Data Article

Amplicon metabarcoding data of prokaryotes and eukaryotes present in ‘Kalamata’ table olives packaged under modified atmosphere

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\textbf{ABSTRACT}
Evaluation of food microbiome is of major importance since it accounts for the product’s organoleptic characteristics and their nutritional value. In this dataset, microbes present in olive samples (‘Kalamata’ variety) stored under modified atmosphere and throughout different time-points of the shelf life of the product are presented, originated after 16S and 18S rRNA sequencing. The different time-points analyzed were: T0 (immediately after packaging), T6 (six months of storage), T12 (12 months of storage) and T18 (six months after the end of shelf life). Sequencing was performed on a MiSeq platform with the MiSeq Reagent Kit v3 (600 cycles). The raw sequence data used for analysis are available in NCBI under the Sequence Read Archive (SRA), with BioProject ID PRJNA688686. Raw reads were analyzed using the QIIME2 pipeline, clustered into Operational Taxonomic Units (OTU) and aligned against SILVA 132 reference database. OTUs are presented in different taxonomic levels for each time-point. These data present valuable information on the microbial communities of table olives, a dynamic niche that affect the final product quality. The data presented are related to the research article “Insights into the evolution of Greek style

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Specifications Table

| Subject                                      | Food Science: Food Microbiology |
|----------------------------------------------|---------------------------------|
| Specific subject area                        | Amplicon metabarcoding analysis of table olives |
| Type of data                                  | Tables and Figures              |
| How data were acquired                       | Sequencing of 16S rRNA and 18S rRNA genes was conducted on an Illumina MiSeq platform using the MiSeq® reagent kit v3 (paired end sequencing). |
| Data format                                  | Raw and analyzed                |
| Parameters for data collection               | Operational Taxonomic Units (OTU) clustering analysis was conducted using the QIIME2 pipeline and the VSEARCH tool. Sequences were clustered into OTUs at 99% sequence similarity against the SILVA 132 database. |
| Description of data collection               | Samples of ‘Kalamata’ olives packaged under modified atmosphere were analyzed throughout different time-points of storage (T0, T6, T12) and after the end of shelf life (T18). |
| Data source location                         | Institution: Institute of Applied Biosciences – Centre for Research and Technology Hellas |
| Data accessibility                           | Country: Greece |
| Data identification number                   | Repository name: NCBI SRA |
| Direct URL to data                           | Data identification number: PRJNA688686 |
| Related research article                     | Direct URL to data: http://www.ncbi.nlm.nih.gov/bioproject/688686, https://www.ncbi.nlm.nih.gov/sra?term=PRJNA688686%20 |

Value of the Data

- This dataset provides information on the microbiome present in olives packaged under modified atmosphere and monitors the changes that take place based on 16S and 18S rRNA amplicon sequencing.
- The data provide useful information on the microbial species present in food, thus, industry and other stakeholders can benefit from the identified microorganisms, by monitoring and evaluating the final product offered to consumers.
- This dataset can serve as a threshold for scientific community regarding the evolution of microorganisms in olive samples stored under modified atmosphere.

1. Data Description

The data reported here, refer to raw reads obtained after sequencing the V3-V4 hypervariable regions of the 16S rRNA gene and the V7-V8 hypervariable regions of the 18S rRNA gene. The raw sequence data (.fastq files) used for analysis are accessible through NCBI’s Sequence Read Archive (SRA), under the BioProject ID PRJNA688686. For prokaryotes, sequencing resulted in 698,220 raw reads (Table 1). After quality and chimera filtering 294,978 reads were obtained for OTU clustering. Details on reads per time-point are presented in Table 1. Filtered reads were
clustered into 2715 unique OTUs. The number of unique OTUs was gradually decreased during storage, with T0 being the most enriched time-point in terms of bacterial diversity (\( N = 1246 \)), whereas T18 was the least diverse, presenting 540 unique bacterial OTUs.

For eukaryotes, sequencing resulted in 788,917 raw reads, which after quality and chimera filtering were reduced to 572,231 and 508,811, respectively (Table 2). Assignment and clustering of these sequences against SILVA 132 database resulted in 1778 unique OTUs. Unlike prokaryotes, the number of observed OTUs was gradually increased from T0 (\( N = 520 \)) to T12 (\( N = 1072 \)), but at T18 a steep decrease was observed (\( N = 302 \)).

Classification of the identified bacterial OTUs showed that Firmicutes was the dominant Phylum with a relative percentage above 71.8% in all time-points (Fig. 1). Proteobacteria, was the second major Phylum identified, with its relative percentages among time-points ranging from 12.84% (T0) to 25.83% (T12). At the Class level, Firmicutes were mainly classified as Bacilli, whereas Proteobacteria were mainly represented by Gammaproteobacteria (Fig. 2).

At the Order taxonomic level bacterial community of olive samples was mainly represented by Lactobacillales with relative percentages ranging from 50.0% at T12 to 65.8% at T0 (Fig. 3).
At the onset of packaging (T0) bacterial profile was slightly differentiated from the subsequent time-points. In particular, although Betaproteobacteria were found at 7.0% at T0, they were gradually displaced by Rhizobiales, Pseudomonadales and Sphingomonadales at the following time-points.

At the Family level, *Lactobacillaceae* dominated the bacterial communities with relative percentages being above 71.7% for all time-points (Fig. 4). *Burkholderiaceae* was found as the second most abundant family with relative percentages being on average 5.6%. Other families that were identified included *Sphingomonadaceae, Caulobacteraceae, Rhodobacteraceae, Stappiaceae, Moraxellaceae, Rhizobiaceae* and *Pseudomonadaceae*. Interestingly, *Bacillaceae* was only identified in T0.

Comparison of the different time points through Principal Component Analysis revealed that T0 demonstrates a well differentiated bacterial profile. The first two principal components (PC1+PC2) accounted for the 98.3%, capturing most of the total genetic variation (Fig. 5). T6 and T12 samples grouped together and in great distance from the other time points, while at the beginning of storage (T0) and after the end of product’s shelf life (T18), samples were found
Fig. 4. Heatmap of relative abundances averaged for olive samples at Family level after 16S rRNA amplicon metabarcoding analysis.

Fig. 5. Principal component analysis for the four time points (T0, T6, T12 and T18) for olive samples based on 16S rRNA amplicon sequencing.
Fig. 6. Analysis of eukaryotic load in olive samples throughout the different time-points. Distribution of the major Phyla. The scale in the y axis reflects the normalized relative abundance percentages (%). Black lines within each bar separate each Phylum into lower taxonomic levels.

Fig. 7. Analysis of eukaryotic communities in olive samples throughout the different time-points at class level. The scale in the y axis reflects the normalized relative abundance percentages (%). Black lines within each bar separate each Class into lower taxonomic levels.

dispersed in axes, demonstrating a differentiated genetic profile based on the 16S rRNA amplicon sequencing.

Evaluation of eukaryotic OTUs revealed that Opisthokonta was the major Phylum identified in all samples with its relative abundance being over 99.67% across all time-points (Fig. 6). Likewise, Nucleomycyea dominated the eukaryotic load at Class level (Fig. 7), whereas for the Order level, Fungi were identified as the dominant eukaryotes (Fig. 8).

At the Family level eukaryotes were mainly represented by members of the Pichiaceae family. In particular, from the onset of packaging (Pichiaceae at T0: 93.1%) a progressive decline on the relative abundance is observed until members of this family are found with a relative abundance of 36.1% at T12. After the end of shelf life (T18) Pichiaceae suddenly increase and dominate in MAP packaging with relative percentage of 93.4% (Fig. 9). Following Pichiaceae, Saccharomycetaceae is the second most abundant family with its members found at 4.7%, 13.1%, 16.4% and 6.6% for T0, T6, T12 and T18, respectively. At similar relative abundances to Saccharomycetaceae, members of Cladosporiaceae family are present but only for T6 (11.8%) and T12 (19.6%). Overall, T0 and T18 show similar profile concerning the eukaryotic load of olive samples compared to T6 and T12. In particular, for T6 and T12 families that are present, but detected in traces at T0 and
Fig. 8. Analysis of eukaryotic communities in olive samples throughout the different time-points at the level of Order. The scale in the y axis reflects the normalized relative abundance percentages (%). Black lines within each bar separate each Order into lower taxonomic levels.

Fig. 9. Analysis of eukaryotic load in olive samples throughout the different time-points. Distribution of the major fungal families. The scale in the y axis reflects the normalized relative abundance percentages (%). Black lines within each bar separate each family into lower taxonomic levels.

T18, are Aspergillaceae, Malasseziaceae, Pleosporaceae and Nectriaceae. In Fig. 9 Cucurbitales, Lami-ales and Spermatophyta are presented, since they could not be classified to the Family level.

Genetic relatedness of samples through PCA based on the identified eukaryotic OTUs did not form any particular clusters among samples. The first two principal components (PC1+PC2) accounted for the 93.9%, capturing most of the total genetic variation (Fig. 10).

2. Experimental Design, Materials and Methods

2.1. Sample description and DNA extraction

In this dataset samples of ‘Kalamata’ table olives are presented packaged under modified atmosphere (30% CO₂ - 70% N₂). Samples were supplied by a private company (Pelopac S.A., Thes- saloniki, Greece). Olives were left to ripe and darken naturally on the tree, and then they were harvested at the stage of full ripeness. ‘Kalamata’ table olives were further processed according to the Greek style; olives were fermented in their natural brine (natural fermentation). Although
the shelf life of MAP pouches was 12 months, analysis was also performed six months after the end of the product’s shelf life (18 months) to document changes in microbial communities after the expiration date. Hence, the four different time points of storage and analysis were: T0 = beginning, T6 = 6 months, T12 = 12 months and T18 = 18 months.

Upon arrival at the laboratory, samples were stored at 4 °C. From each pouch, 20 g of olive tissue were placed in sterile disposable plastic containers and homogenized in a polytron homogenizer (Polytron PT-MR 6100, Kinematica AG, Littau, Switzerland). To enable uniformity in stirring and obtain a homogenous pulp, apart from their natural brine, 5 ml of ddH2O were also added to the container prior to homogenization. Microbial DNA was extracted from ~400 μl of the homogenized pulp, using the ZymoBIOMICS DNA Miniprep Kit (ZYMO RESEARCH; Irvine, CA, USA) according to the manufacturer’s instructions. For sample disruption, the pulp was beaten using the ZR bashing beads provided by the kit, on a TissueLyser (Qiagen, Hilden, Germany) for 5 min at 30Hz (1800 oscillations/minute). Elution was performed in the minimum volume allowed according to the protocol for highly concentrated DNA (V elution = 50 μl). DNA concentration was measured on a Qubit 4.0 Fluorimeter using the Qubit® dsDNA BR assay kit (Invitrogen, Carlsbad, CA, USA).

2.2. Library construction and amplicon sequencing

In line with the method previously described in Michailidou et al. [1], assessment of microbial species was conducted by amplifying different genes. For the identification of bacterial load the V3,V4 region of the 16S rRNA gene (≈460 bp) was amplified and sequenced, whereas fungal diversity was assessed by amplifying and sequencing the V7-V8 hypervariable regions of the 18S rRNA gene (≈350 bp). For the amplification of the 16S rRNA sequences, primers S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 were selected from on Klindworth et al. [2], whereas for the amplification of V7-V8 regions, universal primers FR1 and FF390 were selected from Chemidlin Prevost-Boure et al. [3]. For each primer used, an Illumina overhang adapter nucleotide sequence
was added at the 5’ end of the selected primer. The sequences of the primers used were 16S_F: 5’- TCG TCG GCA GCG TCA GAT GTG TAT AAC AGA CAG CCT AAC GGN GGC WGC AG-3’, 16S_R: 5’- GTC TCG TGG CTC CGG AGA TGT GTA TAA GAG ACA GAA CTA CHV GGG TAT CTA ATC C-3’, 18S_F: 5’-TCG TCG GCA GCG TCA GAT GTG TAT AAC AGA CAG CGA TAA CGA AGC AGA CCT-3’ and 18S_R: 5’-GTC TCG TGG CTC CGG AGA TGT GTA TAA GAG ACA GAN CCA TTC AAT CGG TAN T-3’. All libraries were constructed following the Illumina’s 16S Metagenomic Sequencing Library Preparation (15044223 B) protocol with minor modifications. All PCR reactions were performed on a Rotor-Gene Q thermocycler (Qiagen) and monitored on real-time by adding in each sample a green fluorescent nucleic acid stain. Each PCR reaction was conducted in a final volume of 20 μl, containing 10 μl of 2x KAPA HiFi HotStart Ready enzyme mix (KAPA BIOSYSTEMS, Woburn, MA, U.S.A.), 0.80 μl of 10μM forward and reverse primer mix, 2.50 μl (~5ng/μl) of microbial DNA, 0.25 μl 50 μM SYTO9 (Thermo Fisher Scientific, USA) and 6.45 μl ddH2O. All PCR products and libraries were purified to remove unincorporated primers and primer-dimer sequences using NucleoMag® NGS Bead Suspension (Macherey-Nagel, Düren, Germany) using different ratios of beads (ratio = volume of paramagnetic beads to PCR volume), depending on the target region size. Libraries were initially quantified with a fluorometric quantification using Qubit® dsDNA BR assay kit and their quality was automatically assessed on a Fragment Analyzer system (Agilent Technologies Inc. Santa Clara, United States) using the DNF-477-0500 kit. The final molarity of libraries was evaluated by a quantitative PCR (qPCR), conducted on a Rotor-Gene Q thermocycler (Qiagen, Hilden, Germany) with the KAPA Library Quantification kit for Illumina sequencing platforms (KAPA BIOSYSTEMS, Woburn, MA, U.S.A.). For library quantification, each sample was analyzed in triplicates. Final molarity of libraries was calculated in relation to the size of DNA amplicons after indexing, based on the following equation:

\[ C = \text{pM after qPCR} \times \left( \frac{452 \text{ (size of DNA standard in bp)}}{\text{Average fragment length of library (bp)}} \right) \times \text{relevant dilution factor} \]

Finally, 16S and 18S rRNA libraries were pooled at 12.5pM, mixed at equal percentages and sequenced on a MiSeq platform using the MiSeq® reagent kit v3 (2 × 300 cycles) (Illumina, San Diego, California).

2.3. Bioinformatics and data analysis

Raw sequences (fastq files) were analyzed using the Quantitative Insights into Microbial Ecology 2 (QIME2) pipeline [4]. Analyzes were implemented on a Linux-based HPC cluster assigning one node with 32 cores and 256 GB RAM. Adapters were trimmed from raw sequences using cutadapt plugin [5] with the trim-paired function, joined using the join-pairs function and filtered with the quality-filter q-score-joined command with minimum quality score 28 (p-min-quality). Dereplication of sequences was performed with the derepicate-sequences command and sequences were clustered into operational taxonomic units (OTUs) at 99% sequence similarity, using the open-reference method and the VSEARCH tool [6]. In addition, chimeras and “borderline chimeras” were excluded from downstream analysis, by applying the uchime-denoovo tool and sequences were further aligned against the SILVA 132 reference database [7]. Taxonomy classification was performed with the feature-classifier plugin using the classify-consensus-blast command with a percent identity threshold of 0.99. Finally, from the resulted OTU table Archaea and chlorplastic or mitochondrial sequences were excluded from downstream analyzes.

Further analysis and data visualization was performed in R version 3.6.0 [8]. OTU tables and .biom files were imported and merged in R environment using the import_biom command of the Phyloseq R package [9]. Ampvis2 [10] and ggplot2 [11] R packages were used to visualize OTU abundances as barplots; phyloseq objects were sorted with the sort function, pruned for top OTUs or the desired taxonomy with the prune_taxa function and transformed to percentages with the transform_sample_counts function. Heatmaps and Principal Component Analyzes (PCA) were conducted using the amp_heatmap and amp_ordinate commands of ampvis2 package, respectively, by applying the Euclidean distance method.
Ethics Statement

The work did not involve the use of human subjects, animals, cell lines and endangered species of wild fauna and flora.

CRediT Author Statement

Sofia Michailidou: Conceptualization, Methodology, Software, Writing – review & editing; George Economou Petrovits: Resources, Funding acquisition; Mary Kyritsi: Resources, Funding acquisition; Anagnostis Argiriou: Conceptualization, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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