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# Microbial Biogeography of Greek Olive Varieties Assessed by Amplicon-Based Metagenomics Analysis

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**Abstract :** Table olives are one of the most popular fermented vegetables worldwide, which along with olive oil, have a crucial role in the world economy. They are highly appreciated by the consumers for their characteristic taste and pleasant aromas, while several health and nutritional benefits have been reported as well. Until recently, microbial biogeography, i.e., the study of microbial diversity over time and space, has been mainly associated with wine. However, nowadays, the term 'terroir' has been extended to other crops and food products so as to link the geographical origin and environmental conditions to quality aspects of fermented foods. Taking the above into consideration, the present study focuses on the microbial fingerprinting of the most important olive varieties of Greece with the state-of-the-art amplicon-based metagenomics analysis. Towards this, in 2019, 61 samples from 38 different olive varieties were collected at the final stage of ripening from 13 well spread geographical regions in Greece. For the metagenomics analysis, total DNA was extracted from the olive samples, and the 16S rRNA gene and ITS DNA region were sequenced and analyzed using bioinformatics tools for the identification of bacterial and yeasts/fungal diversity, respectively. Furthermore, principal component analysis (PCA) was also performed for data clustering based on the average microbial composition of all samples from each region of origin. According to the composition, results obtained, when samples were analyzed separately, the majority of both bacteria (such as Pantoea, Enterobacter, Roserbergiella, and Pseudomonas) and yeasts/fungi (such as Aureobasidium, Debaromyces, Candida, and Cladosporium) genera identified were found in all 61 samples. Even though interesting differences were observed at the relative abundance level of the identified genera, the bacterial genus Pantoea and the yeast/fungi genus Aureobasidium were the dominant ones in 35 and 40 samples, respectively. Of note, olive samples collected from the same region had similar fingerprint (genera identified and relative abundance level) regardless of the variety, indicating a potential association between the relative abundance of certain taxa and the geographical region. When samples were grouped by region of origin, distinct bacterial profiles per region were observed, which was also evident from the PCA analysis. This was not the case for the yeast/fungi profiles since 10 out of the 13 regions were grouped together mainly due to the dominance of the genus Aureobasidium. A second cluster was formed for the islands Crete and Rhodes, both of which are located in the Southeast Aegean Sea. These two regions clustered together mainly due to the identification of the genus Toxicocladosporium in relatively high abundances. Finally, the Agrinio region was separated from the others as it showed a completely different microbial fingerprinting. However, due to the limited number of olive samples from some regions, a subsequent PCA analysis with more samples from these regions is expected to yield in a more clear clustering. The present study is part of a bigger project, the first of its kind in Greece, with the ultimate goal to analyze a larger set of olive samples of different varieties and from different regions in Greece in order to have a reliable olives’ microbial biogeography.

**Keywords :** amplicon-based metagenomics analysis, bacteria, microbial biogeography, olive microbiota, yeasts/fungi
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