

## A cryopreservation protocol of microbial consortia from Apulian table olives

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The field of microbiome research has evolved rapidly over the past few decades and has become a topic of great scientific, industrial, and public interest. Exploiting microbiomes as drivers of innovation in food systems and promoting reproducibility in basic, applied and industrial science shed new light on the preservation of microbial communities associated with food fermentation/specific matrices. Therefore, the challenges of optimally preserving microbiome samples are huge, with difficulties ranging from conserving microbial consortia to preserving microbiome integrity/functionality. Fermented table olives represent the most representative and diffused plant-based fermented products in Mediterranean countries, with relevant productions in Italy, in general, and in the Apulian region, in particular. Within the SUS-MIRRI.IT project, a cryopreservation protocol has been developed to preserve the microbiota from typical Apulian table olives cv Leccino, by using glycerol or DMSO as cryoprotectants, and a storage temperature of -135 °C. The microbial consortium was studied before and after mid-term storage using a culture-dependent approach, RNA-based metabarcoding analysis, and metabolic profiling evaluation. Results showed that after six months of cryopreservation, the viability of the microbial consortium slightly decreased regardless of the cryoprotectant used, and no significant changes in the metabolic profile were observed. Also, the metabarcoding analysis showed no significant differences in relative abundances after storing period. Results confirmed the effectiveness of the developed cryopreservation protocol, proper preservation of the microbial consortium, and its functionality during a mid-term storage period. They also pointed out preliminary insights on the possibility of exploiting them in fermenting processes after cryopreservation.

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