

THE BACTERIAL MICROBIOTA OF TABLE OLIVES: A METASTUDY.

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THE MICROBIOTA OF TABLE OLIVES

Table olives are among the most ancient fermented foods and their production is strongly intertwined with the culture of Mediterranean countries. Three main trade preparations exist (alkali treated olives; naturally fermented olives; olives darkened by oxidation) but combinations of alkali treatment, drying, cracking, addition of spices result in a large variety of products, with different ecological conditions (Anagnostopoulos and Tsaltas, 2022, 10.3389/fmicb.2021.797295). The microbial ecology of table olives has been extensively studied, first by cultivation-based approaches and, recently, by metataxonomic and multiomic approaches. Yeasts, lactic acid bacteria and other halophilic *Bacillota* and *Pseudomonadota* dominate the microbiota, with different community structures and successions in different products. Results on table olive microbiota are dispersed in a large number of studies and, even if for several of these raw sequences are available in repositories, direct, quantitative comparison of results is hampered by lack of a coherent metadata structure. **The objective of this work was to develop a Findable, Accessible, Interoperable and Reusable database** which can be potentially useful to researchers in the academia and industry, and even regulators, to:

1. study the occurrence and distribution of bacterial and fungal genera in table olives
2. identify potential starters, beneficial and spoilage organisms
3. identify the core and accessory microbiota of table olives
4. infer microbial association networks
5. evaluate if microbiota can be used to identify selected olive varieties for fraud protection purposes or for the development of quality schemes

THE α -DIVERSITY OF TABLE OLIVES

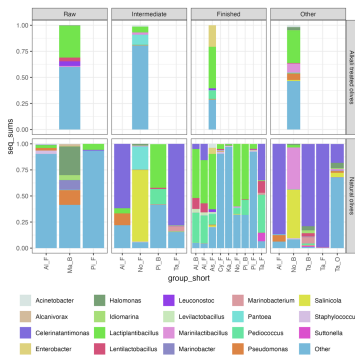


Figure 2. Relative abundance of the top 24 most prevalent and abundant bacterial genera in olives (xx_F), brines (xx_B) or processing environments and materials (xx_O), for **raw** olives, **intermediate** samples during fermentation, or **finished** products at the end of fermentation.

The bacterial diversity in fruit, brines and contact material for 8 varieties (Al: Aloreña; As: Ascolana tenera; Cy: Cypriot; Ka: Kalamata; Ma: Manzanilla; No: Nocellara Etnea; Pi: Picual; Ta Tanche) produced as alkali treated olives and/or naturally fermented is shown in **Figure 2**. After agglomeration and filtering, 158 taxa were retained out of 509. Several genera were found in both alkali treated and naturally fermented olives.

The most important genera include both *Pseudomonadota* (*Celerinatantimonas*, *Idiomarina*, *Marinobacter*, *Halomonas*, *Pseudomonas*, *Salinicola*, *Enterobacter*, *Acinetobacter*) and *Bacillota* (*Lactiplantibacillus*, *Pediococcus*, *Leuconostoc*, *Lentilactobacillus*).

The database, which allowed to easily compare bacterial microbiota over 10 studies will grow rapidly by addition of data from table olive samples (>>250) which are being processed within the METAolive project and we are confident that it will become the most comprehensive data resource on the microbiota of table olives.

Conclusions and future plans

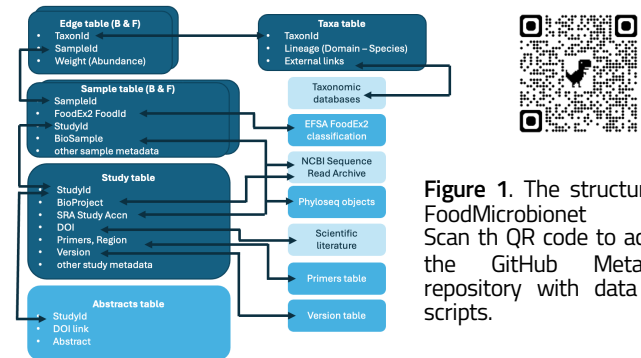


Figure 1. The structure of FoodMicrobionet v5.0. Scan the QR code to access the GitHub Metaolive repository with data and scripts.

Metataxonomic data were extracted from FoodMicrobionet (Parente et al., 2023, 10.1016/j.ijfoodmicro.2022.109696), whose version 5.0 now also contains data on fungal communities. We developed, in collaboration with CSIC, Seville, a set of controlled terms for manually annotating samples, including information on cultivar, trade preparation style, starter addition, spoilage, etc. The dataset for bacteria, covers 10 studies published between 2013 and 2023, and 425 samples of olives, brines and food contact surfaces or material, for 5 countries and 8 olive varieties (both Greek-style and Spanish-style). The database is available as a phyloseq (McMurdie and Holmes, 2013, 10.1371/journal.pone.0061217) object, a format which can be seamlessly used by many R packages for the analysis of microbiome. Extra metadata are available as a tab delimited file which can be easily merged with the phyloseq object.

THE β -DIVERSITY OF TABLE OLIVES

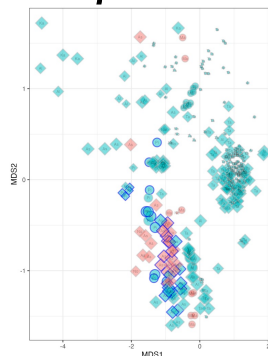


Figure 3. Non-metric multidimensional scaling plot of the Bray-Curtis distance matrix for the composition of bacterial microbiota of table olives and contact materials for **raw** olives (stage 1), **intermediate** samples during fermentation (2), or **finished** products (3).

A simple depiction of the β -diversity of the bacterial communities of table olives, brines, and contact material is shown in **Figure 3** as a multidimensional scaling plot. In most, but not all cases, bacterial microbiota of brines and fruits differed within the same variety (see also Fig. 2). The microbiota of olives from different varieties differed more than those of Greek- or Spanish- style within the same variety. Starter did not necessarily change very much the composition of bacterial communities, compared to non-started products of the same variety. Ordination is connected to higher abundance of halophiles for samples on the right and higher abundance of LAB for samples on the bottom.

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