Microbial innovation in table olive fermentation: a decade of research on *Lactiplantibacillus pentosus* OM13

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Background.

Table olive fermentation is a traditional longstanding biotechnological practice integral to the Mediterranean diet and agri-food sector. Despite its cultural and economic relevance, spontaneous fermentation often suffers from unpredictable microbial dynamics and activities, inconsistent sensory outcomes, and safety concerns due to the presence of spoilage and pathogenic microorganisms. These limitations have generated interest in controlled fermentation strategies based on microbial ecology and starter culture technology.
At the University of Palermo, the food microbiology research group has undertaken a comprehensive programme aimed at improving the quality, safety, and reproducibility of table olive fermentation. Focusing on Sicilian cultivars, particularly Nocellara del Belice, the research group has explored both Spanish-style and Greek-style fermentation methods. This work contributes to a broader scientific effort to understand and manage microbial communities in fermented vegetables, with a special emphasis on the role of lactic acid bacteria (LAB) and yeasts in shaping product quality and safety (https://doi.org/10.1016/j.ijfoodmicro.2022.109670).

Methods.

Central to this research was the application and characterisation of the autochthonous strain *Lactiplantibacillus pentosus* OM13, used consistently across all trials. Originally isolated from Sicilian table olives, OM13 was selected for its robust fermentative capabilities, tolerance to salt and phenolic compounds, and ability to dominate the microbial ecosystem during fermentation. Its performance was assessed under various conditions, including different harvesting methods (manual *vs.* mechanical), irrigation regimes, and fermentation strategies. One of the most innovative approaches followed was the adaptation of the “pied

de cuve" technique, commonly used in winemaking, to table olive fermentation. This involves preparing a small volume of partially fermented brine to inoculate larger batches, promoting early establishment of beneficial microbial populations while reducing the need for high-dose starter culture inoculation. Additional strategies included OM13 acclimatisation in brine before inoculation, supplementation with nutrients to support microbial growth, and co-inoculation with selected yeast strains (*Candida boidinii* and *Candida norvegica*) known for their enzymatic activity and contribution to flavour development. Trials were conducted at both laboratory and industrial scales in collaboration with local producers. Microbial populations were monitored using culture-based methods and molecular techniques such as randomly amplified polymorphic DNA (RAPD)-PCR and 16S rRNA gene sequencing. Physicochemical parameters (pH and salt concentration), volatile organic compounds (VOCs), and sensory attributes were systematically evaluated throughout the fermentation process. Multivariate statistical analyses were used to interpret microbial dynamics and compare fermentation treatments.

Results.

Across all experiments, *L. pentosus* OM13 consistently dominated the fermentation microbiota, rapidly acidified the brine, and suppressed spoilage organisms including Enterobacteriaceae and pseudomonads. Molecular analyses confirmed that LAB isolates from inoculated trials closely matched the OM13 genetic profile. The strain demonstrated strong adaptability to diverse environmental conditions, including high salinity, low pH, and phenolic presence. The "pied de cuve" approach proved particularly effective in promoting native LAB growth while reducing reliance on freeze-dried cultures. This approach preserved microbial diversity and enhanced the expression of cultivar-specific sensory traits. The acclimatisation of OM13 prior to inoculation further improved fermentation performance, accelerating acidification and stabilising microbial communities. Nutrient supplementation supported OM13 persistence and improved fermentation kinetics. Sensory evaluations consistently showed that olives fermented with OM13 scored higher in aroma, flavour complexity, and overall acceptability compared to spontaneous fermentations. OM13 use was associated with a favourable VOC profile, including elevated levels of phenylethyl alcohol, ethyl acetate, and hexanal,compounds linked to freshness and green fruit notes. Co-inoculation with selected yeasts contributed to improved colour retention, pulp firmness, and reduced off-flavours. Inoculated trials demonstrated superior microbiological stability, reduced variability, and enhanced sensory quality across different processing styles and environmental conditions, underscoring the robustness and versatility of OM13 as a starter culture.

Conclusions.

The use of *L. pentosus* OM13 has proven to be a reliable and effective strategy for guiding table olive fermentation. Its consistent dominance, environmental resilience, and positive impact on product quality making it a key element in advancing new methods for improving

table olive fermentation in Sicily. By integrating microbial selection, process optimisation, and sensory science, producers can achieve greater consistency, safety, and authenticity in their products. The development of tailored starter cultures and fermentation aids has advanced microbial biotechnology, offering practical solutions for both artisanal and industrial-scale productions. Moreover, the implementation of OM13 has contributed to the valorisation of local cultivars and traditional methods, enhancing the competitiveness of Sicilian table olives in domestic and international markets. Techniques such as “pied de cuve”, starter acclimatisation, and yeast co-inoculation are replicable and adaptable across the Mediterranean, providing a model for sustainable innovation in fermented vegetable production. This decade-long research effort has significantly deepened scientific understanding of microbial ecology in table olive fermentation while delivering tangible benefits to the agri-food sector. In conclusion, this work serves as a paradigm of how microbiological research can drive innovation, safeguard traditional practices, and contribute to the enhancement of the quality and identity of emblematic Mediterranean fermented foods.

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