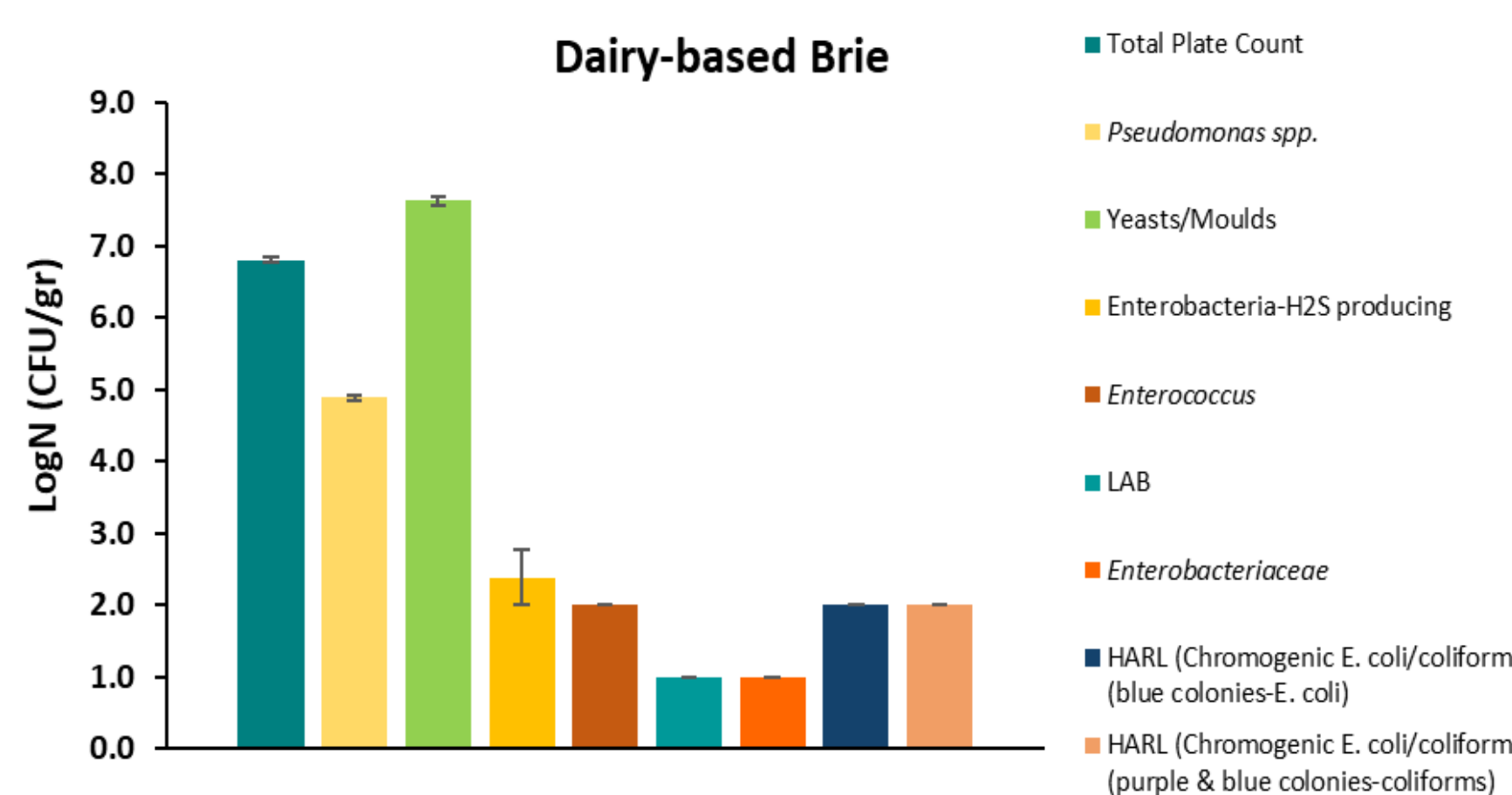


Results

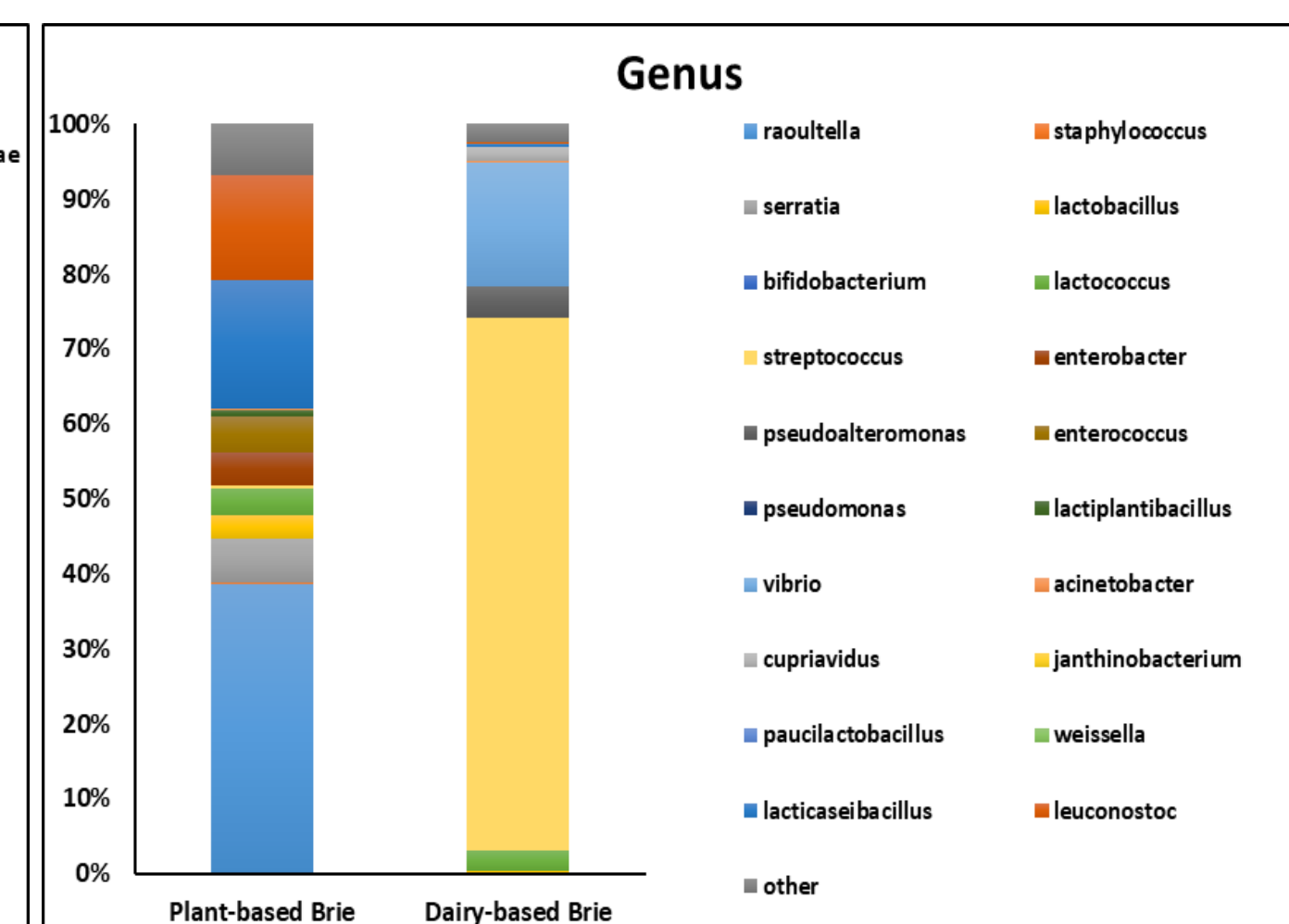
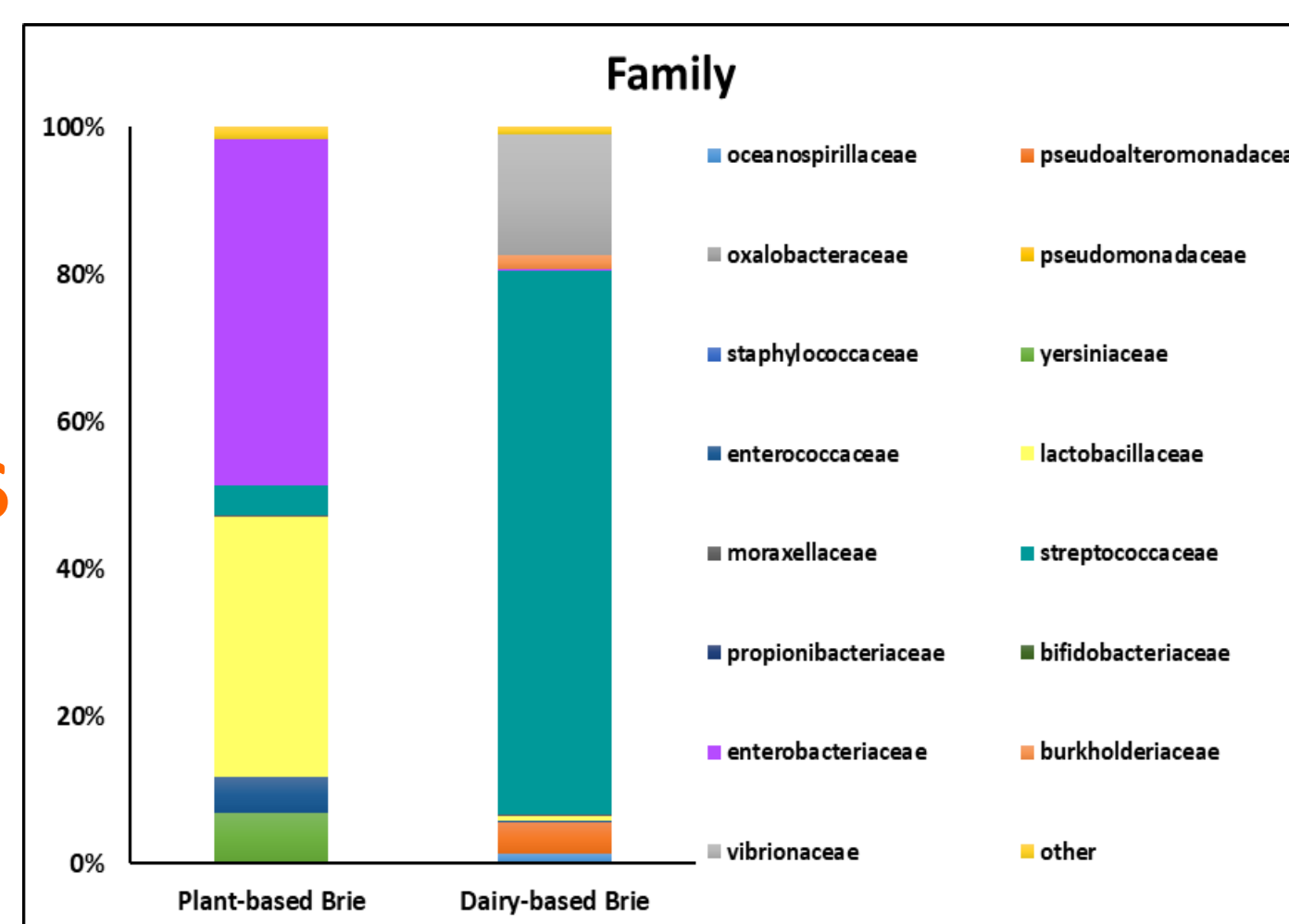
Microbial characterization

Dairy-based Brie

- **pH: 6.73 - 6.97** (near-neutral)
- **a_w : 0.987**
- **Yeasts/molds**: predominant
- **TVC**: substantial populations
- **16S**: dominant *Streptococcus* (73%), *Vibrio* (16%)
- **ITS**: dominant *Penicillium* (94%)

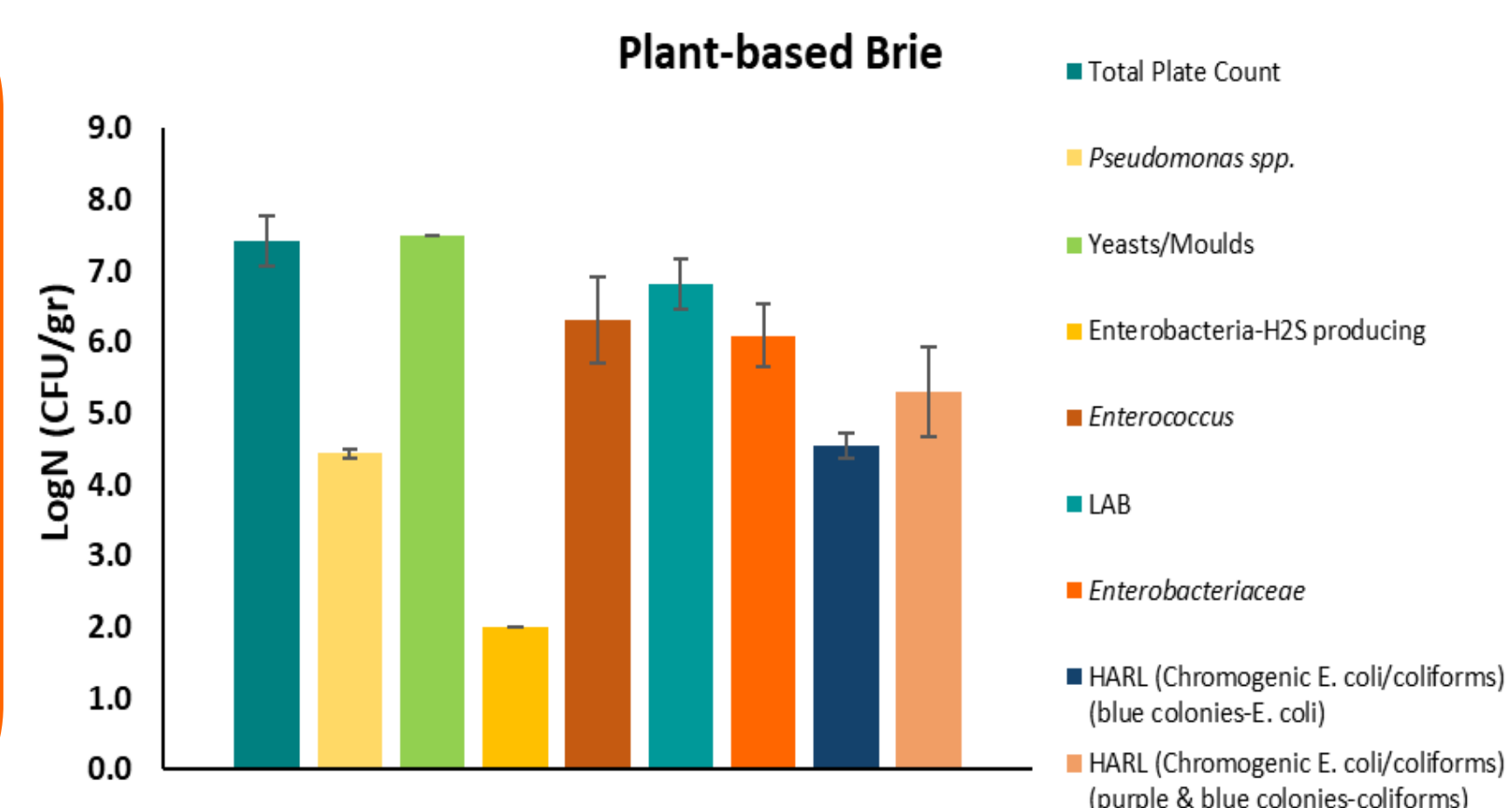


16S

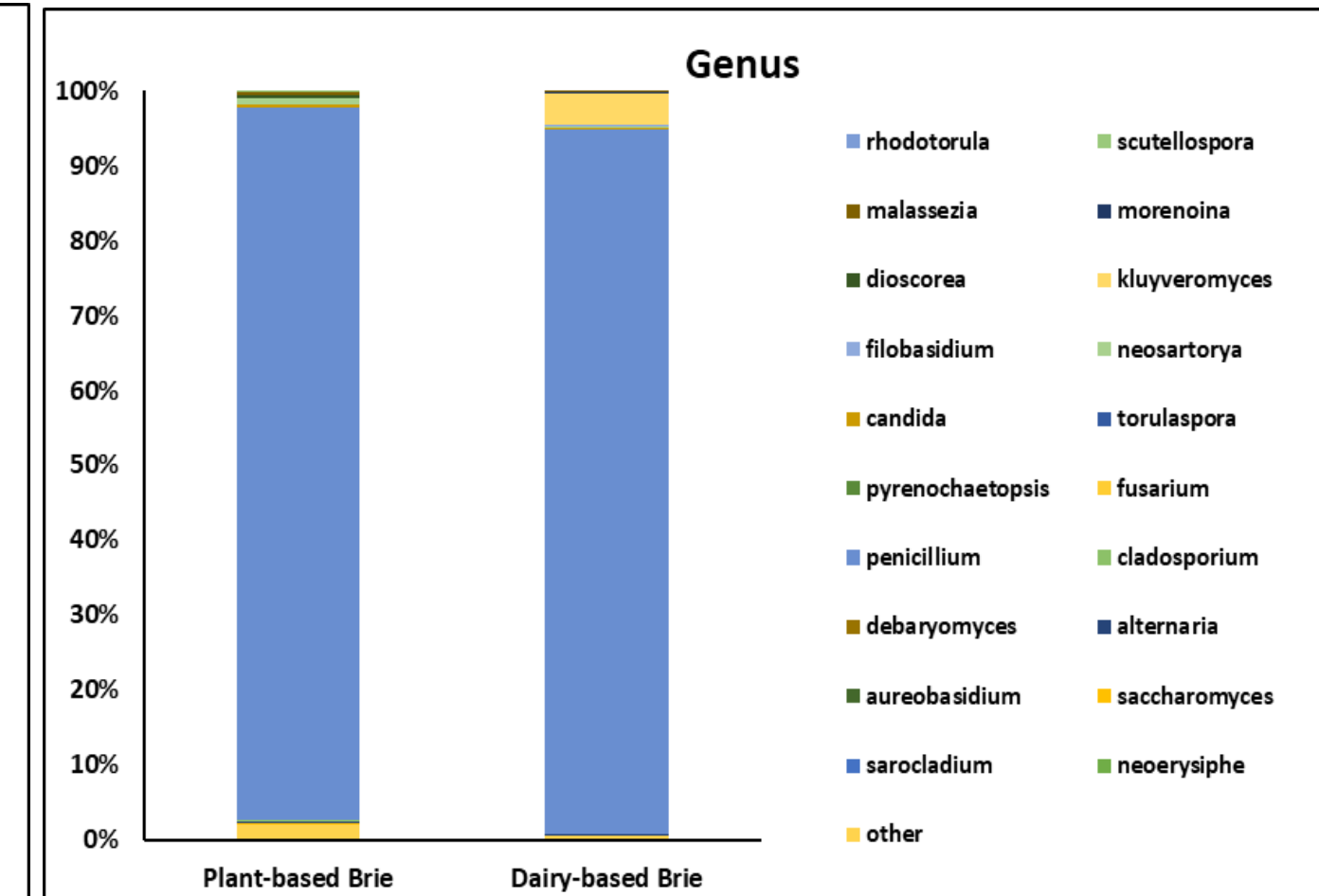
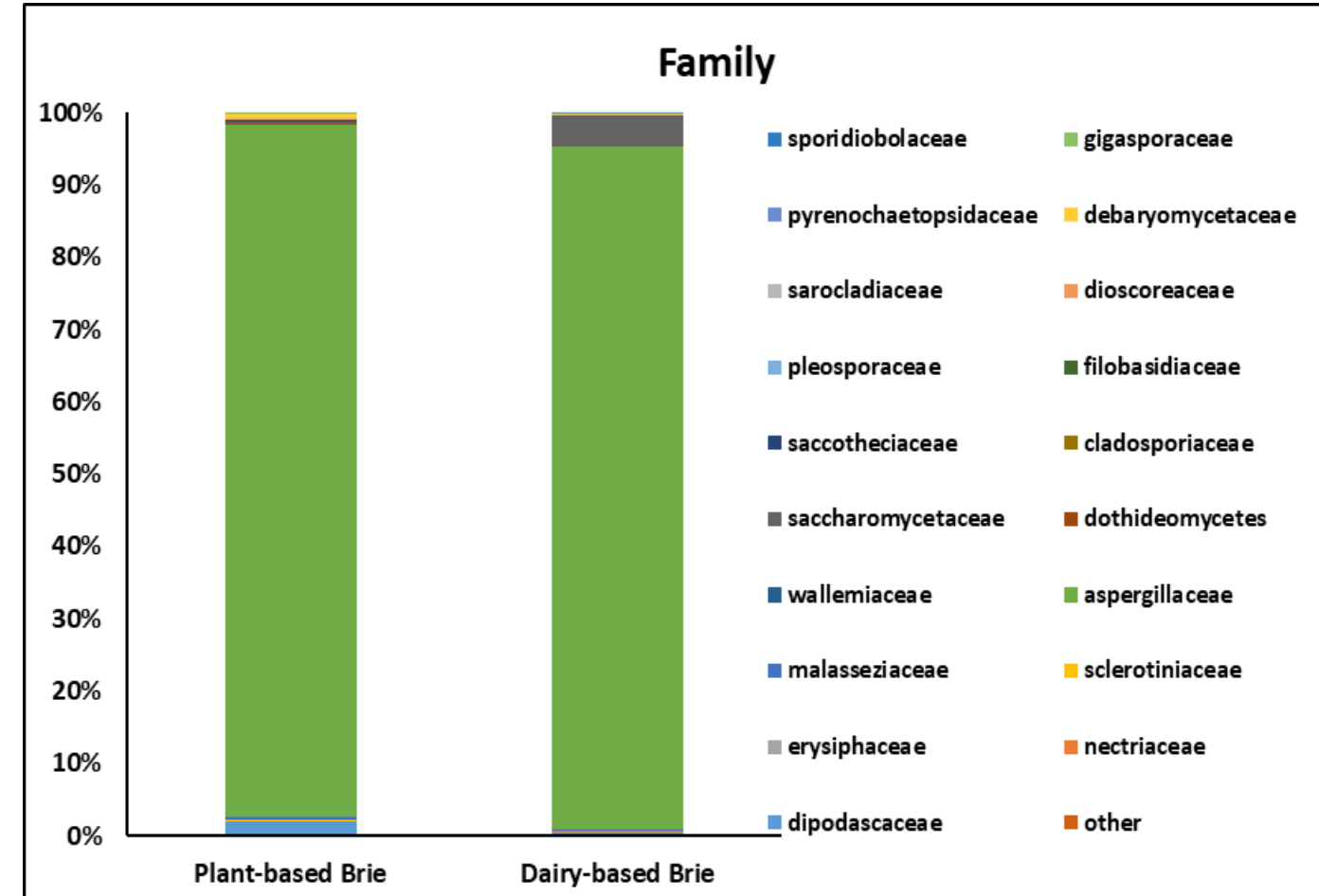


Plant-based Brie

- **pH: 4.9 – 5.3** (acidic)
- **a_w : 0.991**
- **TVC, presumptive yeasts, LAB and *Enterococci***: high populations
- **16S**: *Lactocaseibacillus* (17%) with *Raoultella* (38.6%), *Leuconostoc* (14%), *Enterococcus* (5%)
- **ITS**: dominant *Penicillium* (95%)



ITS



These results highlight the challenges of replicating traditional fermentation in plant-based matrices and the importance of substrate composition in shaping microbial succession and safety

Conclusions

- **Substrate drives distinct microbiomes** in Brie (dairy vs plant-based)
- **Dairy Brie**: near-neutral pH 6.73–6.97, dominant yeasts/molds and TVC, dominance of *Streptococcus* and *Penicillium*

- **Plant-based Brie**: acidic pH 4.9 – 5.3, TVC, presumptive yeasts, LAB and *Enterococci*: high populations; starter *Lactocaseibacillus* with *Raoultella*, *Leuconostoc* and *Enterococcus* detected by NGS
- **Need for integrated microbial monitoring** in plant-based cheesemaking to ensure product quality, stability, and safety

References

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