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Exploring microbial diversity of naturally fermented PDO Nyons table olives

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► To cite this version:

Marine Penland, Stéphanie-Marie Deutsch, Hélène Falentin, Christophe Le Meur, Emmanuel Coton, et al.. Exploring microbial diversity of naturally fermented PDO Nyons table olives. FEMS – 8th Congress of European Microbiologists, Jul 2019, Glasgow, United Kingdom. <hal-03343668>

HAL Id: hal-03343668

<https://hal.science/hal-03343668v1>

Submitted on 14 Sep 2021

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Context & experimental design

Artisanal fermented foods constitute a significant part of the Mediterranean diet and represent a cultural heritage that needs to be preserved and protected. PDO Nyons table olives are produced according to a traditional process by a slow spontaneous fermentation in brine (Figure 1). The manufacture and unique sensorial properties of these olives only relies on autochthonous complex microbiota.

This study aimed at unravelling the microbial ecology and dynamics of Nyons table olives during a yearlong spontaneous fermentation (Figure 2). The core microbial species were determined for potential future directed fermentations.



Figure 1. Nyons olive fermentation process

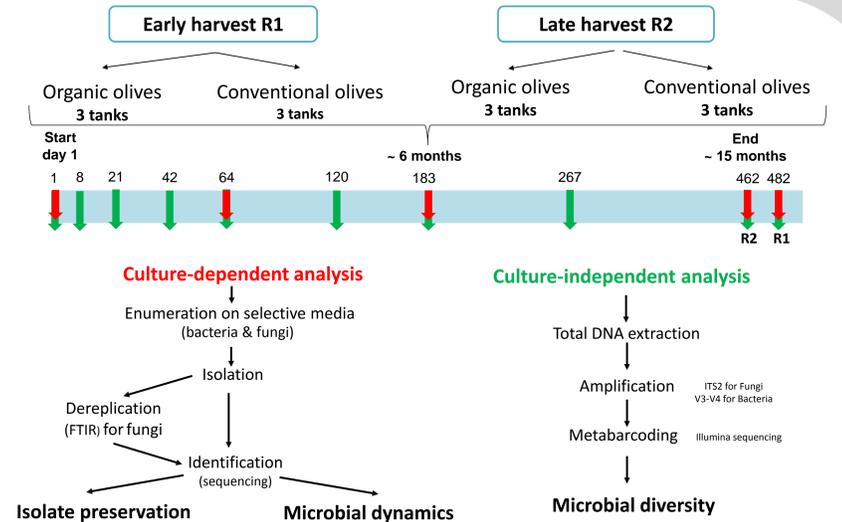


Figure 2. Experimental design and strategy to study microbial ecology

Microbial populations were stable during the studied fermentations

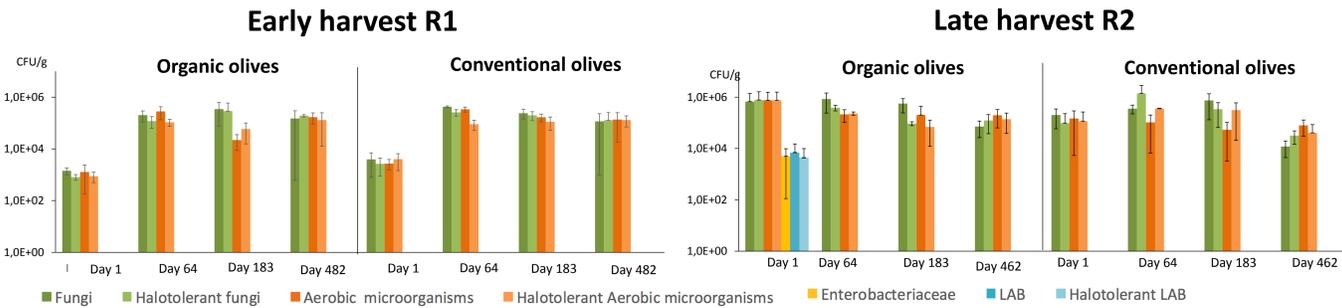


Figure 3. Microbial counts in CFU/g and diversity encountered on different media. Average values and standard deviation based on triplicates

- Results suggest that Nyons table olive fermentations mainly rely on yeasts.
- Lactic acid bacteria were only detected in fresh organic olive fruits (second harvest) whereas fungal populations remained steady throughout fermentation.
- Microbial populations were higher for the late harvest for both olive types.

Culture-dependent analyses revealed diversified fungal populations and species dynamics...

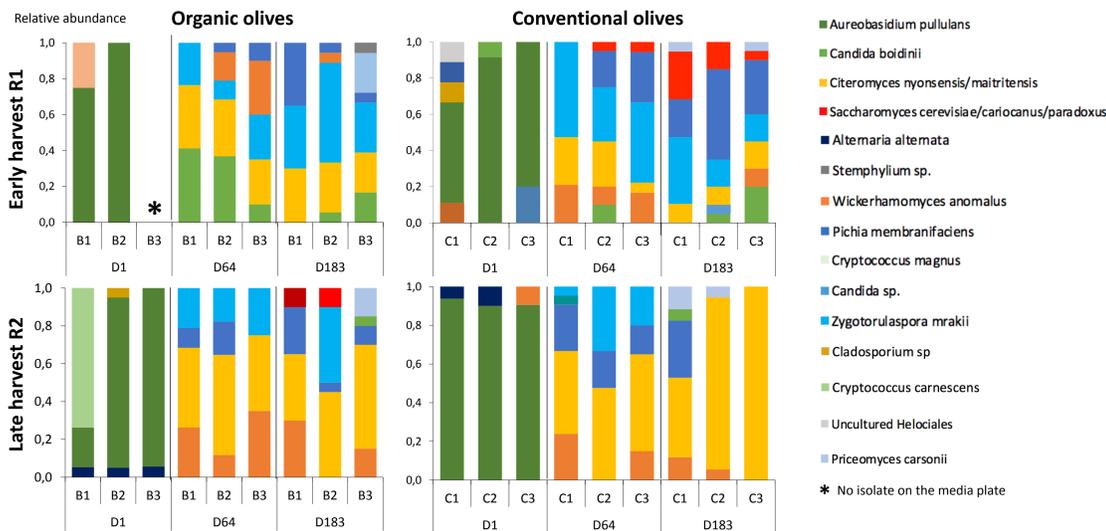


Figure 4. Fungal diversity encountered on fresh fruit (day 1), day 64 and day 183 of fermentation for the two monitored fermentation R1 and R2. Isolates were taken after plating on YGC+5% NaCl. Data and isolate identification were obtained after clustering via Fourier Transform InfraRed spectroscopy analysis and 26S rDNA gene sequencing of representative isolates.

- *Aureobasidium pullulans* was dominant on fresh fruit in all 4 fermentations, although other species were encountered in some replicates in smaller abundances.
- After 2 months of fermentation, fungal diversity increased and four yeast species remained dominant forming the core microbiota (*W. anomalus*, *C. nyonsensis*, *P. membranifaciens*, *Z. mrakii*). However, relative abundance varied between replicates and harvests. Among them, only *Wickerhamomyces anomalus* was previously isolated from fresh fruits but in low abundance, highlighting the importance of the environmental flux.
- Differences in species diversity between harvests were not substantial except for *Candida boidinii* in the early harvest fermentations.

Conclusion & perspectives

Four different Nyons table olive fermentations were monitored using culture-dependent and -independent analyses. Results suggest that yeasts are the key actors of the process with four species consistently found in high abundances. Metabarcoding analyses performed at early days of fermentation were well correlated and provided complementary information about fungal and bacterial communities. To better understand microbial dynamics, metagenetic analyses will be performed on the remaining sampling points. Moreover, volatiles, sugars and organic acids at the same stages of fermentation are currently being analysed to link microbial diversity and dynamics with olive aroma profiles and biochemical changes.

...confirmed by preliminary metagenetic analyses

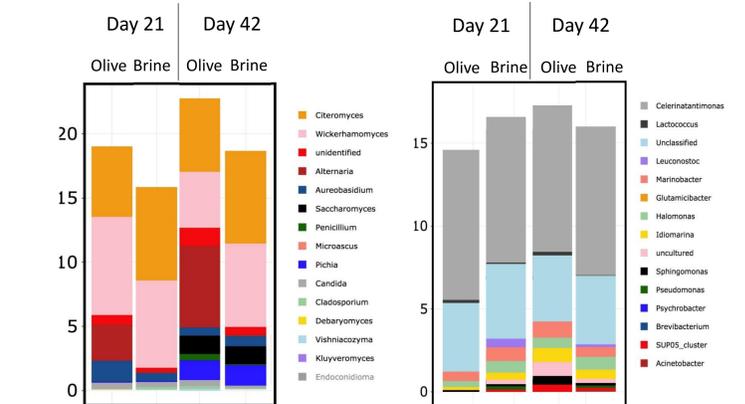


Figure 5. Fungal diversity in the samples at the genus level. Data were analysed using an internal Qiime ITSx pipeline and the UNITE_Fungi database

- At day 21 and 42 of fermentation, fungal communities were dominated by *Citeromyces* and *Wickerhamomyces* genera and in olives, *Alternaria*. Some genera such as *Pichia* and *Saccharomyces* were only present at day 42.
- These results were consistent with culture-dependent analyses and bring complementary information on fungal dynamics.
- Bacterial communities were dominated by *Celerinatantimonas*, a diazotrophic halotolerant and fermentative bacteria, suggesting a potential role during fermentation.
- Lactic acid bacteria such as *Lactococcus* and *Leuconostoc* genera were detected at very low abundances which might explain the culture-dependent counts.

Acknowledgements

We would like to thank our olive producer partner, cooperative VIGNOLIS (Nyons, France) for collaborating in this project and providing the samples.

PROMEDFOODS is a project funded through the ARIMNet2 2017 Call by the following funding agencies: ANR, ELGO-DEMETER, MESRS, MIPAF, INIA and MHESR. ARIMNet2 (2014-2017) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 618127.