PhD DISSERTATION PROJECTS

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Evaluation, characterization and conservation of microbial diversity in complex ecosystems of agri-food interest

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This PhD thesis project aims at developing long-term storage protocols to preserve the taxonomic stability, cell viability and metabolic activity of complex microbial communities (microbiomes) isolated from food matrices. In addition, the isolated microbial species, and the associated genetic and metabolic information, will be functional for the implementation of the Microbial Collection of the University of Sassari (MBDS-UNISS-CC). The PhD research work is carried out within the framework of the activities of the SUS-MIRRI project 'Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy' and the technical-scientific collaboration agreement within the 'Hermàion 2.0' project.

**Valutazione, caratterizzazione e conservazione della diversità microbica in ecosistemi complessi di interesse agroalimentare**

Questo progetto di tesi di dottorato mira alla messa a punto di un protocollo sperimentale atto a garantire la conservazione, la stabilità e la vitalità di comunità microbiche complesse da matrici alimentari. Le specie microbiche isolate e le informazioni ad esse associate andranno ad incrementare la Collezione Microbica dell’Università di Sassari (MBDS-UNISS-CC) e il relativo Database. Il lavoro di ricerca del dottorato si svolge nell’ambito delle attività del progetto SUS-MIRRI “Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy” e dell’accordo di collaborazione tecnicoscientifica nell’ambito del progetto “Hermàion 2.0”.

# **1. State-of-the-Art**

Microbial resources play a central role in biotechnological innovations, and their use in food, industry, medicine, environment and agriculture has been widely discussed in the literature. To meet the growing demand for fermented foods, the use of microbial starters ensure that high quality standards are achieved and maintained. However, their control over the microbiota naturally associated with the raw material can lead to a significant reduction of biodiversity and sensorial complexity of the final products. Indeed, it is now widely accepted that microbial communities should be considered as a whole, as the activity of a single component is modified and regulated by those of other community members. Microbial communities, considered as dynamic ecological niches, are central to food microbiologists, especially when considering their evolution and fluctuations during the fermentation processes. The number and diversity of microorganisms in fermented foods and drinks varies according to the geographical area, climatic factors, environment, raw materials used and their preparation methods (M.Walsh et al.,2023). Therefore, the use of complex microbial communities in traditional fermentation processes (e.g sourdough) has become increasingly important. In addition, the use of traditional microbial communities combined with modern production processes leads to the production of new fermented foods and drinks enriched in taste and aroma (Giraffa, 2004). A key point in the availability of complex microbial starters for scientific and industrial applications is to assure the long-term maintenance of their taxonomic and metabolic characteristics. The conservation of complex microbial communities is thus a challenge for microbiologists, due to the lack of standardized and effective storage programs to optimally preserve both tolerant and sensitive microorganisms. This is of particular importance for Culture collections (CCs) and Microbial Resource Centers (CRMs), which currently base their activities on the conservation and maintenance of microorganisms mainly in pure culture. Several techniques are widely used to preserve microbial resources ex situ (De Vero et al., 2019). According to the guidelines of the World Culture Collection (WFCC) and Organization for Economic Cooperation and Development (OECD), at least two techniques should be used to maintain each microbial culture. Particularly, cryopreservation and lyophilization are the most reliable and widely used methods for long-term preservation of many biological resources. Also, to reduce the risk of accidental loss, additional storage should be established in different places on site and preferably with different storage techniques to ensure the long-term preservation of the harvest (Prakash et al., 2013). In this context, the stability of taxonomic, genetic, metabolic and functional characteristics of complex microbial communities in food matrices will be determined by means of NGS techniques, Phenotype microarray, RNA-seq, following different community preservation methods.

# **2. PhD Thesis Objectives and Milestones**

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 1:

A1) **Validation of SOPs for the sampling of microbiome in different ecosystems** (Definition and validation of Standard Operation Procedures (SOPs) for the sampling of microbiomes from different sources (A1.1, A1.2)).

A2) **Test performance study for the microbiome analysis** (SOPs for genetic characterisation of microbiomes with Hig-throughput sequencing (HTS) technologies, including sample preparation, sequencing protocol, databases and bioinformatic pipelines).

A3) **Optimization of analytical methods to follow the quality of microbiomes during storage** (Intermediate results of the optimization of analytical methods for quality and compliance of the microbiomes (A3.1)).

A4) **Definition of conditions and protocols for long-term preservation of microbiomes** (Optimisation of suitable methods to control the quality of the microbiomes during storage (after 6 and 12 month) (A4.1, A4.3). Definition of the best conditions to safely store microbiomes. Design of appropriate methodologies to propagate the conserved microbiome prior to utilisation and proof of concept related to safe deposit of microbiomes (A4.2).

A5) **Application of the knowledge acquired to safely store and reutilize microbiomes from different sources.**

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 1*** *Gantt diagram for this PhD thesis project.*



# **3. Selected References**

De Vero L, Boniotti MB, Budroni M, Buzzini P, Cassanelli S, Comunian R & Varese GC (2019). Preservation, characterization and exploitation of microbial biodiversity: The perspective of the italian network of culture collections. *Microorganisms*, *7*(12), 685.

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Prakash O, Nimonkar Y, Shouche YS (2013) Practice and prospects of microbial preservation. *FEMS microbiology letters*, *339*(1), 1-9.

Walsh AM, Leech J, Huttenhower C, Delhomme-Nguyen H, Crispie F, Chervaux, C, Cotter PD (2023) Integrated molecular approaches for fermented food microbiome research. *FEMS Microbiology Reviews*, *47*(2), fuad001.