

IN-DEPTH MICROBIAL ANALYSIS OF **SANT'AGOSTINO** TABLE OLIVES FLAVORED WITH **WILD FENNEL** (*Foeniculum vulgare* Mill., 1768)

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INTRODUCTION

This study investigates the microbial ecology of Sant'Agostino green table olives, a traditional Apulian product green flavored with wild fennel (*Foeniculum vulgare*), employing a polyphasic approach that integrates both culture-dependent and culture-independent

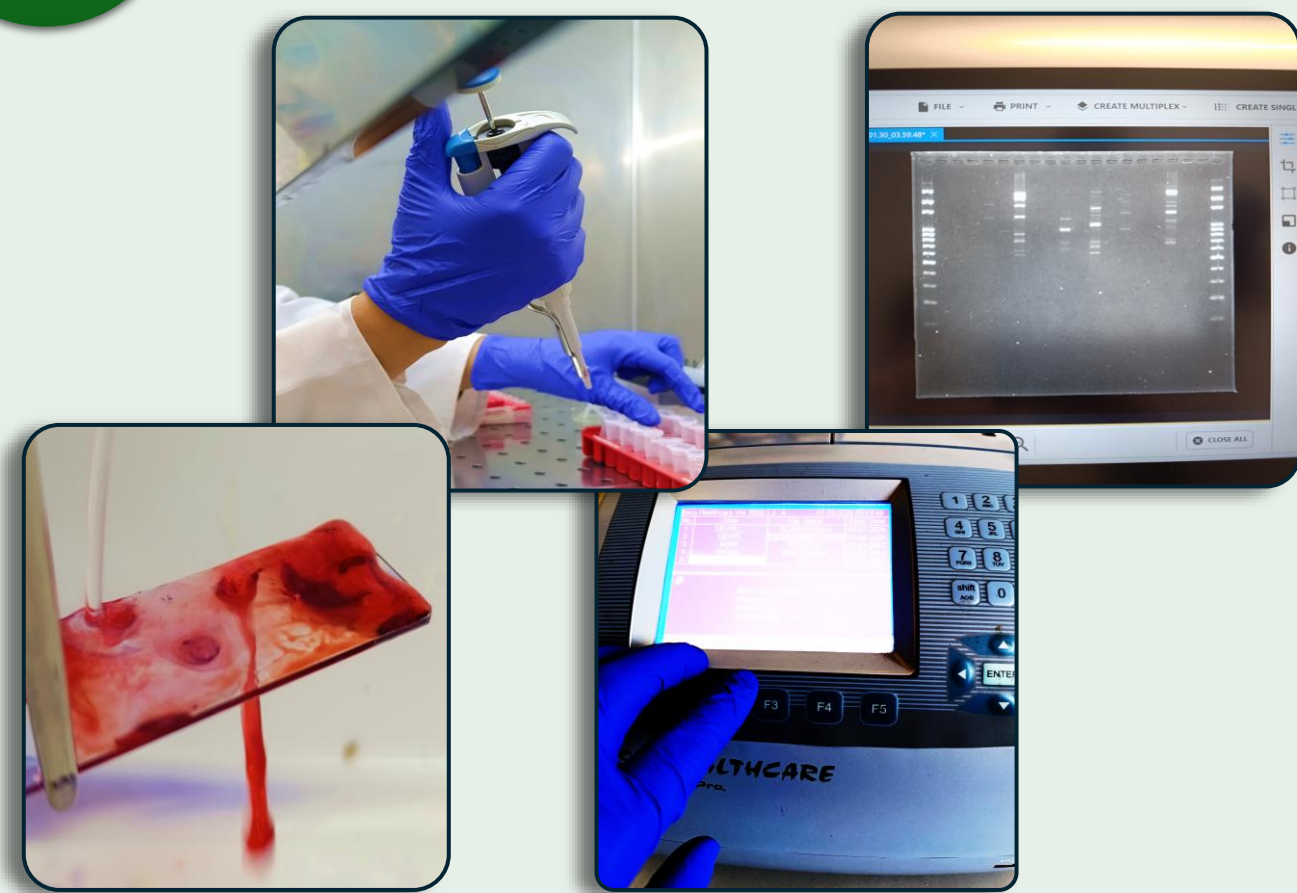
methodologies.

The olives underwent spontaneous fermentation. Both fruit and brine samples were analysed to characterise cultivable and non-cultivable microbial communities.

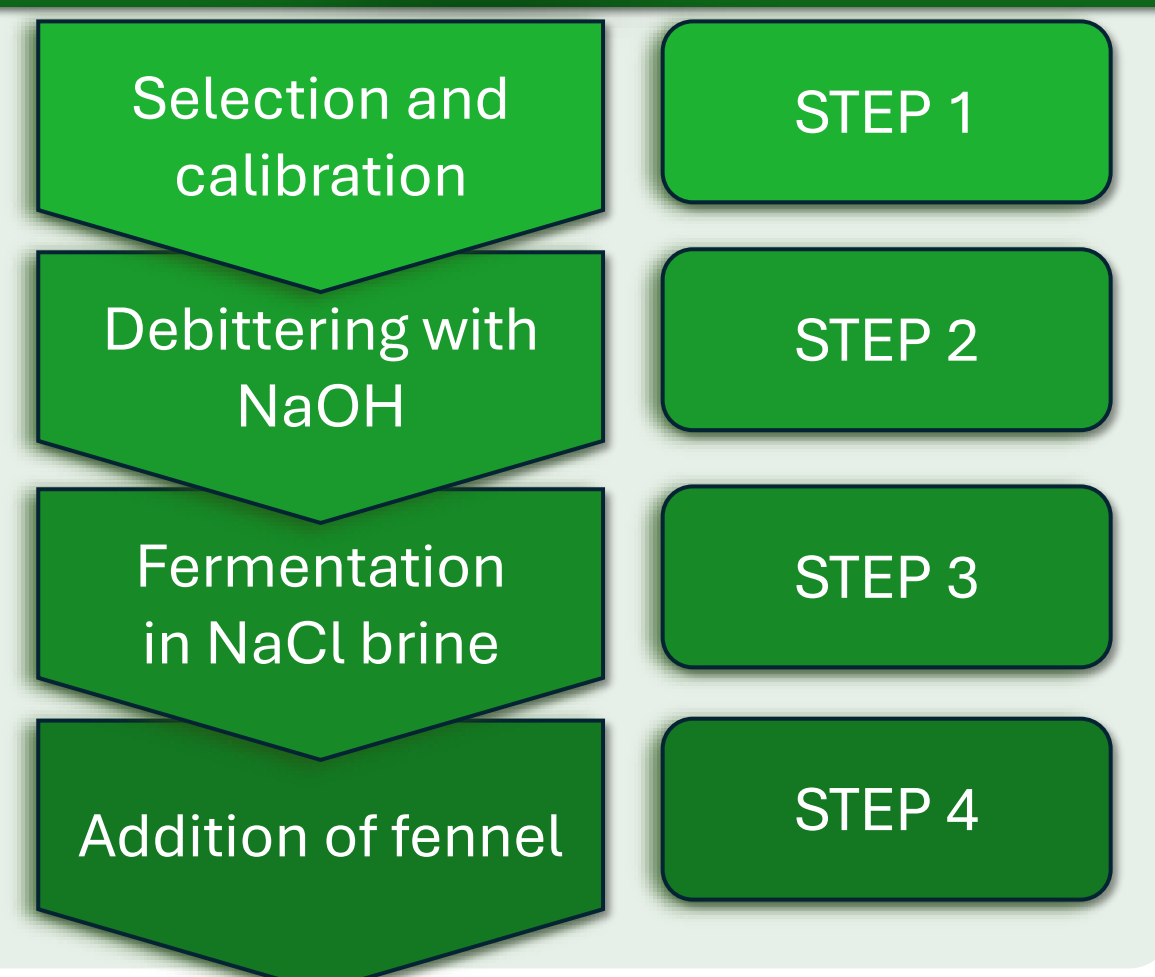


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METHODOLOGY



- ❖ Table olive processing and sampling.
- ❖ pH measurement.
- ❖ Microbiological analysis.
- ❖ Isolation, phenotypic and genotypic characterisation of LAB and spore-forming bacteria.
- ❖ Isolation, phenotypic and genotypic characterisation of yeast.
- ❖ Extraction of the DNA and preparation of the MiSeq library.



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RESULTS AND DISCUSSION

Microbial enumeration revealed lactic acid bacteria (LAB) and yeasts as the dominant groups. LAB counts reached 7.3 Log CFU/g in fruit and 6.4 Log CFU/mL in brine, whereas yeast populations were higher in brine (5.6–5.9 Log CFU/mL) than in fruit (4.5–4.9 Log CFU/g). *Enterococcus faecium* and *Candida tropicalis* were the most frequently isolated species.

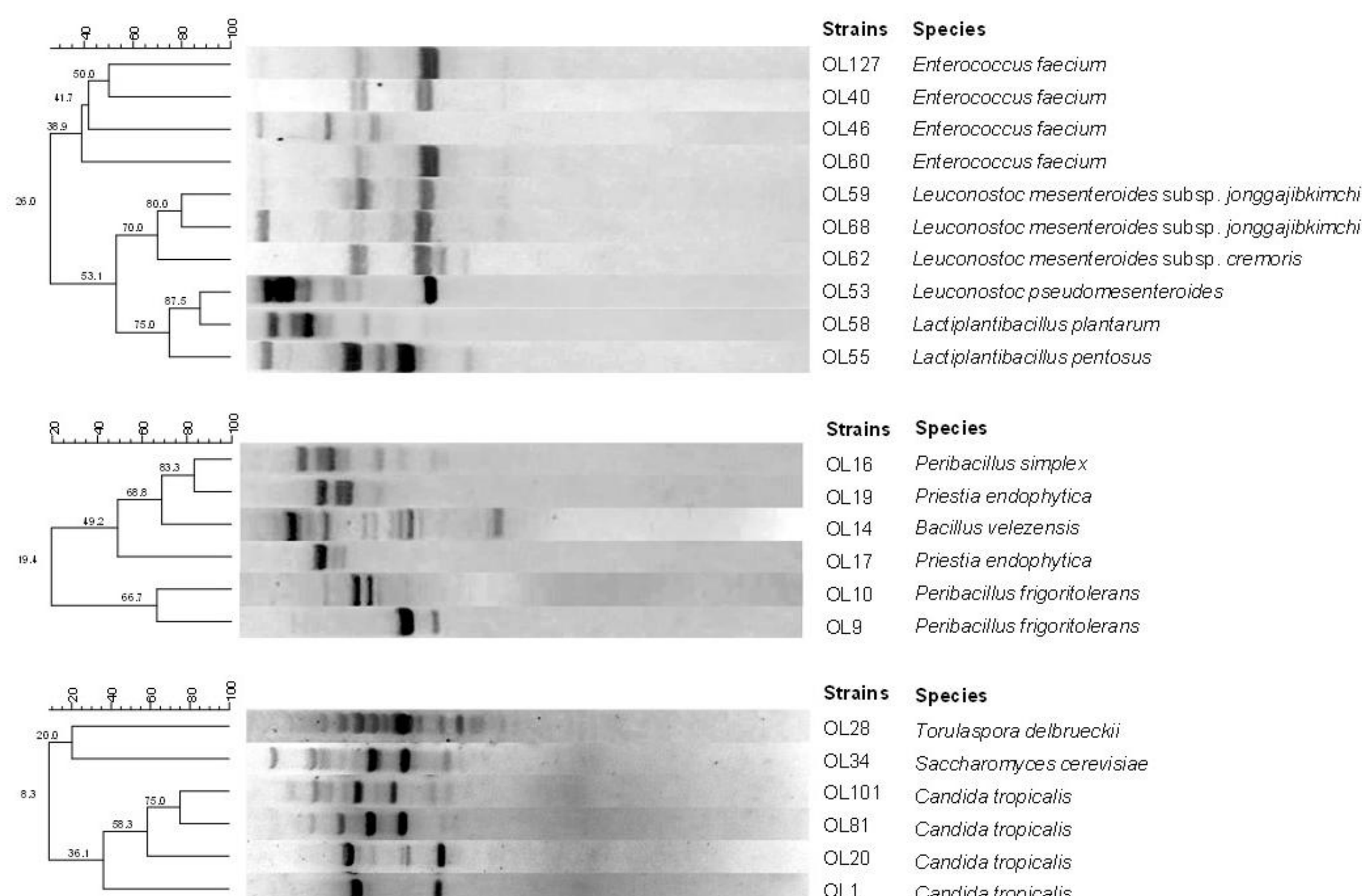
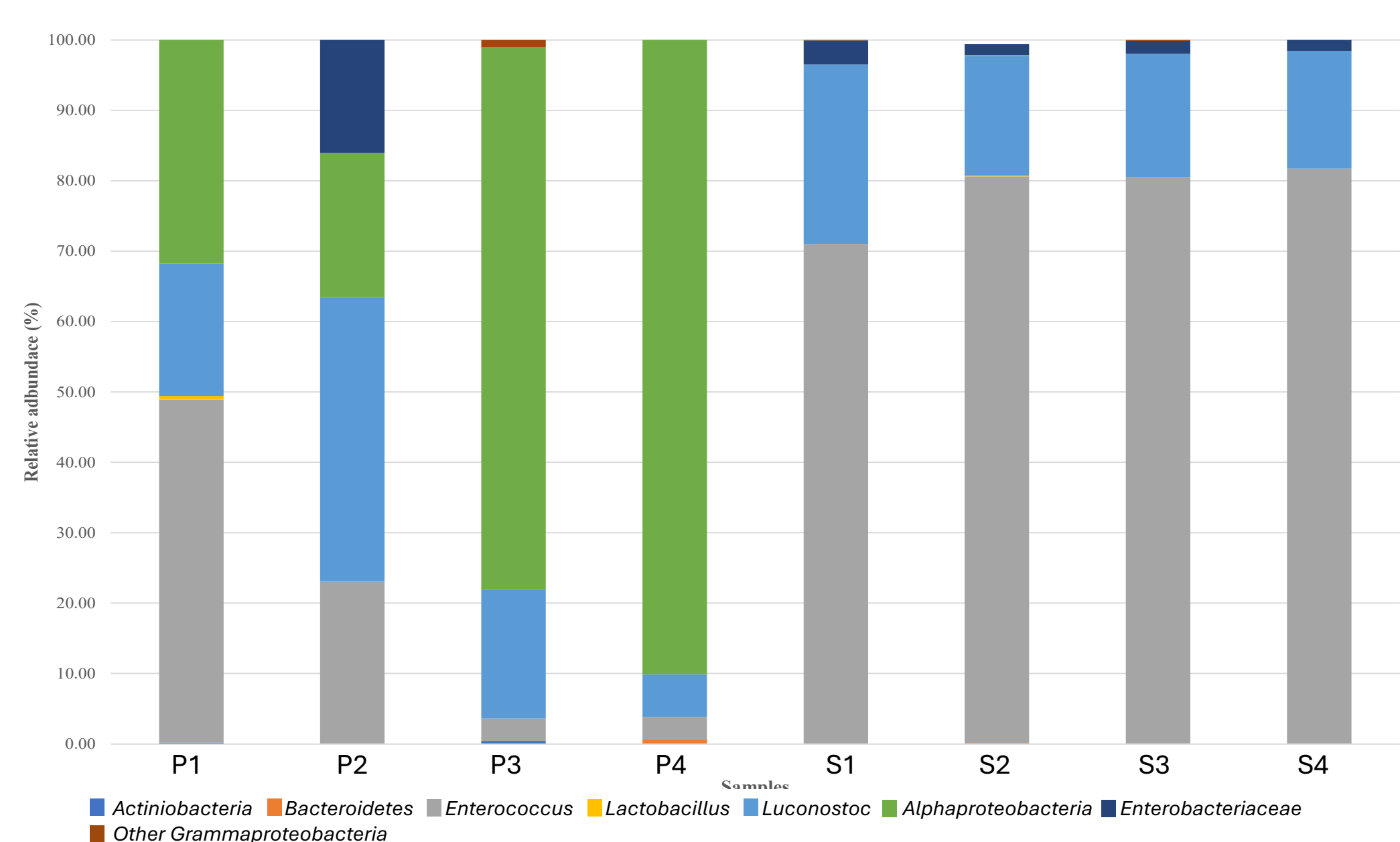
Among the spore-forming bacteria detected exclusively in the brine samples across all four batches, none of the identified species have

been previously associated with table olives.

Their endophytic nature suggests a possible origin from the stems, leaves, or seeds of the wild fennel used as a flavouring agent. Illumina sequencing enabled the detection of non-culturable bacterial communities in olive fruit and brine, revealing eight taxa including *Enterococcus*, *Leuconostoc*, and Alphaproteobacteria.

Enterococcus and *Leuconostoc* dominated both matrices, with notable variability in relative abundance across samples.

LAB species	Fruit samples				Brine samples			
	P1	P2	P3	P4	S1	S2	S3	S4
<i>Enterococcus faecium</i>		■	■	■	■	■	■	■
<i>Lactiplantibacillus plantarum</i>						■		
<i>Lactiplantibacillus plantarum</i>	■						■	
<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	■		■		■	■	■	
<i>Leuconostoc mesenteroides</i> subsp. <i>jonggajibkimchi</i>		■	■	■				■
<i>Leuconostoc pseudomesenteroides</i>						■		
Sporogenic bacteria								
<i>Bacillus velezensis</i>						■		
<i>Peribacillus frigiditolerans</i>					■			■
<i>Peribacillus simplex</i>							■	
<i>Priestia endophytica</i>							■	■
Yeast species								
<i>Candida tropicalis</i>	■	■	■	■	■	■	■	■
<i>Saccharomyces cerevisiae</i>		■	■	■				
<i>Torulopsis delbrueckii</i>					■			



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CONCLUSION

The final product, obtained through spontaneous fermentation, exhibited significant microbial diversity.

Enterococcus faecium was the dominant LAB, while *C. tropicalis* was the most frequently isolated yeast. The absence of viable pathogenic microorganisms suggested that,

despite the artisanal production and spontaneous fermentation, the four analysed batches do not pose a microbiological risk to consumers. Nonetheless, further research is required to comprehensively assess the microbiota of this specific production process.

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