CRYOPRESERVATION AND REVITALIZATION OF MICROBIAL CONSORTIA ISOLATED FROM APULIAN TABLE OLIVES

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Background and aims

The *ex-situ* preservation of microbial resources as pure cultures in specific microbial Biological Research Centres (mBRCs) is crucial to ensure scientific research and promote biotechnological innovation. However, in light of the interest in microbiomes, studying how to preserve complex microbial communities in mBRCs becomes fundamental. In the food sector, microbial communities and microbiomes represent treatable model systems for microbial ecology studies and bioresources to support fermentation processes and improve the sustainability, quality and safety of food products.

Methods

Within the SUS-MIRRI.IT project, this study aims to preserve the microbiota from typical Apulian table olives cv *Leccino*, and to evaluate the effectiveness of a cryopreservation protocol by using glycerol and DMSO as cryoprotectants and a storage temperature of-135 °C (Fig.1).

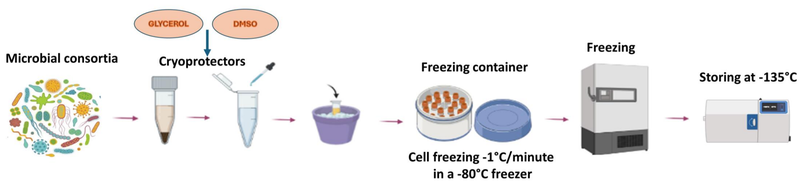

Figure 1: Scheme of cryopreservation protocol.

The microbial population was sampled one month after spontaneous fermentation and studied before short and mid-term storage using a culture-dependent approach, RNA-based metabarcoding analysis, and metabolic profiling evaluation by Biolog Ecoplate® (Fig,2). Furthermore, a revitalization protocol of the stored microbial consortia has been tested by using a synthetic brine (Bleve *et al.,* 2014[1]) to define appropriate methodologies to propagate the preserved microbiome after cryopreservation.

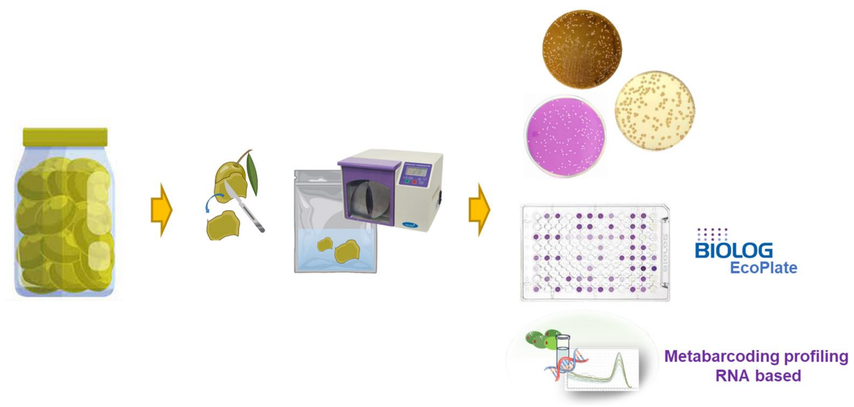

Figure 2: Scheme of sampling and analysis procedures.

[1]Bleve, G., Tufariello, M., Durante, M., Perbellini, E., Mita, G., Ramires, F., Grieco, F., Logrieco, A. (2014). Method for production of fermented table olives. European Patent Application.

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Results

Results indicated that after one and six months of cryopreservation, the **viability** of the microbial consortia slightly decreased regardless of the cryoprotectant used (Fig.3).

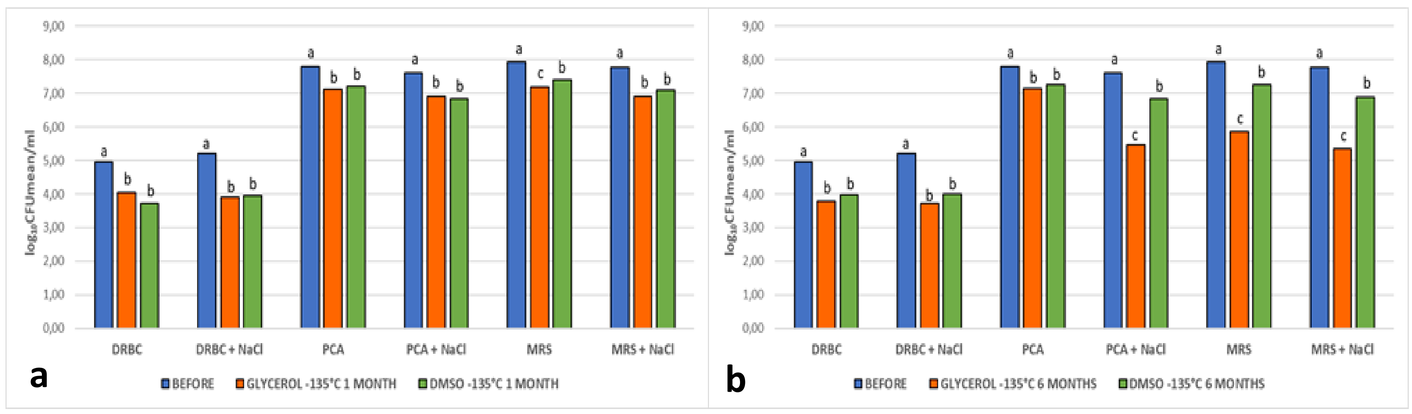

Figure 3: Evaluation of prokaryotes and eukaryotes viable cells expressed as log_{10}CFU/ml before and after the one-month (a) and six-month (b) storing period. Letters indicate significant differences according to ANOVA Tukey HSD test (P≤0.05).

No significant changes in the **metabolic profile** were observed after one and six months of cryopreservation, indicating potential functionality maintenance (Tab.1).

144h	BEFORE	GLYCEROL -135°C		DMSO -135°C	
		1 MONTH	6 MONTHS	1 MONTH	6 MONTHS
AWCD	0.417	0.466	0.542	0.516	0.458
S	13.333 ± 1.155	12.333 ± 0.577	13.667 ± 0.577	12.667 ± 0.577	12.667 ± 1.528
H'	2.783 ± 0.035	2.749 ± 0.053	2.921 ± 0.027	2.749 ± 0.041	2.827 ± 0.051
E	1.077 ± 0.051	1.094 ± 0.018	1.117 ± 0.028	1.083 ± 0.004	1.117 ± 0.041

Table 1: Microbial metabolic activity before and after one-six months of cryopreservation: average well color development (**AWCD**); Richness (**S**);Shannon diversity index (**H'**); Shannon's evenness index (**E**) after 144h of incubation.

Also, the **metabarcoding analysis** of prokaryotes showed no significant differences in relative aboundances after storing periods (Fig.4).

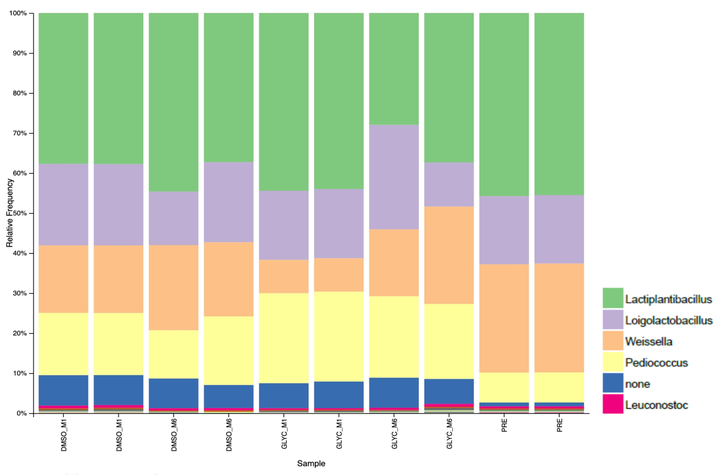

Figure 4: Metabarcoding profile by **16S** sequencing of microbiota before and after one and six months storing period.

The effect of cryopreservation was more evident on eukaryotic population regardless of the cryoprotectant used, even if preliminary evidences indicate that the dominant genera were preserved better (Fig.5).

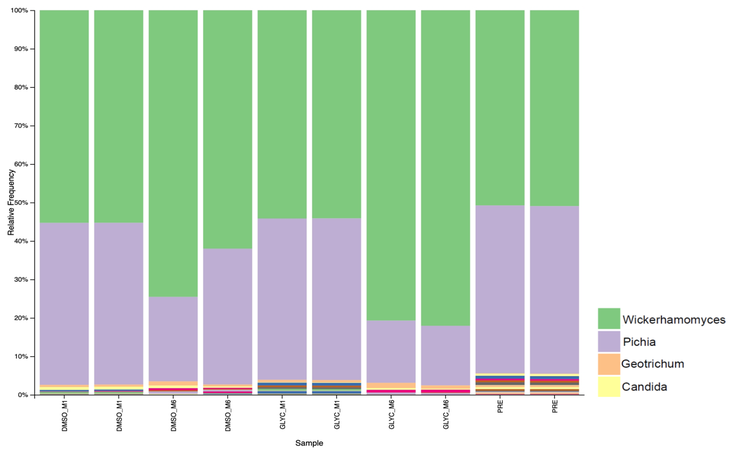

Figure 5: Metabarcoding profile by **ITS2** sequencing of microbiota before and after one and six months storing period.

Preliminary results indicate that the use of a synthetic brine could support the revitalization of microbial consortia after six months of cryopreservation. However, more deep investigation are needed to further study the functional integrity of revitalized consortium.

Conclusions

Results confirmed the proper preservation of the microbial consortium and its functionality after a short and mid-term storage period.

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