Determining the core bacterial and fungal genera in table olive fermentations.

Annamaria Ricciardi[1], Francisco Noé Arroyo López[2], Marilisa Giavalisco[1], Rocchina Pietrafesa[1], Eugenio Parente[1*]

[1] Dipartimento di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy.
[2] Food Biotechnology Department. Instituto de la Grasa, Consejo Superior de Investigaciones Cientificas (CSIC), Seville, Spain.

*Corresponding author: Eugenio Parente.
Dipartimento di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Viale dell'Ateneo lucano 10, Potenza, Italy.
E-mail: eugenio.parente@unibas.it

Abstract
Table olives are among the most ancient and important fermented foods of the Mediterranean basin. Their production is still strongly related to traditional practices, and the lack of thermal treatments, the reliance on natural contamination and on selective factors (NaCl, anaerobiosis, occurrence of food phenolics, etc.) determine the dynamics of the microbial community. Lactic acid bacteria (LAB) and yeasts have a pivotal role in table olive microbial communities, but several halophilic and alkalophilic microorganisms may also contribute, positively or negatively, to the quality.
We have use metataxonomic data extracted from the FoodMicrobionet database to provide quantitative insights on the structure of bacterial and fungal microbial communities of table olvies and to identify core genera in different trade preparations.
A set of bacterial genera may be representative of the dominating core microbiota in table olives, with Celerinatantimonas and Lactiplantibacillus as the most prevalent genera, followed by several LAB, halophilic and alkalophilic lactic acid bacteria (HALAB) and Gram negatives, including non-halophilic species. Similarly, 3 fungal genera (Pichia, Candida and Wickerhamomyces) were the most abundant and prevalent among those representing the core microbiota of table olives.
The distribution of both bacteria and yeasts varied significantly in different olive varieties, among olives, brines and contact surfaces or materials, and at different production stages, and no clear grouping related to the combination of ripeness and trade preparation was found, although HALAB were characteristically abundant in Spanish style green olives.
Addition of starter cultures affected the composition and dynamics of microbial communities to a variable extent.

Keywords
Table olives; core microbiota; fungal community; bacterial community

Highlights

- Data from 16 metataxonomic studies on table olives, including 833 samples, were combined
- Enhanced metadata facilitated the comparison among different studies
- Composition of the microbiota of table olives was highly variable
- *Celerinatantimonas* and *Lactiplantibacillus* dominated the core bacterial microbiota
- *Pichia*, *Candida* and *Wickermahomyces* were the most prevalent and abundant yeasts

1. Introduction

Table olives are the result of the processing of the fruit of selected varieties of *Olea europea* L. that have been treated to remove their natural bitterness and preserved by fermentation, heat treatment, drying or salting (IOOC, 2004). The preservation of olives in brines dates back to 7,000 BP (Langgut and Garfinkel, 2022). Nowadays, they are still produced mostly in the Mediterranean Basin, but the production and export from the Americas is increasing. World production for the crop year 2023/24 is estimated at 2,828,500 t, and is concentrated in Mediterranean countries such as Spain, Egypt, Turkey, Morocco, Italy, and Greece (source: https://www.internationaloliveoil.org/world-market-of-olive-oil-and-table-olives-data-from-december-2024/, last accessed March 2025).

While the level of innovation in industrial production may be high (Campus et al., 2018), table olive production is still strongly related to traditional practices, and, as for many other vegetable fermentations (Gänzle, 2022; Hernández-Velázquez et al., 2024; Tamang et al., 2020), the lack of thermal treatments of raw material, the reliance on natural contamination and on selective factors (NaCl, anaerobiosis, occurrence of food phenolics, etc.) determine the dynamics of the microbial community and could affect the quality and safety of the final product (Anagnostopoulos and Tsaltas, 2022; Perpetuini et al., 2020; Vaccalluzzo et al., 2020).

IOOC recognizes 5 main trade preparation groups for table olives: i) treated olives (often referred to as Spanish style olives, treated by alkali, prior to fermentation in brine); ii) natural olives (Greek style olives, naturally fermented in brine with no alkali treatment); iii) dehydrated and/or shrivelled olives (which may or may not be treated in brines); iv) olives darkened by oxidation (California style; which may be fermented prior to oxidation treatment); and v) other specialties (which do not fall in previous categories) (https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-OT-NC1-2004-Eng.pdf). Microorganisms play an important role in the production, safety and quality of all these products (Anagnostopoulos and Tsaltas, 2022; Vaccalluzzo et al., 2020).

Several PDO and PGI table olive varieties are recognized in the European Union (https://ec.europa.eu/agriculture/eambrosia/geographical-indications-register/) and their microbiota may contribute to aspects related to identity and quality (Anagnostopoulos and Tsaltas, 2022). Additionally, the composition of the microbiota may be used to prevent frauds or identify the geographical origin of the product (Argyri et al., 2020; Kalogiouri et al., 2020; Kamilari et al., 2019).

Natural contamination from the olives, use of salt, spices, water and food contact surfaces and, in some cases, the addition of starter cultures, determine the assembly of the initial microbial community (Lanza, 2013; Penland et al., 2021), whose evolution is affected by a large number of factors, including initial pH, scarcity of nutrients (due to the repeated washing needed to remove alkali residuals in some varieties), high phenolic concentration, high salt concentration (brines with 6-12% NaCl are used, although there is a trend for reduction of salt concentration), temperature (which may be low in some areas at the beginning of fermentation) and anaerobiosis (Anagnostopoulos and Tsaltas, 2022; Benítez-Cabello et al., 2023; Blekas et al., 2002; Khalil et al., 2023; Perpetuini et al., 2018).

Due to the diversity of contamination sources, to the lack of heat treatments and to the variety and variability of ecological factors influencing this fermentation process, the microbial communities of table olives are complex and dynamic.

As for many other food products, -omic approaches are increasingly being used to complement or even replace cultivation-based approaches (Anagnostopoulos and Tsaltas, 2022; Ferrocino et al., 2022; De Filippis et al., 2018; Tsoungos et al., 2023). Two main microbial groups, lactic acid bacteria (LAB) (Giavalisco et al., 2024; Portilha-Cunha et al., 2020) and yeasts (Arroyo-López et al., 2012, 2008), are recognized as having a pivotal role in table olive microbial communities, but several halophilic and alkalophilic microorganisms may also contribute, positively or negatively, to the quality of table olives (de Castro et al., 2022; Lucena-Padrós and Ruiz-Barba, 2016; Posada-Izquierdo et al., 2021).

The microbiology of table olives has been reviewed multiple times, including recently (Anagnostopoulos and Tsaltas, 2022; Campus et al., 2018; Heperkan, 2013; Perpetuini et al., 2020; Portilha-Cunha et al., 2020; Tsoungos et al., 2023; Vaccalluzzo et al., 2020). These reviews provide qualitative data on the occurrence, dynamics and abundance of beneficial, functional and spoilage microorganisms in table olive fermentation, but they usually lack quantitative insights nor provide re-usable data.

At the time of writing this article, there were 36 studies which investigated the microbiota of table olives using amplicon targeted approaches (Table 1). In most cases, studies focused on one or few olive varieties and trade preparations; while the most frequent approaches include targeting of regions the 16S rRNA gene or ITS region using the Illumina platform, the type of samples used (fruits, brines, or both; environmental samples) varies among studies. The quality of metadata is also highly variable, as are the experimental designs, although the comparison of started/unstarted fermentation or the evaluation of the effect of salt concentration is rather frequent. Qualitative lists of genera found in most studies have been presented in recent reviews (Tsoungos et al., 2023) but they are of little help in evaluating the variability in prevalence and abundance of the different species or in the identification of core microbial populations of fermented olives in general or of selected processes.

In this article, we leverage on the FoodMicrobionet database (Parente et al., 2016, 2022; Parente and Ricciardi, 2024) to provide quantitative insights on the structure of bacterial and fungal microbial communities of table olives and to identify core genera as a function of table olive elaboration. Even if an obvious shortcoming of our approach is the heterogeneity of data available (in terms of olive varieties, sampling strategies and experimental designs, gene targets and primers, sequencing platforms), we feel that the use of a standardized pipeline for raw sequence processing and an enhanced metadata structure will in part mitigate this problem and provide a transparent and reusable dataset to the scientific community.

Table 1. Metataxonomic studies on the microbial communities of table olives (in inverse chronological order).

Olive varieties	Olive trade preparation	Platform	Targeted region(s)	Description	Reference
Kalamata	Greek style olives, black	Oxford Nanopore	16S rRNA gene	Use of probiotic LAB starters in the production of Kalamata black olives	(Vougiouklaki et al., 2024)
Manzanilla	Spanish style olives, green	Illumina	ITS region	Impact of the addition of LAB starters on the fungal microbiota	(López-García et al., 2024)
Gordal, Manzanilla, Hojiblanca	Greek style olives, green	Illumina	16S rRNA gene, ITS region	Impact of wild yeast starters on the volatilome	(Ruiz-Barba et al., 2024)
Taggiasca	Greek style olives, turning color	Illumina	26S rRNA gene	Dynamics of yeast species during natural fermentation	(Traina et al., 2024)
Thassos	Naturally dry salted olives, black	Illumina	26S rRNA gene	Composition of yeast microbiota in dry salted olives	(Gounari et al., 2023)
Cypriot, Picual, Kalamata	Greek style, green or black	Illumina	16S rRNA gene, ITS region	Bacterial and fungal microbiota of three varieties, collected in different locations	(Kamilari et al., 2023)
Halkidikis, Thassou, Kalamon, Amfissis, and Konservolia	Greek style, Spanish style, dry salted	Illumina	16S rRNA gene, 18S rRNA gene	Composition of bacterial and yeast microbiota in market samples of dry olives	(Mougiou et al., 2023)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene, ITS region	Fungal and bacterial microbiota in laboratory fermented olives inoculated with LAB and yeasts	(López-García et al., 2023)
Manzanilla	Spanish or Greek style, green	Illumina	16S rRNA gene, ITS region	Bacterial and yeast microbiota in uninoculated olive fermentation	(Ruiz-Barba et al., 2023a)
Gordal, Hojiblanca and Manzanilla	Greek style, green	Illumina	16S rRNA gene, ITS region	Bacterial and yeast microbiota in uninoculated natural green olive fermentation	(Ruiz-Barba et al., 2023b)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene, ITS region	Optimization of a pretreatment technique for recovery of LAB and yeasts from biofilms	(López-García et al., 2022)
Manzanilla, Aloreña, Hojiblanca, Gordaln,	Various	Illumina	ITS region	Fungal communities of packaged olives	(Benítez-Cabello et al., 2022)

Morona, Verdial, Empeltre, Empeltre Mallorquina, Kalamata, Conservolea, Galega, Picholine, Criolla, Lucques du Languedoc, Azapa, Arauco					
Nyons	Greek style, black	Illumina	16S rRNA gene, ITS region	Effect of salt reduction and inoculation with a complex consortium or backslopping on microbiota dynamics	(Penland et al., 2022)
Hojiblanca	Spanish style, green	Illumina	16S rRNA gene	Effect of inoculation with LAB on the dynamics of bacterial microbiota	(Correa-Galeote et al., 2022)
Gordal, Hojiblanca and Manzanilla	Spanish style, green	Illumina	16S rRNA gene	Investigation of the role of *Celerinatantimonas* in gas pocket defect	(de Castro et al., 2022)
Ascolana tenera	Spanish and Greek style, green	Illumina	16S rRNA gene	Impact of starter addition on the microbiota in laboratory scale fermentation	(Maoloni et al., 2022)
Halkidiki, Conservolea	Spanish and Greek style, green	Illumina	ITS region	Fungal microbiota of green olives stored in two types of pouches	(Tzamourani et al., 2022)
Nyons	Greek style, black	Illumina	16S rRNA gene, ITS region	Source tracking of desirable and spoilage microorganisms	(Penland et al., 2021)
Aloréña de Malaga	Greek style, green, cracked	Illumina	16S rRNA gene, ITS region	Microbiota of traditionally packed olives during storage	(López-García et al., 2021)
Kalamata	Greek style, black	Illumina	16S rRNA gene, 18S rRNA gene	Evolution of microbiota during storage in modified atmosphere	(Michailidou et al., 2021)
Unknown/Unspecified	Greek style, green, cracked	Illumina	Unknown	Composition of the microbiota fungal and bacterial microbiota in brine of an unspecified Turkish variety	(Demirci et al., 2021)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene	Diversity of the bacterial microbiota in spoiled industrial fermentations	(Arroyo-López et al., 2021)
Manzanilla	Spanish style, green	Illumina	16S rRNA gene	Impact of a LAB and yeast starter on	(Benítez-Cabello et al., 2020a)

				bacterial communities	
Kalamata	Greek style, black	Illumina	16S rRNA gene, ITS region	Bacterial and fungal microbiota of olives and brines of industrially fermented Kalamata olives	(Kazou et al., 2020)
Nyons	Greek style, black	Illumina	16S rRNA gene, ITS region	Dynamics of fungal and bacterial microbiota during fermentation	(Penland et al., 2020)
Picual	Greek style, green	Illumina	16S rRNA gene	Dynamics of bacterial community in uninoculated and inoculated (LAB) fermentation	(Anagnostopoulos et al., 2020)
Konservolia and Halkidiki	Greek style, black and Spanish style, green	Ion Torrent (bacteria), Illumina (fungi)	16S rRNA gene, ITS region	Bacterial and fungal communities in samples from different regions	(Argyri et al., 2020)
Manzanilla, Aloreña, Hojiblanca, Gordal, Morona, Verdial, Empeltre, Empeltre Mallorquina, Kalamata, Conservolea, Galega, Picholine, Criolla, Lucques du Languedoc, Azapa, Arauco	Various	Illumina	16S rRNA gene	Bacterial communities of commercial packaged olives	(Benítez-Cabello et al., 2020b)
Manzanilla, Hojiblanca	California style	Illumina	16S rRNA gene	Bacterial communities at different stages of processing	(Medina et al., 2018)
Manzanilla, Gordal	Spanish style, green	Illumina	16S rRNA gene, ITS region	Bacterial and fungal microbiota of spoiled olives	(de Castro et al., 2018)
Nocellara Etnea	Greek style, green	Ion Torrent	16S rRNA gene	Bacterial microbiota dynamics in LAB started and spontaneous fermentation	(Randazzo et al., 2017)
Aloreña de Malaga	Greek style, green	Illumina	16S rRNA gene	Bacterial microbiota in cured or cracked olives, with or without heat shock	(Rodríguez-Gómez et al., 2017)
Nocellara del Bélice	Spanish style and Picholine, green	Illumina	16S rRNA gene	Effect of salt reduction and type of processing on bacterial microbiota	(Zinno et al., 2017)
Aloreña de Malaga	Greek style, green	F454	ITS region	Dynamics of fungal microbiota in brines and fruit	(Arroyo-López et al., 2016)

Aloreña de Malaga	Greek style, green	F454	16S rRNA gene	Dynamics of bacterial microbiota in brines and fruit	(Medina et al., 2016)
Nocellara Etnea	Spanish and Greek style, green	F454	16S rRNA and rRNA gene	Effect of type of processing on bacterial microbiota	(Cocolin et al., 2013a)

ITS internal transcribed spacer

2. Materials and methods

2.1 Database analysis

FoodMicrobionet v 5.01 (Parente and Ricciardi, 2024) includes 16 different studies (with a total of 833 samples) on table olives (Table 2) whose raw sequences are available from NCBI Short Read Archive (SRA). The sequences were processed using the scripts available in FoodMicrobionet repository (https://github.com/ep142/FoodMicrobionet) using the approaches described elsewhere (Parente et al., 2022; Parente and Ricciardi, 2024).
ST253 is an unpublished study from our group, dealing with the effect of starter culture (*Lactiplantibacillus pentosus* O17) and timing of salt addition to the brine on the manufacture of black Itrana olives (Giavalisco et al., 2023). Microbial DNA was extracted and purified from microbial biomass separated from brines by centrifugation using Qiagen DNeasy PowerFood Microbial kit and sent for sequencing (Illumina NovaSeq) of the V3-V4 region of the 16S RNA gene and of the ITS2 region to an external service (Novogene UK Ltd). Sequences were deposited in NCBI SRA with accession number PRJNA1034814.
Studies were extracted from FoodMicrobionet using the extract_studies.R script available in the Metaolive repository (https://github.com/ep142/Metaolive) and saved as phyloseq objects (McMurdie and Holmes, 2013). Further metadata (olive cultivar, olive variety, olive trade preparation, style, spoilage, starter addition, etc.), obtained directly form metadata deposited in SRA or inferred from the papers describing the study, were added to the sample tables.
Graphical and statistical analysis of phyloseq data was carried out using own R 4.3.2 (R core team, 2024) scripts (available in the GitHub repository). Taxonomic re-assignment was carried out using the assignTaxonomy() and addSpecies() functions of the dada2 package (Callahan et al., 2016), using Silva 138.1 (Quast et al., 2013) and UNITE (general release 04/04/2024) (Abarenkov et al., 2023) taxonomic reference databases. Statistical and graphical treatment of the results included:

1. sample filtering (by removing samples with < 1000 sequences)
2. primary taxa filtering: sequences to mitochondria, and chloroplasts were removed from bacterial datasets; sequences assigned to fungi were removed from bacterial datasets, and viceversa;
3. taxonomic agglomeration was performed at the genus level;
4. application of a very lenient prevalence (threshold for removal < 0.01) and abundance (threshold for removal < 0.005) filter (taxa were retained if they passed both thresholds).
5. calculation of alpha diversity

6. production of a variety of boxplots and bar plots for alpha diversity
7. ordination using non-monotonic multidimensional scaling of the Bray-Curtis distance calculated on taxa abundance data

To this end, functions of libraries phyloseq, vegan (Oksanen et al., 2022) and tidyverse (Wickham et al., 2019) were used.

Table 2. List of studies on table olives included in FoodMicrobionet 5.0.1 (Parente and Ricciardi, 2024) which were used in the study.

study	target	region	Read length	bioproject	samples	reference
ST14	16S RNA gene and 16S RNA	V1-V3	323	PRJNA192807	20	(Cocolin et al., 2013b)
ST133	16S RNA gene	V3-V4	270	PRJNA600153	18	(Anagnostopoulos et al., 2020)
ST141	16S RNA gene	V3-V4	426	PRJEB3989	215	(Penland et al., 2020)
ST182	ITS region and 16S RNA gene	ITS1 and V3-V4	427	PRJEB43241	15	(López-García et al., 2021)
ST183	16S RNA gene	V3-V4	404	PRJNA688686	4	(Michailidou et al., 2021)
ST226	ITS region and 16S RNA gene	ITS1 and V3-V4	427	PRJNA878774	12	(Kamilari et al., 2023)
ST229	16S RNA gene	V3-V4	427	PRJNA811759	48	(Maoloni et al., 2022)
ST236	ITS region and 16S RNA gene	ITS1 and V3-V4	427	PRJEB61009	17	(Ruiz-Barba et al., 2023a)
ST239	ITS region	ITS2	374	PRJEB39898	208	(Penland et al., 2020)
ST240	16S RNA gene	V3-V4	425	PRJEB40675	47	(Penland et al., 2021)
ST246	ITS region	ITS1 + ITS2	339	PRJNA317749	28	(Arroyo-López et al., 2016)
ST247	ITS region	ITS2	422	PRJEB40674	47	(Penland et al., 2021)
ST248	ITS region	ITS1	268	PRJEB50357	69	(Benítez-Cabello et al., 2022)
ST251	16S RNA gene	V2-V3	275	PRJNA315418	28	(Medina et al., 2016)
ST252	16S RNA gene	V3-V4	330	PRJNA761998	8	(Vaccalluzzo et al., 2022)
ST253	ITS region and 16S RNA gene	ITS2 and V3-V4	322	PRJNA1034814	49	(Giavalisco et al., 2023)

Detailed metadata are available in our GitHub repository (https://github.com/ep142/Metaolive).
Of the 16 studies available, 8 included data for bacteria only, 4 for fungi only and 4 for both bacteria and fungi. However, due to inconsistencies in the deposit of sequences in SRA (in several cases the same sample was deposited with two separate biosample accessions

and/or data for bacteria and fungi were deposited with different bioproject or study accessions), the same samples might be present in two studies, one for bacteria and one for fungi (by convention in FoodMicrobionet a study must have a unique bioproject accession and a sample a unique biosample accession). The number of samples per study varied from 4 to 251, with a median value of 28.

3. Results and Discussion

3.1 The bacterial microbiota of table olives

Data on bacterial communities of table olives and their production environments were found in 12 studies, whose number of samples was highly unbalanced (Supplementary Table 1). The most common target and platform are the V3-V4 region of the 16S RNA gene and Illumina, respectively; data on 16S RNA were found in one study but were removed from the analysis.
Data were available for olives produced in 5 countries (France, Spain, Italy, Greece, and Cyprus; Supplementary Figure 1).
Samples were available for fruits and/or brine, or contact surface and raw materials (Supplementary Table 2 and 3). After removing samples with less than 1,000 sequences, a total of 411 samples were left for analysis (Supplementary Table 3).
The largest number of samples was available for Nyons (Penland et al., 2020, 2021) and Itrana naturally fermented black olives (Giavalisco et al., 2023).
Alpha diversity indices were calculated before taxa filtering. Chao1 ranged from 2 to 100.
The variability of Chao1 as a function of olive variety, stage of fermentation (0 environmental samples, 1 raw material, 2 samples during fermentation, samples at the end of fermentation), ripeness, trade preparation, sample type is shown in Figure 1.

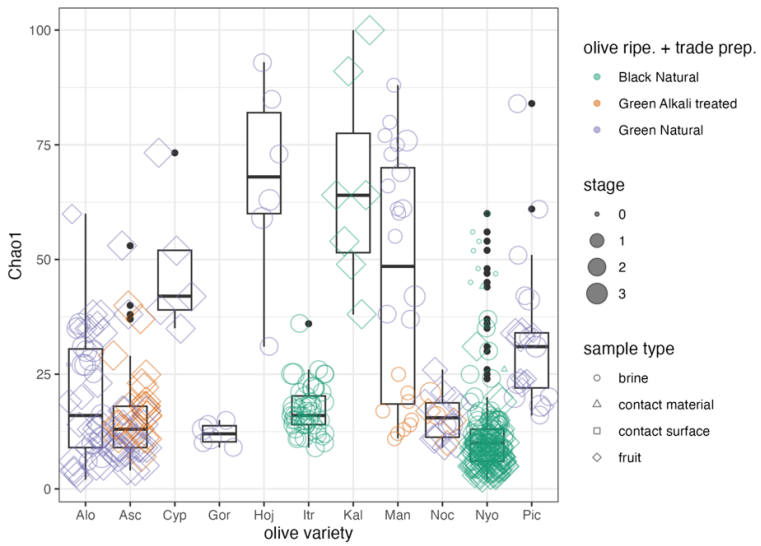

Figure 1. Distribution of Chao1 for bacterial communities from studies on table olives (Table 2) by olive variety, fermentation stage (1 raw materials, 2 samples during fermentation, 3 samples at the end of fermentation), olive ripeness and trade preparation, sample type.

Due to the differences in methods and experimental designs, only very general conclusions can be drawn:

- there is no evidence of a relationship between number of sequences per sample and Chao1
- olive variety, degree of ripening and process are almost always confounded, therefore comparisons need to be done within the same variety
- for the few studies for which the same olive variety was processed according to different trade preparations (usually Green Natural and Green Spanish style) the diversity is not consistently higher for either type
- for the few studies for which inoculated and inoculated olives were compared, we found no significant (Kolmogorov-Smirnov two sample test) evidence of lower diversity in inoculated fermentations; median Chao1 was even higher in some cases for inoculated fermentations (see Supplementary Figure 2)
- there is no clear evidence of fermentation stage affecting the diversity (see Figure 1), i.e. diversity does not apparently decrease with increasing fermentation time due to the selection of a less diverse community adapted, for example, to high salt / high polyphenol / low nutrient conditions, although in some environmental samples (see Nyons olives) diversity is much higher than in olives or brines. The reasons for this are not clear. One reason is that, although a single study (Cocolin et al., 2013a) included both 16S RNA (which may depict the active fraction within the community) and the 16S RNA gene (which may depict the structure of both the active and inactive parts of the fermentation), the number of samples was too low to make any conclusion, it is impossible to decide which portion of the microbial community as determined by amplicon targeted sequencing was active. This problem is common to many other fermentations and there is no consensus on the best approach to be used (Emerson et al., 2017; Erkus et al., 2013, 2016; Yap et al., 2021) to solve it.

After this stage taxonomic filtering and agglomeration was applied. As a result, the number of taxa was reduced from 929 (pre-taxonomic agglomeration) to 157, although the proportion of sequences retained was 99.5%. A prevalence and abundance plot is shown in Supplementary Figure 3. Bacterial genera in table olives and their production environments belonged to 24 phyla, but the majority belonged to *Bacillota, Pseudomonadota, Actinobacteriota*, and *Bacteroidota*. The proportion of sequences removed varied significantly among samples: this was mostly due to the poor quality of taxonomic assignment for some samples (data not shown). However, 75% of the samples retained more than 86% sequences after filtering.
We realize that the very unequal sample number per study may have significantly affected the results of filtering and the calculation of average prevalence and abundance: the majority of samples were in fact Nyons olives and their production environments (Penland et al., 2020, 2021), and Itrana olives (Giavalisco et al., 2023) (see Table 2). Given the nature of our composite dataset, this is inevitable, and subsamplig to an even number of samples would greatly reduce the size of the data set and hide part of the observed diversity. Since this analysis is, in its nature, repeatable, this problem will be mitigated as the number and variety of samples for table olives in FoodMicrobionet increases. Prevalence and abundance for different genera varied within different combinations of olive ripeness and trade

preparation (data not shown), but a set of 31 bacterial genera whose relative abundance exceeded 0.01 in at least one sample and whose prevalence was >0.1 in at least one olive group (Green Alkali treated, Green Natural, and Black Natural) may be representative of the dominating core microbiota in table olives (Figure 2, and Supplementary Table 4).

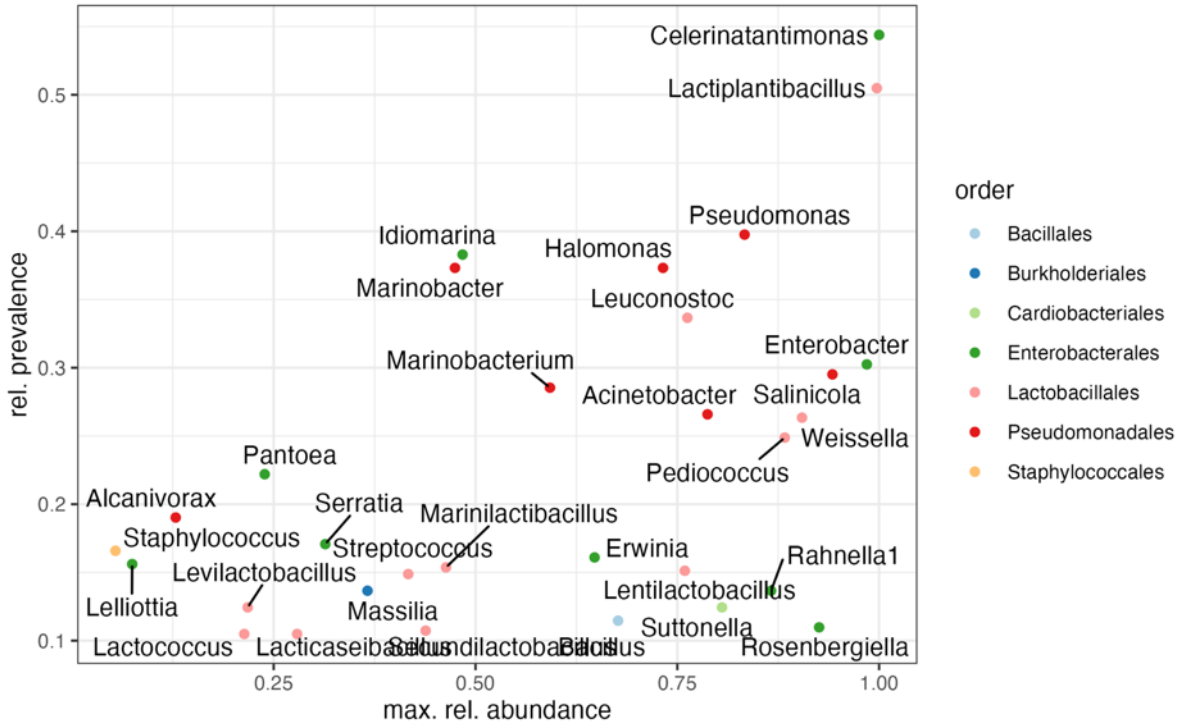

Figure 2. Relative prevalence and maximum relative abundance of the dominating bacterial genera in the Olive FMBN data set.

These bacteria belong to two main groups: Lactobacillales, including Halophilic and Alkalophilic Lactic Acid Bacteria (HALAB), and Gram-negative and Gram-positive halophiles (Enterobacterales, Pseudomonadales, Burkholderiales, Staphylococcales).
Their distribution varied significantly in different olive varieties, among olives, brines and contact surfaces or materials, and at different production stages (raw materials, olives during fermentation, products at the end of fermentation). A bar plot showing the average composition of bacterial communities for olives and brines of different varieties and different trade preparations and at different stages of production is shown in Supplementary Figure 4. The 31 most abundant and dominant genera covered more than 50% of the bacterial communities for most, but not all varieties. Notable exceptions were Kalamata, Manzanilla, Aloreña, Nocellara Etnea, Hojiblanca, and Picual, in which some microorganisms which were not prevalent in other varieties occurred at relatively high abundance. In Kalamata olives from Cyprus a large number of sequences was assigned to species of the genera *Streptococcus* and *Lactobacillus* (Kamilari et al., 2023) which are more frequently found in dairy products rather than in table olives. In Kalamata olives from Greece (Michailidou et al., 2021) an unusually high proportion of Alpha- and Gammaproteobacteria was found. In Green Spanish style Manzanilla olives, a high proportion of *Alkalibacterium* was found. This genus was found in these olives in several studies (Arroyo-López et al., 2021; Benítez-Cabello et al., 2019; Ruiz-Barba et al., 2023a). In Nocellara Etnea green alkali treated olives (Cocolin et al., 2013a) members of the genus *Kosakonia, Citrobacter,* and more

occasionally *Pantoea* and *Cobetia*, were also abundant (rel. abundance >0.10). In Aloreña de Malaga green natural olives some Actinobacteria (*Cutibacterium, Modestobacter*, *Pseudokineococcus*) were occasionally found with relative abundances >0.2 (López-García et al., 2021; Medina et al., 2018). In Picual olives from Cyprus (Anagnostopoulos et al., 2020; Kamilari et al., 2023), *Sediminibacterium* was abundant (>0.3 rel. abundance in some samples).

The lack of clear differences among different groups defined by combinations of ripeness and olive trade preparation is confirmed by the non-monotonic Multidimensional Scaling ordination of the Bray-Curtis distance matrix shown in Supplementary Figure 5. Rather, groups of samples within the same study (see samples for Nocellara Etnea, Itrana, Ascolana Tenera and Nyons olives for studies ST14, (Cocolin et al., 2013b); ST253 (Giavalisco et al., 2023); ST239-240, (Penland et al., 2020, 2021); and ST229, (Maoloni et al., 2022) are generally close among them. Addition of starter cultures affects the sample position in the ordination to a variable extent: differences are larger in some study (ST253; Itana olives) than in others (ST229, Ascolana Tenera, (Maoloni et al., 2022); ST133, Picual, (Anagnostopoulos et al., 2020) possibly reflecting different abilities of the starters in colonizing fruit and brines and in affecting directly and indirectly other members of the microbiota. Spoilage does also often cause notable changes in the composition of the microbiota, as shown in the ordination of Supplementary Figure 6.

The variability in the relative abundance and distribution of LAB and HALAB in table olives is shown in Figure 3. In this and in the other boxplots samples in which a given genus was absent are shown at the bottom of the graph. The significance of 0s in metataxonomic data has always been an issue: they could reflect lack of sensitivity (due to shallow sampling) or real absence (Lutz et al., 2022). Due to differences in sequencing depth in our dataset (we did not to apply any rarefaction; McMurdie and Holmes, 2014) we decided not to replace 0s with dummy values. As a consequence, the quantiles reflect the abundance of a given genus solely in the sample in which it was found, while its prevalence is shown in Supplementary Table 4.

Homofermentative genera, with *Lactiplantibacillus* as the most prevalent and abundant genus, followed by members of the genera *Lacticaseibacillus, Pediococcus* and *Latilactobacillus,* are frequently found by amplicon targeted metagenomics in several varieties of olives (Portilha-Cunha et al., 2020; Tsoungos et al., 2023), and they are sometimes used as starters (Giavalisco et al., 2024). Other minor homofermentative genera (*Schleiferilactobacillus*, *Loigolactobacillus*) are far less prevalent but can sometimes be subdominant. Some heterofermentative genera (*Leuconostoc*, *Lentilactobacillus, Levilactobacillus, Ligilactobacillus, Secundilactobacillus, Weissella)* frequently occur at relative abundances >0.01 and can occasionally be abundant in table olives: their role is unclear (Portilha-Cunha et al., 2020; Tsoungos et al., 2023), and both *Lentilactobacillus (Lentil. parafarraginis* and *Lentil. buchneri* have been found in brines of spoiled Nyons olives; (Penland et al., 2021). Enterococci, streptococci and lactococci are occasionally found as abundant members of microbiota in some table olive samples, but it is unclear if some of them are actually contaminating sequences. While *Lactococcus lactis* is in fact commonly isolated from vegetable sources, *Streptococcus thermophilus* has low salt tolerance and is preeminently associated to dairy products. Other genera (homofermentative *Atopostipes*, *Companilactobacillus*; heterofermentative *Paucilactobacillus*) are usually below 0.01.

HALAB (*Alkalibacterium*, *Marinilactobacillus*, but also *Halolactobacillus*) are characteristically abundant in green, alkali treated olives (Lucena-Padrós and Ruiz-Barba, 2019, 2016; Portilha-

Cunha et al., 2020). *Alkalibacterium* have been found to be abundant in spoiled olive samples (Arroyo-López et al., 2021)

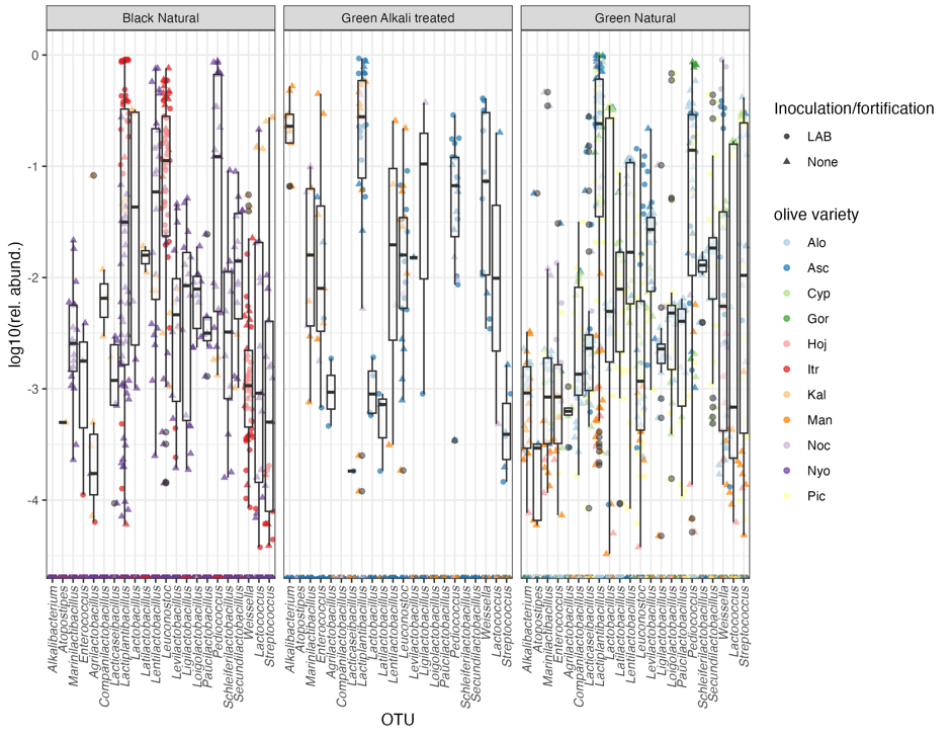

Figure 3. Distribution of the relative abundance of Lactic Acid Bacteria (including Halophilic and Alkalophilic LAB) in table olives. See Supplementary Table 3 for the abbreviations of table olives varieties.

The distribution of the abundance of other halophilic genera is shown in Figure 4.
A number of halophilic Pseudomonadota are found in table olives, sometimes with high abundance and prevalence. The most notable genus is *Celerinatantimonas* which has been found to be abundant in Nyons black olives (Penland et al., 2021, 2020) where they are not associated with spoilage, and is also often found at relatively high abundances in green naturally fermented olives (Benítez-Cabello et al., 2020b; López-García et al., 2021; Medina et al., 2016; Rodríguez-Gómez et al., 2017). *Cel. diazotrophica* has been specifically associated with the spoilage of Manzanilla and Gordal olives (de Castro et al., 2022; Ruiz-Barba et al., 2022). Most of the other species are associated with salt and brines.
Vibrio have been isolated from table olives (Lucena-Padrós et al., 2015) but several *Vibrio* species are apparently unable to grow in olive fermentation brines, possibly because of inhibition by phenolic compounds (Posada-Izquierdo et al., 2021). However, they are found among the dominant members of the microbiota of Hojiblanca green Spanish style olives and might be favored by alkaline conditions with high salt; their presence in brine is not desirable (Correa-Galeote et al., 2022).
Other halophilic Pseudomonadota (*Alidiiomarina*, *Halomonas*, *Idiomarina*, *Marinomonas*, *Salinicola*) are also abundant in green natural olives and in some black natural olives (Nyons olives), but less so in green alkali treated olives (Cocolin et al., 2013a; Randazzo et al., 2017; Ruiz-Barba et al., 2023b, 2023a). Fresh brines are a likely source of some of these species (Penland et al., 2021).

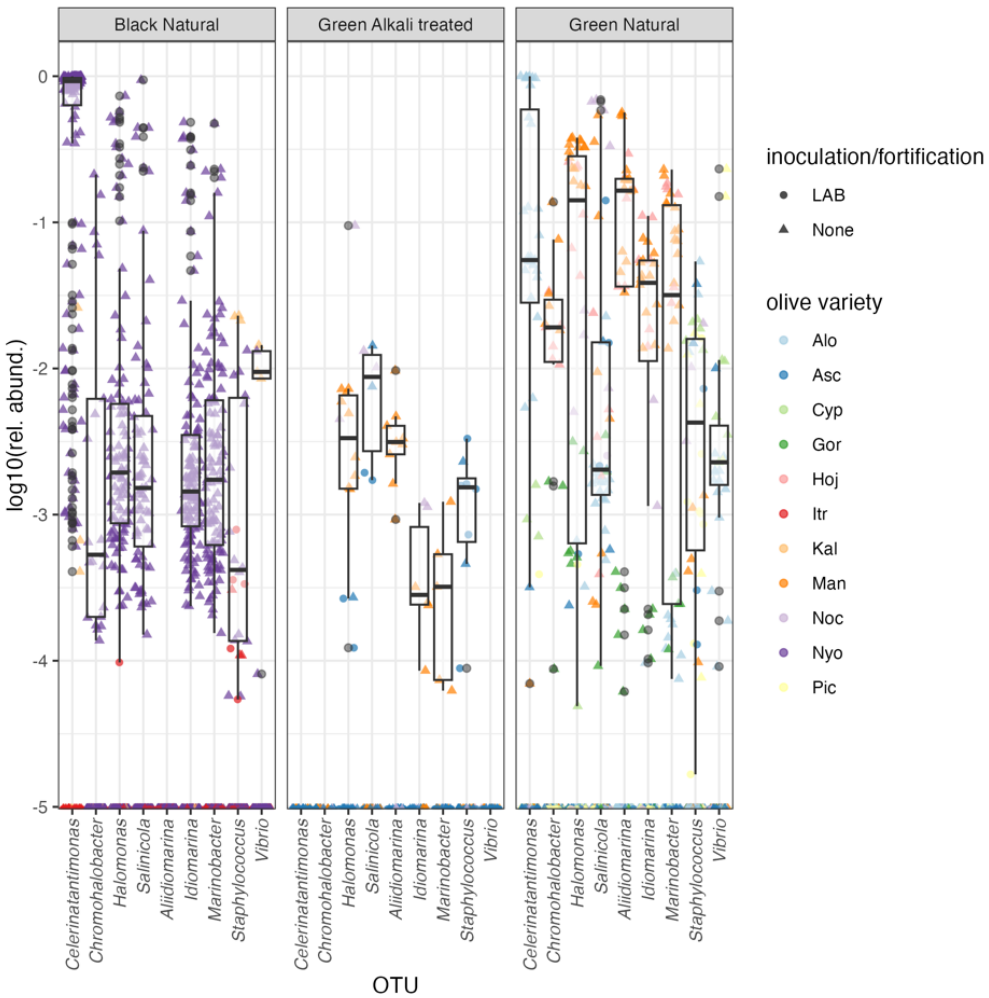

Figure 4. Distribution of the relative abundance of halophilic genera (other than HALAB) in table olives. See Supplementary Table 3 for abbreviations of table olive varieties.

Because of low pH, relatively high NaCl and presence of polyphenols, table olives are a safe food (with the possible exception of olives darkened by oxidation, which need to be submitted to a botulinum cook (Campus et al., 2018) and, to our knowledge, they have never been implicated in a food borne outbreak. Short reads used in amplicon targeted metagenomics seldom allow the resolution of closely related species and, in our study, we choose to agglomerate data at the genus level. A boxplot showing the distribution of the abundance of some genera which include human pathogens is shown in Supplementary Figure 7. Sequences assigned to *Brucella* and *Campylobacter* were only occasionally found and only at very low levels (relative abundance <0.001). On the other hand, sequences assigned to the genera *Salmonella* and *Yersinia* were found in Itrana, Ascolana Tenera, Aloreña and Hojiblanca, sometimes with relative abundances >0.01. *Listeria* was never found. The significance of these findings is unclear. First of all, there is no way of ascertaining if these sequences come from live microorganisms. Secondly, almost none of the studies in our dataset included information which could have allowed the removal of contaminating sequences (blanks, DNA measurements, mock communities) using, for example, package decontam (Davis et al., 2018). The samples of Ascolana tenera were produced with addition of herbs and spices (Maoloni et al., 2022), which may be a source of pathogenic microorganisms, but the potential source of potential pathogens in other samples is unclear.

3.2 The fungal microbiota of table olives

Data on fungal communities for table olives are less available in public sequence repositories compared to those on bacteria. This is rather surprising, considering the importance of yeast in table olive quality and spoilage (Arroyo-López et al., 2012; Benítez-Cabello et al., 2020a; Ciafardini and Zullo, 2019; Tsoungos et al., 2023; Tufariello et al., 2019). They are available in FoodMicrobionet for 8 studies (Supplementary Table 5) from 6 countries (Cyprus, France, Greece, Italy, Perú, and Spain; Supplementary Figure 8) with a total of 453 samples (including fruits, brine, fruit+brine, raw materials and contact surfaces). The number of samples per study was highly unbalanced (12-208, median 37.5) and, as for bacterial communities, most were contributed by two studies, one on black natural Nyons olives (Penland et al., 2020, 2021) and one on Itrana olives (Giavalisco et al., 2023). Samples were available for fruits and/or brine, or contact surface and raw materials. After removing samples with less than 1,000 sequences, 390 samples were left for analysis (Supplementary Table 6).
Alpha diversity indices were calculated before taxa filtering. Chao1 ranged from 2 to 100. The variability of Chao1 as a function of olive variety, stage of fermentation (0 environmental samples, 1 raw material, 2 samples during fermentation, samples at the end of fermentation), ripeness, trade preparation, sample type is shown in Figure 5.

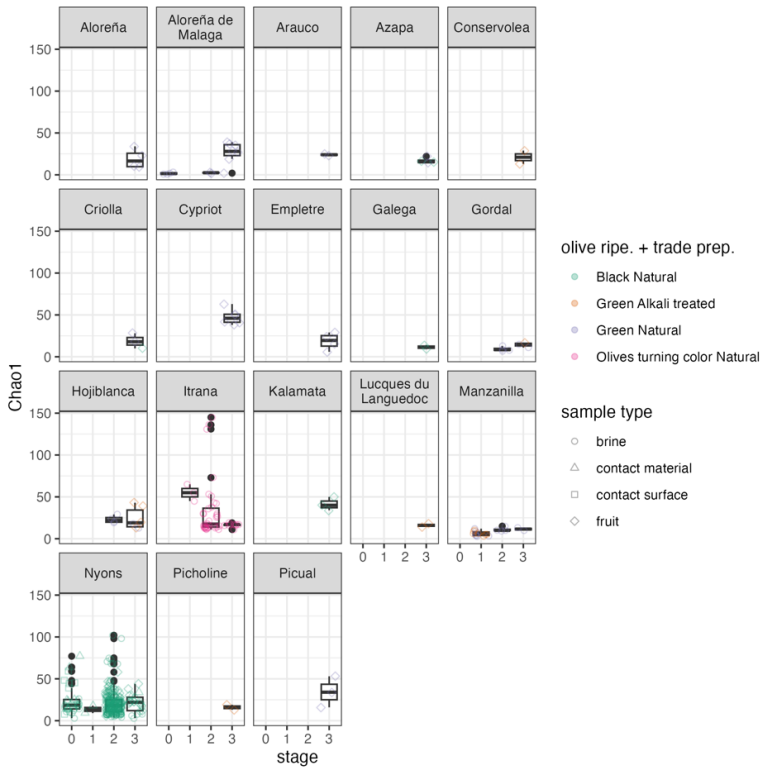

Figure 5. Distribution of Chao1 for fungal communities from studies on table olives (Table 2) by olive variety, fermentation stage (1 raw materials, 2 samples during fermentation, 3 samples at the end of fermentation, 0 other, including environmental samples), olive ripeness and trade preparation, sample type.

For black natural olives of the Itrana variety (Giavalisco et al., 2023) there was a clear trend for decrease of diversity from raw olives to the end of fermentation (Supplementary Figure 9), but this is almost entirely due to the higher diversity in raw, uninoculated olives and before day 25, while no significant differences were found between started and uninoculated brines (data not shown).
However, there is no evidence of a similar pattern for the few other studies for which time-course data are available (ST182, ST236, ST239), even when duration of fermentation was very long (ST239).
Taxonomic filtering and agglomeration at the genus level was performed as described for bacteria. Even if our pipeline allowed taxonomic assignment at the species level, given the differences in targets used in this study and the potential biases in taxonomic assignment related to different regions of ITS used for metataxonomic analysis (Hakimzadeh et al., 2023; Kauserud, 2023; Rué et al., 2023) the use of genus level assignment may be more prudent when comparing several studies. As a result of taxonomic filtering and agglomeration, the number of taxa was reduced from 535 to 140, and the proportion of sequences retained was 99.5%. A prevalence and abundance plot is shown in Supplementary Figure 10. Most of the genera passing the filters belonged to the phyla Ascomycota and Basidiomycota.
Prevalence and abundance for different genera varied within different combinations of olive ripeness and trade preparation (data not shown), but a set of 31 genera whose relative abundance exceeded 0.01 in at least one sample and whose prevalence was >0.1 in at least one olive group (Green Alkali treated, Green Natural, and Black natural) may be representative of the dominating core fungal microbiota in table olives (Figure 6, Supplementary Table 7).

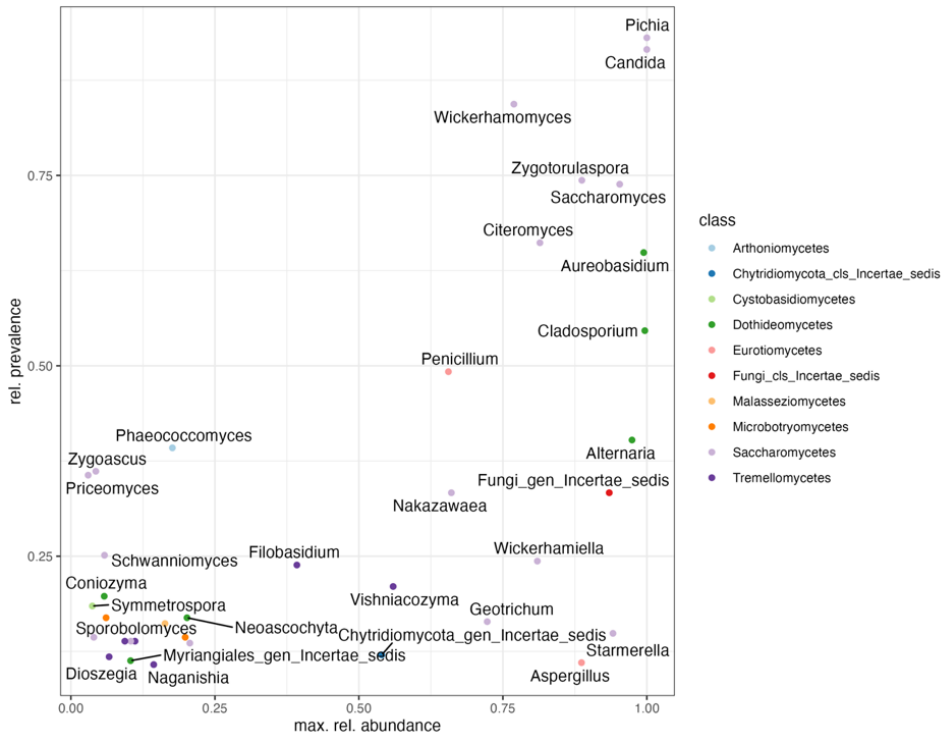

Figure 6. Relative prevalence and max relative abundance of the dominating fungal in the Olive FMBN data set

The relative abundance and prevalence of these genera varies in different group of olives. Given the high number of olive varieties it is impossible to show them in a single plot. The distribution of the core fungal genera is shown in Figure 7, while data on individual varieties are shown in the Supplementary Figures 11-13.

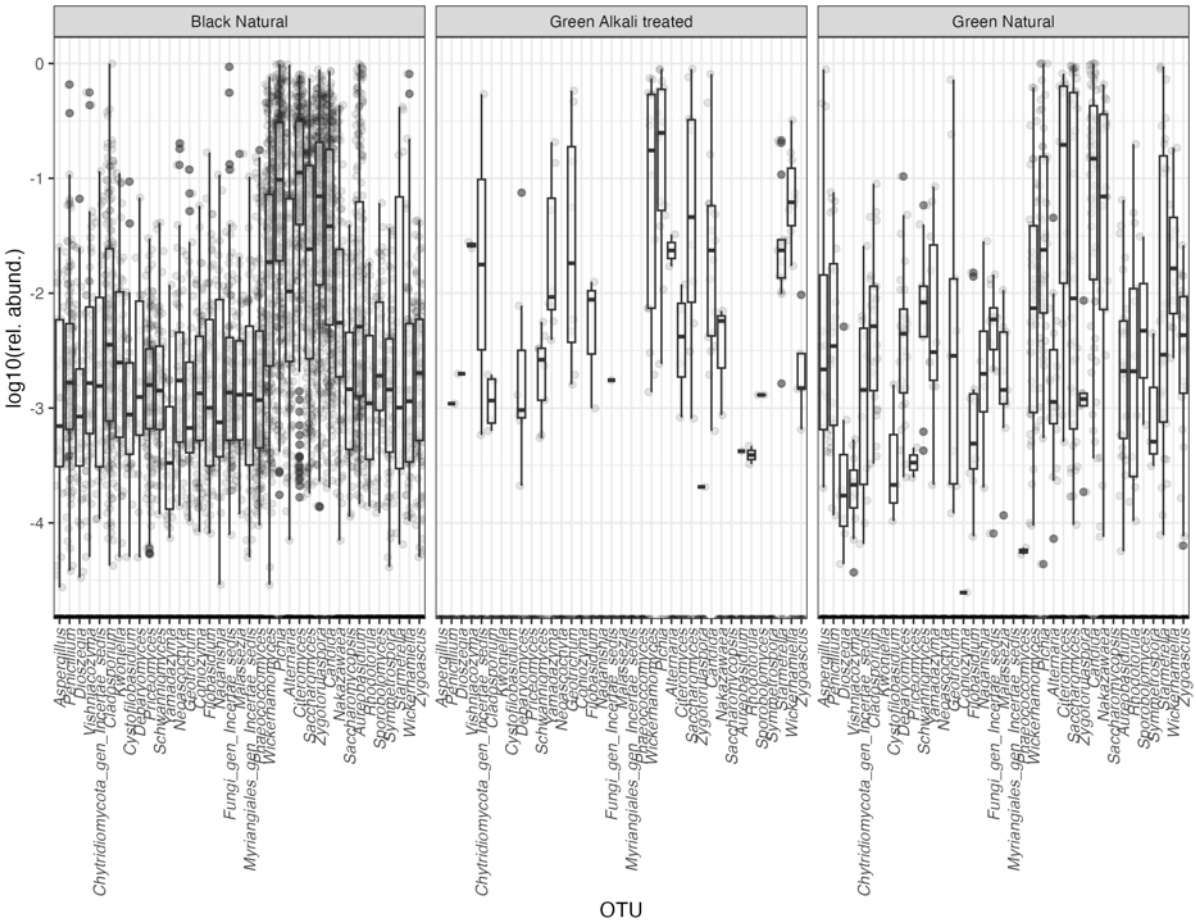

Figure 7. Distribution of the dominating fungal genera in the different types of table olive processing.

The distribution of abundance of fungi in table olives and their brines and food production environments was highly variable among olive groups and varieties (Supplementary Figure 14). The largest diversity was found in black natural olives, in which all core genera were found (although only *Pichia* and *Cyteromyces* had a median relative abundance > 10^{-1}). The variety of core genera found in green natural and green alkali treated olives was lower, and the most abundant genera were different (*Pichia* and *Wickerhamomyces* in green alkali treated olives, and *Pichia*, *Cyteromyces,* and *Candida* in green natural olives). The most abundant and prevalent fungal genera include members of the genus *Candida* (species level taxonomic assignment included *C. boidinii*, the species with the highest average relative abundance and prevalence*, C. diddensiae,* and *C. spencermartinsiae*), *Pichia* (with the two closely related species *P. membranifaciens* and *P. manshurica*) and *Wickerhamomyces* (*W. anomalus*). These species have been regularly found in culture dependent and independent studies on the fungal communities of olives, both during fermentation and in commercial

packages (Anagnostopoulos and Tsaltas, 2022; Arroyo-López et al., 2012; Tsoungos et al., 2023). Members of the genus *Saccharomyces* (*S. cerevisiae*) are also prevalent and may be abundant in some varieties (Nyons, Penland et al., 2021, 2020; Itrana, Giavalisco et al., 2023). They have been frequently reported as dominating and subdominating members of the fungal microbiota of table olives (Arroyo-López et al., 2016; Kamilari et al., 2023; Kazou et al., 2020; Ruiz-Barba et al., 2023a, 2023b; Tsoungos et al., 2023; Tzamourani et al., 2022). *Cyteromyces* (*Cyt. nyonsensis*) was found in both black and green natural olives with relatively high abundance (see below). It was first described in Nyons olives (Casaregola et al., 2013) , but is also found in Taggiasca olives (Traina et al., 2024) and in black dry salted olives (Gounari et al., 2023).
Other yeast genera which have been commonly associated to table olives and olive brines (Arroyo-López et al., 2012; Tsoungos et al., 2023) like *Dekkera, Nakazawea, Zygotorulaspora, Wickerhamiella, Starmerella, Schwanniomyces*, are much less abundant and prevalent. Other like *Dioszegia*, *Kwoniella*, and *Vishniacozyma* are found occasionally and at lower abundances and may be associated to the phylloplane rather than to the fermentation (Ferluga et al., 2024; Wang et al., 2008; Zhu et al., 2021) (see also the Omnicrobe database https://omnicrobe.migale.inrae.fr/index).
Yeast-like fungi (*Aureobasidium*) and molds (*Penicillium, Cladosporium, Aspergillus*) may have the same origin, although they are also associated with spoilage (Arroyo-López et al., 2016; Sánchez et al., 2022).
Overall, these figures confirm that there is very little in terms of common patterns of the composition of fungal microbiota among different combinations of olive ripeness and olive trade preparation. Although some genera (*Pichia, Candida, Wickerhamomyces, Saccharomyces*) tend to be more abundant and prevalent than others in almost all varieties, their abundance can be highly variable among different varieties in the same group and, for the few cases in which data are available, large changes are observed between the earliest stages of fermentation (raw olives, olives at the beginning of fermentation) and intermediate stages. The latter tend to be similar to late stages. In addition, in the few cases in which data for both fruits and brine are available, differences existed between the two niches.
This is confirmed by the ordination plots shown in Supplementary Figures 15 and 16.

4. Conclusions

In this paper we have used, in a transparent and reproducible manner, public metagenomic data sets on the composition and dynamics of microbial communities in table olives. Even if the microbiota of table olives has been thoroughly reviewed in recent years (Anagnostopoulos and Tsaltas, 2022; Campus et al., 2018; Corsetti et al., 2012; Perpetuini et al., 2020; Portilha-Cunha et al., 2020; Tsoungos et al., 2023), we feel that the quantitative insights provided in this paper significantly advance our knowledge of the core microbiota (if any) of different trade preparations of table olives and points out to future research needs. Table olive fermentation is, in most cases, an artisanal process. This may explain the variability of the composition of the microbiota even within the same variety. The latter may be controlled, in part, by modern technologies for decontamination of the fruits, improved control of the fermentation process and starter addition (Campus et al., 2018). However, in general, we feel that larger and more structured studies are needed to identify more reliably the microbiota associated with the fermentation of individual varieties and to relate its composition and dynamics to contamination and dispersal and to selective forces operating

during the fermentation. More, and more diverse data would also be needed for the development of machine-learning approaches for the identification of specific subsets of the microbiota which are reliably associated to specific varieties or to distinguish between PDO and non-PDO processes, or to identify the microbial spoilage associations causing a given type of spoilage (Arroyo-López et al., 2021; Montaño et al., 1992; Penland et al., 2021).
Part of the variability observed in our study may be due to the diversity of type of samples (brines, whole olives, homogenized olives, combinations of olives and brines), to differences in DNA extraction and purification procedures and other differences in wet lab approaches and sequencing platform (here, a common analysis pipeline was used for the analysis of raw sequences). It is well known that the microbiota of the surface of the olives and of the brines may differ (López-García et al., 2022) and it would be highly desirable if the scientific community working on table olives would agree on common protocols and sampling approaches.
Use of LAB and yeast starters does indeed improve the quality of table olives, as pointed out in several recent reviews (Anagnostopoulos and Tsaltas, 2022; Campus et al., 2018). However, their impact on the structure of microbial communities, as measured by amplicon targeted approaches, may be limited (see Supplementary Figures 5 and 15). On the other hand, when cultivation based, strain typing approaches are used (Martorana et al., 2015) population dynamics are evident. This clearly points out to several of the limitations of metataxonomic studies based on short read approaches. Long read, 3rd generation sequencing approaches can greatly improve the taxonomic resolution of metataxonomic studies (Srinivas et al., 2025). One recent, small study, has been published on the use of Oxford Nanopore Technology for the study of the microbiota of Kalamata black olives (Vougiouklaki et al., 2024). More data are needed to understand if the cost/benefit ratio of using this approach is favourable.
Evaluation of the microbial diversity of table olive fermentations would be extremely important to understand strain level dynamics and the role of selection and drift in shaping, over time, the metagenome of table olive fermentations. Amplicon targeted approaches do not have enough resolution for this purpose. The number of shotgun metagenomic studies on food microbiota has been rapidly increasing and large databases are being created to allow the exploitation of this wealth of information (Carlino et al., 2024). To our knowledge, a single study reporting shotgun metagenomic data on table olives exists (Soto-Giron et al., 2021) and more data are clearly needed. Finally, comparative metagenomic studies on the main actors of table olive fermentations, such as *Lactiplantibacillus pentosus* (Page et al., 2023) would be extremely useful in analyzing the patterns of domestication of this species and understanding the relationships, if any, between selective patterns associated with specific olive fermentation processes and the dominance of individual strains.
Another major issue is the detection of active fractions of the microbial community. To our knowledge, a single study used both rRNA and the 16S RNA gene as a target (Cocolin et al., 2013b), and concluded that the composition of the bacterial communities detected with both methods was similar, with the exception of one fruit sample. It is impossible to say if this is the case for all fermentations and it is well known in other food fermentations that the structure of active and inactive microbial communities may differ (Erkus et al., 2016, 2013).

Acknowledgements.
This work was carried out within the PRIN 2022 Project METAOlive 2022NN28ZZ and received funding from the European Union Next-GenerationEU, CUP C53D23005460006 (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1048 14/07/2023).

5. References

Abarenkov, K., Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., May, T.W., Frøslev, T.G., Pawlowska, J., Lindahl, B., Põldmaa, K., Truong, C., Vu, D., Hosoya, T., Niskanen, T., Piirmann, T., Ivanov, F., Zirk, A., Peterson, M., Cheeke, T.E., Ishigami, Y., Jansson, A.T., Jeppesen, T.S., Kristiansson, E., Mikryukov, V., Miller, J.T., Oono, R., Ossandon, F.J., Paupério, J., Saar, I., Schigel, D., Suija, A., Tedersoo, L., Kõljalg, U., 2023. The UNITE database for molecular identification and taxonomic communication of fungi and other eukaryotes: sequences, taxa and classifications reconsidered. Nucleic Acids Res. 52, D791–D797. https://doi.org/10.1093/nar/gkad1039.

Anagnostopoulos, D.A., Kamilari, E., Tsaltas, D., 2020. Evolution of Bacterial Communities, Physicochemical Changes and Sensorial Attributes of Natural Whole and Cracked Picual Table Olives During Spontaneous and Inoculated Fermentation. Front. Microbiol. 11, 1128. https://doi.org/10.3389/fmicb.2020.01128.

Anagnostopoulos, D.A., Tsaltas, D., 2022. Current Status, Recent Advances, and Main Challenges on Table Olive Fermentation: The Present Meets the Future. Front. Microbiol. 12, 797295. https://doi.org/10.3389/fmicb.2021.797295.

Argyri, K., Doulgeraki, A.I., Manthou, E., Grounta, A., Argyri, A.A., Nychas, G.-J.E., Tassou, C.C., 2020. Microbial Diversity of Fermented Greek Table Olives of Halkidiki and Konservolia Varieties from Different Regions as Revealed by Metagenomic Analysis. Microorganisms 8, 1241. https://doi.org/10.3390/microorganisms8081241.

Arroyo-López, F.N., Benítez-Cabello, A., Romero-Gil, V., Rodríguez-Gómez, F., Garrido-Fernández, A., 2021. Delving into the bacterial diversity of spoiled green Manzanilla Spanish-style table olive fermentations. Int. J. Food Microbiol. 359, 109415. https://doi.org/10.1016/j.ijfoodmicro.2021.109415.

Arroyo-López, F.N., Medina, E., Ruiz-Bellido, M.Á., Romero-Gil, V., Montes-Borrego, M., Landa, B.B., 2016. Enhancement of the Knowledge on Fungal Communities in Directly Brined Aloreña de Málaga Green Olive Fermentations by Metabarcoding Analysis. PLoS One 11, e0163135. https://doi.org/10.1371/journal.pone.0163135.

Arroyo-López, F.N., Querol, A., Bautista-Gallego, J., Garrido-Fernández, A., 2008. Role of yeasts in table olive production. Int. J. Food Microbiol. 128, 189–196. https://doi.org/10.1016/j.ijfoodmicro.2008.08.018.

Arroyo-López, F.N., Romero-Gil, V., Bautista-Gallego, J., Rodriguez-Gómez, F., Jiménez-Díaz, R., Garcia, P.G., Querol, A., Fernandez, A.G., 2012. Yeasts in table olive processing:

desirable or spoilage microorganisms? Int. J. Food Microbiol. 160, 42–49. https://doi.org/10.1016/j.ijfoodmicro.2012.08.003.

Benítez-Cabello, A., Calero-Delgado, B., Rodríguez-Gómez, F., Bautista-Gallego, J., Garrido-Fernández, A., Jiménez-Díaz, R., Arroyo-López, F.N., 2020a. The use of multifunctional yeast-lactobacilli starter cultures improves fermentation performance of Spanish-style green table olives. Food Microbiol. 91, 103497. https://doi.org/10.1016/j.fm.2020.103497.

Benítez-Cabello, A., Calero-Delgado, B., Rodríguez-Gómez, F., Garrido-Fernández, A., Jiménez-Díaz, R., Arroyo-López, F.N., 2019. Biodiversity and Multifunctional Features of Lactic Acid Bacteria Isolated From Table Olive Biofilms. Front. Microbiol. 10, 836. https://doi.org/10.3389/fmicb.2019.00836.

Benítez-Cabello, A., Delgado, A.M., Quintas, C., 2023. Main Challenges Expected from the Impact of Climate Change on Microbial Biodiversity of Table Olives: Current Status and Trends. Foods 12, 3712. https://doi.org/10.3390/foods12193712.

Benítez-Cabello, A., Ramiro-García, J., Romero-Gil, V., Medina, E., Arroyo-López, F.N., 2022. Fungal biodiversity in commercial table olive packages. Food Microbiol. 107, 104082. https://doi.org/10.1016/j.fm.2022.104082.

Benítez-Cabello, A., Romero-Gil, V., Medina-Pradas, E., Garrido-Fernández, A., Arroyo-López, F.N., 2020b. Exploring bacteria diversity in commercialized table olive biofilms by metataxonomic and compositional data analysis. Sci Rep-uk 10, 11381. https://doi.org/10.1038/s41598-020-68305-7.

Blekas, G., Vassilakis, C., Harizanis, C., Tsimidou, M., Boskou, D.G., 2002. Biophenols in Table Olives. J. Agric. Food Chem. 50, 3688–3692. https://doi.org/10.1021/jf0115138.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Campus, M., Değirmencioğlu, N., Comunian, R., 2018. Technologies and Trends to Improve Table Olive Quality and Safety. Front. Microbiol. 9, 617. https://doi.org/10.3389/fmicb.2018.00617.

Carlino, N., Blanco-Míguez, A., Punčochář, M., Mengoni, C., Pinto, F., Tatti, A., Manghi, P., Armanini, F., Avagliano, M., Barcenilla, C., Breselge, S., Cabrera-Rubio, R., Calvete-Torre, I., Coakley, Mairéad, Cobo-Díaz, José F., De Filippis, F., Dey, H., Leech, J., Klaassens, E.S., Knobloch, S., O'Neil, D., Quijada, N.M., Sabater, C., Skírnisdóttir, S., Valentino, V., Walsh, L., Alvarez-Ordóñez, A., Asnicar, F., Fackelmann, G., Heidrich, V., Margolles, A., Marteinsson, V.T., Stabelli, O.R., Wagner, M., Ercolini, D., Cotter, P.D., Segata, N., Pasolli, E., Alvarez-Ordóñez, A., Alvarez, P., Antonielli, L., Arendt, E., Armanini, F., Aubry, A., Baelum, J., Barcenilla, C., Belanche, A., Benavent-Gil, Y., Blake, T., Blanco-Míguez, A., Bongoni, R., Boyer, M., Brennan, F., Breselge, S., Briem, H., Butler, D., Calvete-Torre, I., Carballo, O.C., Cardinal, M., Carlino, N., Chervaux, C., Chopin, C., Clotaire, N., Coakley,

Mairead, Cobo-Díaz, José Francisco, Codd, J., Conroy, S., Corral-Jara, K.F., Corral-Jara, K.-F., Cotter, P.D., Coyne, G., Coyne, G., Creevey, C., Cuevas, P.D., Curran, B., Delgado, S., Derde, L., Derrien, M., Ercolini, D., Exposito, R.G., Fernández, M.M.L., De Filippis, F., Fordham, D., Galy, H., Gavriilidou, A., Gunnarsson, O., Hanson, B., Hermes, G., Huang, R., Huws, S., Ikoyi, I., Jaeger, A., Jeffery, I., Jérôme, M., Juan, P.-A., Kenny, D., Kers, A., Khinouche, K.-F., Kirwan, S., Klaassens, E.S., Knobloch, S., Kolbeinsson, K., Kolypczuk, L., Kostic, T., Ledda, F., Leech, J., Lehmann, D., Leroi, F., Lewis, E., Ley, J., Lucic, E., Lynch, K., Mace, S., MacLaren-Lee, I., Sanchez, L.M. de, Marchart, J., Margolles, A., Marteinsson, V.T., Masetti, G., McGovern, F., McHugh, N., McLoughlin, S., Meehan, D., Mølbak, L., Monin, T., Moreno, J., Morgavi, D., Morrison, S., Müench, S., Neves, A.R.R., Neylon, E., Nyhan, L., O'Kelly, R., O'Neil, D., O'Toole, P., Ortiz-Chura, A., Palma, J.M., Pasolli, E., Passerini, D., Pastar, M., Pinto, F., Pirovano, W., Plans, O., Policht, M., Pop, A., Pop, B., Popova, M., Prieto, M., Quijada, N.M., Reiss, A., Romero, P., Ruas-Madiedo, P., Rubino, F., Rubio, R.C., Ruiz, L., Ryan, A., Ryan, C., Sabater, C., Sahin, A., Salaun, C., Santos, F.G., Schneider, C., Segata, N., Selberherr, E., Sessitsch, A., Skírnisdóttir, S., Smidt, H., Smith, P., Sprenger-Haussels, M., Tapio, I., Tap, J., Valentino, V., Wagner, M., Walsh, A., Walsh, L., Waters, S.M., Willcocks, S., Yáñez-Ruiz, D.R., Yan, T., Yap, M., Zannini, E., Zuliani, V., 2024. Unexplored microbial diversity from 2,500 food metagenomes and links with the human microbiome. Cell. https://doi.org/10.1016/j.cell.2024.07.039.

Casaregola, S., Jacques, N., Louis-Mondesir, C., Coton, M., Coton, E., 2013. *Citeromyces nyonsensis* sp. nov., a novel yeast species isolated from black olive brine. Int. J. Syst. Evol. Microbiol. 63, 3086–3090. https://doi.org/10.1099/ijs.0.049296-0.

de Castro, A., Ruiz-Barba, J.L., Romero, C., Sánchez, A.H., García, P., Brenes, M., 2022. Formation of gas pocket defect in Spanish-style green olives by the halophile *Celerinatantimonas* sp. Food Control 136, 108868. https://doi.org/10.1016/j.foodcont.2022.108868.

de Castro, A., Sánchez, A.H., López-López, A., Cortés-Delgado, A., Medina, E., Montaño, A., 2018. Microbiota and Metabolite Profiling of Spoiled Spanish-Style Green Table Olives. Metabolites 8, 73. https://doi.org/10.3390/metabo8040073.

Ciafardini, G., Zullo, B.A., 2019. Use of selected yeast starter cultures in industrial-scale processing of brined Taggiasca black table olives. Food Microbiol. 84, 103250. https://doi.org/10.1016/j.fm.2019.103250.

Cocolin, L., Alessandria, V., Botta, C., Gorra, R., De Filippis, F., Ercolini, D., Rantsiou, K., 2013a. NaOH-Debittering Induces Changes in Bacterial Ecology during Table Olives Fermentation. PLoS One 8, e69074. https://doi.org/10.1371/journal.pone.0069074.s004.

Cocolin, L., Alessandria, V., Botta, C., Gorra, R., De Filippis, F., Ercolini, D., Rantsiou, K., 2013b. NaOH-Debittering Induces Changes in Bacterial Ecology during Table Olives Fermentation. Plos One 8, e69074. https://doi.org/10.1371/journal.pone.0069074.

Correa-Galeote, D., Ghomari, I., Asehraou, A., González-López, J., 2022. Revealing the bacterial abundance and diversity in brines from started Spanish-style green table olives. LWT 160, 113212. https://doi.org/10.1016/j.lwt.2022.113212.

Corsetti, A., Perpetuini, G., Schirone, M., Tofalo, R., Suzzi, G., 2012. Application of starter cultures to table olive fermentation: an overview on the experimental studies. Front Microbiol. 3, 248. https://doi.org/10.3389/fmicb.2012.00248.

Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome 6, 226. https://doi.org/10.1186/s40168-018-0605-2.

De Filippis, F., Parente, E., Ercolini, D., 2018. Recent Past, Present, and Future of the Food Microbiome. Annu. Rev. Food Sci. Technol. 9, 589–608. https://doi.org/10.1146/annurev-food-030117-012312.

Demirci, H., Kurt-Gur, G., Ordu, E., 2021. Microbiota profiling and screening of the lipase active halotolerant yeasts of the olive brine. World J. Microbiol. Biotechnol. 37, 23. https://doi.org/10.1007/s11274-020-02976-2.

Emerson, J.B., Adams, R.I., Román, C.M.B., Brooks, B., Coil, D.A., Dahlhausen, K., Ganz, H.H., Hartmann, E.M., Hsu, T., Justice, N.B., Paulino-Lima, I.G., Luongo, J.C., Lymperopoulou, D.S., Gomez-Silvan, C., Rothschild-Mancinelli, B., Balk, M., Huttenhower, C., Nocker, A., Vaishampayan, P., Rothschild, L.J., 2017. Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. Microbiome 5, 86. https://doi.org/10.1186/s40168-017-0285-3.

Erkus, O., Jager, V.C. de, Spus, M., Alen-Boerrigter, I.J. van, Rijswijck, I.M. van, Hazelwood, L., Janssen, P.W., Hijum, S.A. van, Kleerebezem, M., Smid, E.J., 2013. Multifactorial diversity sustains microbial community stability. Isme J 7, 2126–2136. https://doi.org/10.1038/ismej.2013.108.

Erkus, O., Jager, V.C.L. de, Geene, R.T.C.M., Alen-Boerrigter, I. van, Hazelwood, L., Hijum, S.A.F.T. van, Kleerebezem, M., Smid, E.J., 2016. Use of propidium monoazide for selective profiling of viable microbial cells during Gouda cheese ripening. Int. J. Food Microbiol. 228, 1–9. https://doi.org/10.1016/j.ijfoodmicro.2016.03.027.

Ferluga, M., Avesani, M., Lorenzini, M., Zapparoli, G., 2024. Assessing variability among culturable phylloplane basidiomycetous yeasts from Italian agroecosystems. World J. Microbiol. Biotechnol. 40, 335. https://doi.org/10.1007/s11274-024-04147-z.

Ferrocino, I., Rantsiou, K., Cocolin, L., 2022. Microbiome and -omics application in food industry. Int. J. Food Microbiol. 377, 109781. https://doi.org/10.1016/j.ijfoodmicro.2022.109781.

Gänzle, M., 2022. The periodic table of fermented foods: limitations and opportunities. Appl Microbiol. Biotechnol. 106(8), 2815-2826. https://doi.org/10.1007/s00253-022-11909-y.

Giavalisco, M., Zotta, T., Parente, E., Siesto, G., Capece, A., Ricciardi, A., 2023. Effect of oil-born yeasts on the quality of extra-virgin olive oils of Basilicata region. Int. J. Food Microbiol. 3862, 110041. https://doi.org/10.1016/j.ijfoodmicro.2022.110041.

Giavalisco, M., Lavanga, E., Ricciardi, A., Zotta, T., 2024. Starter Cultures for the Production of Fermented Table Olives: Current Status and Future Perspectives. Fermentation 10, 351. https://doi.org/10.3390/fermentation10070351.

Gounari, Z., Bonatsou, S., Ferrocino, I., Cocolin, L., Papadopoulou, O.S., Panagou, E.Z., 2023. Exploring yeast diversity of dry-salted naturally black olives from Greek retail outlets with culture dependent and independent molecular methods. Int. J. Food Microbiol. 398, 110226. https://doi.org/10.1016/j.ijfoodmicro.2023.110226.

Hakimzadeh, A., Asbun, A.A., Albanese, D., Bernard, M., Buchner, D., Callahan, B., Caporaso, J.G., Curd, E., Djemiel, C., Durling, M.B., Elbrecht, V., Gold, Z., Gweon, H.S., Hajibabaei, M., Hildebrand, F., Mikryukov, V., Normandeau, E., Özkurt, E., Palmer, J.M., Pascal, G., Porter, T.M., Straub, D., Vasar, M., Větrovský, T., Zafeiropoulos, H., Anslan, S., 2023. A pile of pipelines: An overview of the bioinformatics software for metabarcoding data analyses. Mol. Ecol. Resour. 24(5), e13847. https://doi.org/10.1111/1755-0998.13847.

Heperkan, D., 2013. Microbiota of table olive fermentations and criteria of selection for their use as starters. Front. Microbiol. 4, 143. https://doi.org/10.3389/fmicb.2013.00143.

Hernández-Velázquez, R., Flörl, L., Lavrinienko, A., Sebechlebská, Z., Merk, L., Greppi, A., Bokulich, N.A., 2024. The future is fermented: Microbial biodiversity of fermented foods is a critical resource for food innovation and human health. Trends Food Sci. Technol. 150, 104569. https://doi.org/10.1016/j.tifs.2024.104569.

Kalogiouri, N.P., Aalizadeh, R., Dasenaki, M.E., Thomaidis, N.S., 2020. Authentication of Greek PDO Kalamata Table Olives: A Novel Non-Target High Resolution Mass Spectrometric Approach. Molecules 25, 2919. https://doi.org/10.3390/molecules25122919.

Kamilari, E., Anagnostopoulos, D.A., Tsaltas, D., 2023. Fermented table olives from Cyprus: Microbiota profile of three varieties from different regions through metabarcoding sequencing. Front. Microbiol. 13, 1101515. https://doi.org/10.3389/fmicb.2022.1101515.

Kamilari, E., Tomazou, M., Antoniades, A., Tsaltas, D., 2019. High Throughput Sequencing Technologies as a New Toolbox for Deep Analysis, Characterization and Potentially Authentication of Protection Designation of Origin Cheeses? Int. J. Food. Sci. 2019, 5837301. https://doi.org/10.1155/2019/5837301.

Kauserud, H., 2023. ITS alchemy: On the use of ITS as a DNA marker in fungal ecology. Fungal Ecol. 65, 101274. https://doi.org/10.1016/j.funeco.2023.101274.

Kazou, M., Tzamourani, A., Panagou, E.Z., Tsakalidou, E., 2020. Unraveling the Microbiota of Natural Black cv. Kalamata Fermented Olives through 16S and ITS Metataxonomic Analysis. Microorganisms 8, 672. https://doi.org/10.3390/microorganisms8050672.

Khalil, A.A., Rahman, Md.M., Rauf, A., Islam, Md.R., Manna, S.J., Khan, A.A., Ullah, S., Akhtar, M.N., Aljohani, A.S.M., Abdulmonem, W.A., Simal-Gandara, J., 2023. Oleuropein: Chemistry, extraction techniques and nutraceutical perspectives-An update. Crit. Rev. Food Sci. Nutr. 64, 9933-9954. https://doi.org/10.1080/10408398.2023.2218495.

International Olive Oil Council, 2004. Trade standard applying to table olives. COI/OT/NC no. 1. December 2004.

Langgut, D., Garfinkel, Y., 2022. 7000-year-old evidence of fruit tree cultivation in the Jordan Valley, Israel. Sci. Rep. 12, 7463. https://doi.org/10.1038/s41598-022-10743-6.

Lanza, B., 2013. Abnormal fermentations in table-olive processing: microbial origin and sensory evaluation. Front. Microbiol. 4, 91. https://doi.org/10.3389/fmicb.2013.00091.

López-García, E., Benítez-Cabello, A., Martín-Arranz, V., Garrido-Fernández, A., Jiménez-Díaz, R., Arroyo-López, F.N., 2022. Optimisation of working parameters for lactic acid bacteria and yeast recovery from table olive biofilms, preserving fruit integrity and reducing chloroplast recovery. LWT 166, 113787. https://doi.org/10.1016/j.lwt.2022.113787.

López-García, E., Benítez-Cabello, A., Ramiro-García, J., Romero-Gil, V., Rodríguez-Gómez, F., Arroyo-López, F.N., 2021. New Insights into Microbial Diversity of the Traditional Packed Table Olives Aloreña de Málaga through Metataxonomic Analysis. Microorganisms 9, 561. https://doi.org/10.3390/microorganisms9030561.

López-García, E., Benítez-Cabello, A., Vilches, N., Garrido-Fernández, A., Martín-Arranz, V., Arroyo-López, F.N., 2023. Delving into the study of lactic acid bacteria and yeasts distribution in table olive biofilms using a non-destructive procedure. Food Microbiol. 113, 104250. https://doi.org/10.1016/j.fm.2023.104250.

López-García, E., Romero-Gil, V., Arroyo-López, F.N., Benítez-Cabello, A., 2024. Impact of lactic acid bacteria inoculation on fungal diversity during Spanish-style green table olive fermentations. Int. J. Food Microbiol. 417, 110689. https://doi.org/10.1016/j.ijfoodmicro.2024.110689.

Lucena-Padrós, H., González, J.M., Caballero-Guerrero, B., Ruiz-Barba, J.L., Maldonado-Barragán, A., 2015. *Vibrio olivae* sp. nov., isolated from Spanish-style green-olive fermentations. Int. J. Syst. Evol. Microbiol. 65, 1895–1901. https://doi.org/10.1099/ijs.0.000196.

Lucena-Padrós, H., Ruiz-Barba, J.L., 2019. Microbial biogeography of Spanish-style green olive fermentations in the province of Seville, Spain. Food Microbiol. 82, 259–268. https://doi.org/10.1016/j.fm.2019.02.004.

Lucena-Padrós, H., Ruiz-Barba, J.L., 2016. Diversity and enumeration of halophilic and alkaliphilic bacteria in Spanish-style green table-olive fermentations. Food Microbiol. 53, 53–62. https://doi.org/10.1016/j.fm.2015.09.006.

Lutz, K.C., Jiang, S., Neugent, M.L., Nisco, N.J.D., Zhan, X., Li, Q., 2022. A Survey of Statistical Methods for Microbiome Data Analysis. Front. Appl. Math. Stat. 8, 884810. https://doi.org/10.3389/fams.2022.884810.

Maoloni, A., Cardinali, F., Milanović, V., Osimani, A., Garofalo, C., Ferrocino, I., Corvaglia, M.R., Cocolin, L., Aquilanti, L., 2022. Microbial dynamics and key sensory traits of laboratory-scale co-fermented green olives (*Olea europaea* L. cv. Ascolana tenera) and sea fennel (*Crithmum maritimum* L.). Food Biosci. 50, 102077. https://doi.org/10.1016/j.fbio.2022.102077.

Martorana, A., Alfonzo, A., Settanni, L., Corona, O., Croce, F.L., Caruso, T., Moschetti, G., Francesca, N., 2015. An innovative method to produce green table olives based on "pied de cuve" technology. Food Microbiol. 50, 126–140. https://doi.org/10.1016/j.fm.2015.03.008.

McMurdie, P.J., Holmes, S., 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Comput. Biol. 10, e1003531. https://doi.org/10.1371/journal.pcbi.1003531.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217. https://doi.org/10.1371/journal.pone.0061217.

Medina, E., Brenes, M., García-García, P., Romero, C., de Castro, A., 2018. Microbial ecology along the processing of Spanish olives darkened by oxidation. Food Control 86, 35–41. https://doi.org/10.1016/j.foodcont.2017.10.035.

Medina, E., Ruiz-Bellido, M.A., Romero-Gil, V., Rodríguez-Gómez, F., Montes-Borrego, M., Landa, B.B., Arroyo-López, F.N., 2016. Assessment of the bacterial community in directly brined Aloreña de Málaga table olive fermentations by metagenetic analysis. Int. J. Food Microbiol. 236, 47–55. https://doi.org/10.1016/j.ijfoodmicro.2016.07.014.

Michailidou, S., Trikka, F., Pasentsis, K., Petrovits, G.E., Kyritsi, M., Argiriou, A., 2021. Insights into the evolution of Greek style table olives microbiome stored under modified atmosphere: Biochemical implications on the product quality. Food Control 130, 108286. https://doi.org/10.1016/j.foodcont.2021.108286.

Montaño, A., de Castro, A., Rejano, L., Sánchez, A.-H., 1992. Analysis of zapatera olives by gas and high-performance liquid chromatography. J. Chromatogr. A 594, 259–267. https://doi.org/10.1016/0021-9673(92)80338-u.

Mougiou, N., Tsoureki, A., Didos, S., Bouzouka, I., Michailidou, S., Argiriou, A., 2023. Microbial and Biochemical Profile of Different Types of Greek Table Olives. Foods 12, 1527. https://doi.org/10.3390/foods12071527.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard,. D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B, Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J, 2022. Vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan.

Page, C.A., Pérez-Díaz, I.M., Pan, M., Barrangou, R., 2023. Genome-Wide Comparative Analysis of Lactiplantibacillus pentosus Isolates Autochthonous to Cucumber Fermentation Reveals Subclades of Divergent Ancestry. Foods 12, 2455. https://doi.org/10.3390/foods12132455.

Parente, E., Cocolin, L., De Filippis, F., Zotta, T., Ferrocino, I., O'sullivan, O., Neviani, E., De Angelis, M., Cotter, P.D., Ercolini, D., 2016. FoodMicrobionet: A database for the visualisation and exploration of food bacterial communities based on network analysis. Int. J. Food Microbiol. 219, 28–37. https://doi.org/10.1016/j.ijfoodmicro.2015.12.001.

Parente, E., Ricciardi, A., 2024. A Comprehensive View of Food Microbiota: Introducing FoodMicrobionet v5. Foods 13, 1689. https://doi.org/10.3390/foods13111689.

Parente, E., Zotta, T., Ricciardi, A., 2022. FoodMicrobionet v4: A large, integrated, open and transparent database for food bacterial communities. Int. J. Food Microbiol. 372, 109696. https://doi.org/10.1016/j.ijfoodmicro.2022.109696.

Penland, M., Deutsch, S.-M., Falentin, H., Pawtowski, A., Poirier, E., Visenti, G., Meur, C.L., Maillard, M.-B., Thierry, A., Mounier, J., Coton, M., 2020. Deciphering Microbial Community Dynamics and Biochemical Changes During Nyons Black Olive Natural Fermentations. Front. Microbiol. 11, 586614. https://doi.org/10.3389/fmicb.2020.586614.

Penland, M., Mounier, J., Pawtowski, A., Tréguer, S., Deutsch, S.-M., Coton, M., 2021. Use of metabarcoding and source tracking to identify desirable or spoilage autochthonous microorganism sources during black olive fermentations. Food Res. Int. 144, 110344. https://doi.org/10.1016/j.foodres.2021.110344.

Penland, M., Pawtowski, A., Pioli, A., Maillard, M.-B., Debaets, S., Deutsch, S.-M., Falentin, H., Mounier, J., Coton, M., 2022. Brine salt concentration reduction and inoculation with autochthonous consortia: Impact on Protected Designation of Origin Nyons black table olive fermentations. Food Res. Int. 155, 111069. https://doi.org/10.1016/j.foodres.2022.111069.

Perpetuini, G., Caruso, G., Urbani, S., Schirone, M., Esposto, S., Ciarrocchi, A., Prete, R., Garcia-Gonzalez, N., Battistelli, N., Gucci, R., Servili, M., Tofalo, R., Corsetti, A., 2018. Changes in Polyphenolic Concentrations of Table Olives (cv. Itrana) Produced Under Different Irrigation Regimes During Spontaneous or Inoculated Fermentation. Front. Microbiol. 9, 1287. https://doi.org/10.3389/fmicb.2018.01287.

Perpetuini, G., Prete, R., Garcia-Gonzalez, N., Alam, M.K., Corsetti, A., 2020. Table Olives More than a Fermented Food. Foods 9, 178. https://doi.org/10.3390/foods9020178.

Portilha-Cunha, M.F., Macedo, A.C., Malcata, F.X., 2020. A Review on Adventitious Lactic Acid Bacteria from Table Olives. Foods 9, 948. https://doi.org/10.3390/foods9070948.

Posada-Izquierdo, G.D., Valero, A., Arroyo-López, F.N., González-Serrano, M., Ramos-Benítez, A.M., Benítez-Cabello, A., Rodríguez-Gómez, F., Jimenez-Diaz, R., García-Gimeno, R.M., 2021. Behavior of Vibrio spp. in Table Olives. Front. Microbiol. 12, 650754. https://doi.org/10.3389/fmicb.2021.650754.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590-6. https://doi.org/10.1093/nar/gks1219.

Randazzo, C.L., Todaro, A., Pino, A., Pitino, I., Corona, O., Caggia, C., 2017. Microbiota and metabolome during controlled and spontaneous fermentation of Nocellara Etnea table olives. Food Microbiol. 65, 136–148. https://doi.org/10.1016/j.fm.2017.01.022.

R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rodríguez-Gómez, F., Ruiz-Bellido, M.Á., Romero-Gil, V., Benítez-Cabello, A., Garrido-Fernández, A., Arroyo-López, F.N., 2017. Microbiological and Physicochemical Changes in Natural Green Heat-Shocked Aloreña de Málaga Table Olives. Front. Microbiol. 8, 2209. https://doi.org/10.3389/fmicb.2017.02209.

Rué, O., Coton, M., Dugat-Bony, E., Howell, K., Irlinger, F., Legras, J.-L., Loux, V., Michel, E., Mounier, J., Neuvéglise, C., Sicard, D., 2023. Comparison of metabarcoding taxonomic markers to describe fungal communities in fermented foods. Nucleic Acids Res. . 3. https://doi.org/10.24072/pcjournal.321.

Ruiz-Barba, J.L., de Castro, A., Romero, C., Sánchez, A.H., García, P., Brenes, M., 2022. Study of the factors affecting growth of Celerinatantimonas sp. and gas pocket formation in Spanish-style green olives. Food Control 141, 109208. https://doi.org/10.1016/j.foodcont.2022.109208.

Ruiz-Barba, J.L., Cortés-Delgado, A., Sánchez, A.H., López-López, A., Montaño, A., 2024. Impact of selected wild yeasts starters on the volatilome and phenolic contents of Gordal, Manzanilla and Hojiblanca naturally fermented green olives. LWT 195, 115811. https://doi.org/10.1016/j.lwt.2024.115811.

Ruiz-Barba, J.L., Sánchez, A.H., López-López, A., Cortés-Delgado, A., Montaño, A., 2023a. Microbial community and volatilome changes in brines along the spontaneous fermentation of Spanish-style and natural-style green table olives (Manzanilla cultivar). Food Microbiol. 113, 104286. https://doi.org/10.1016/j.fm.2023.104286.

Ruiz-Barba, J.L., Sánchez, A.H., López-López, A., Cortés-Delgado, A., Montaño, A., 2023b. Microbial and Chemical Characterization of Natural-Style Green Table Olives from the Gordal, Hojiblanca and Manzanilla Cultivars. Foods 12, 2386. https://doi.org/10.3390/foods12122386.

Sánchez, R., Pérez-Nevado, F., Montero-Fernández, I., Lozano, J., Meléndez, F., Martín-Vertedor, D., 2022. Application of Electronic Nose to Discriminate Species of Mold Strains in Synthetic Brines. Front. Microbiol. 13, 897178. https://doi.org/10.3389/fmicb.2022.897178.

Soto-Giron, M.J., Kim, J.-N., Schott, E., Tahmin, C., Ishoey, T., Mincer, T.J., DeWalt, J., Toledo, G., 2021. The Edible Plant Microbiome represents a diverse genetic reservoir with functional potential in the human host. Sci Rep-uk 11, 24017. https://doi.org/10.1038/s41598-021-03334-4.

Srinivas, M., Walsh, C.J., Crispie, F., O'Sullivan, O., Cotter, P.D., Sinderen, D. van, Kenny, J.G., 2025. Evaluating the efficiency of 16S-ITS-23S operon sequencing for species level resolution in microbial communities. Sci. Rep. 15, 2822. https://doi.org/10.1038/s41598-024-83410-7.

Tamang, J.P., Cotter, P.D., Endo, A., Han, N.S., Kort, R., Liu, S.Q., Mayo, B., Westerik, N., Hutkins, R., 2020. Fermented foods in a global age: East meets West. Compr Rev Food Sci F 19, 184–217. https://doi.org/10.1111/1541-4337.12520.

Traina, C., Ferrocino, I., Bonciolini, A., Cardenia, V., Lin, X., Rantsiou, K., Cocolin, L., 2024. Monitoring the yeasts ecology and volatiles profile throughout the spontaneous fermentation of Taggiasca cv. table olives through culture-dependent and independent methods. Int. J. Food Microbiol. 417, 110688. https://doi.org/10.1016/j.ijfoodmicro.2024.110688.

Tsoungos, A., Pemaj, V., Slavko, A., Kapolos, J., Papadelli, M., Papadimitriou, K., 2023. The Rising Role of Omics and Meta-Omics in Table Olive Research. Foods 12, 3783. https://doi.org/10.3390/foods12203783.

Tufariello, M., Anglana, C., Crupi, P., Virtuosi, I., Fiume, P., Terlizzi, B.D., Moselhy, N., Attay, H.A., Pati, S., Logrieco, A.F., Mita, G., Bleve, G., 2019. Efficacy of yeast starters to drive and improve Picual, Manzanilla and Kalamàta table olive fermentation. J. Sci.. Food Agric. 99, 2504–2512. https://doi.org/10.1002/jsfa.9460.

Tzamourani, A.P., Kasimati, A., Karagianni, E., Manthou, E., Panagou, E.Z., 2022. Exploring microbial communities of Spanish-style green table olives of Conservolea and Halkidiki cultivars during modified atmosphere packaging in multi-layered pouches through culture-dependent techniques and metataxonomic analysis. Food Microbiol. 107, 104063. https://doi.org/10.1016/j.fm.2022.104063.

Vaccalluzzo, A., Celano, G., Pino, A., Calabrese, F.M., Foti, P., Caggia, C., Randazzo, C., 2022. Metagenetic and Volatilomic Approaches to Elucidate the Effect of *Lactiplantibacillus*

plantarum Starter Cultures on Sicilian Table Olives. Front. Microbiol. 12, 771636. https://doi.org/10.3389/fmicb.2021.771636.

Vaccalluzzo, A., Pino, A., Russo, N.,De Angelis, M., Caggia, C., Randazzo, C.L., 2020. FoodOmics as a new frontier to reveal microbial community and metabolic processes occurring on table olives fermentation. Food Microbiol. 92, 103606. https://doi.org/10.1016/j.fm.2020.103606.

Vougiouklaki, D., Letsiou, S., Mavrokefalidou, I., Tsakali, E., Akkermans, S., Impe, J.F.M.V., Houhoula, D., 2024. Fermentation of Kalamata Natural Black Olives Using Selected Lactic Acid Bacteria as Starters. Fermentation 10, 53. https://doi.org/10.3390/fermentation10010053.

Wang, Q.-M., Jia, J.-H., Bai, F.-Y., 2008. Diversity of basidiomycetous phylloplane yeasts belonging to the genus *Dioszegia* (Tremellales) and description of *Dioszegia athyri* sp. nov., *Dioszegia butyracea* sp. nov. and *Dioszegia xingshanensis* sp. nov. Antonie van Leeuwenhoek 93, 391–399. https://doi.org/10.1007/s10482-007-9216-9.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the tidyverse. J. Open Source Softw., 4 (43), 1686. https://doi.org/10.21105/joss.01686.

Yap, M., Ercolini, D., Álvarez-Ordóñez, A., O'Toole, P.W., O'Sullivan, O., Cotter, P.D., 2021. Next-Generation Food Research: Use of Meta-Omic Approaches for Characterizing Microbial Communities Along the Food Chain. Annu. Rev. Food Sci. Technol. 13, 1–24. https://doi.org/10.1146/annurev-food-052720-010751.

Zhu, L., Li, T., Xu, X., Shi, X., Wang, B., 2021. Succession of Fungal Communities at Different Developmental Stages of Cabernet Sauvignon Grapes From an Organic Vineyard in Xinjiang. Front. Microbiol. 12, 718261. https://doi.org/10.3389/fmicb.2021.718261.

Zinno, P., Guantario, B., Perozzi, G., Pastore, G., Devirgiliis, C., 2017. Impact of NaCl reduction on lactic acid bacteria during fermentation of Nocellara del Belice table olives. Food Microbiol. 63, 239–247. https://doi.org/10.1016/j.fm.2016.12.001.