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PhD DISSERTATION PROJECTS

Biotechnological innovations aimed at debittering and quality improvement of table olives

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This PhD project aims to microorganisms with acidifying, debittering, and bioprotective properties in table olive production. The initial step involves the technological selection of specific microorganisms, including yeasts and lactic acid bacteria (LAB). Promising strains will then be cultured in experimental batches to assess improvements in microbiological safety, aroma, and sensory attributes. By incorporating debittering strains, the reliance on soda can be reduced, while bioprotective strains can help manage energy costs during cold storage, enhancing overall sustainability in the production process.

Innovazioni biotecnologiche mirate alla deamarizzazione e al miglioramento qualitativo delle olive da tavola

Questo progetto di dottorato mira a identificare e utilizzare microrganismi con proprietà acidificanti, deamarizzanti e bioprotettive nella produzione di olive da tavola. Il primo step prevede la selezione tecnologica di specifici microrganismi, tra cui lieviti e batteri lattici (BL). I migliori ceppi verranno quindi coltivati in lotti sperimentali per valutare miglioramenti in termini di sicurezza microbiologica, aroma e attributi sensoriali. Incorporando ceppi ad attività deamarizzante, si può ridurre l'impiego della soda, mentre i ceppi con attività bioprotettiva possono contribuire a diminuire i costi energetici durante la conservazione a freddo, migliorando complessivamente la sostenibilità nel processo di produzione.

1. State-of-the-Art

Table olives represent a popular fermented product in the Mediterranean region (Perpetuini et al., 2020). The European production is basically concentrated in three countries: Spain, Greece and Italy (Speranza et al., 2022). However, table olives undergo alterations during the debittering process, frequently of microbial origin. To extend shelf life, industrial producers use preservatives (Casado et al., 2010). Recently, the increasing consumer demand for a quality product has led producers to seek alternative processing and preservation methods. Bioprotective microorganisms can counteract undesirable microbe development, safeguarding table olive safety (Vignolo et al.,2012).
The bioprotective role in table olives is carried out through various mechanisms (Siedler et al.,2019). The most common is the ability to compete for nutrients and survive under extreme conditions (Shi et al.,2022). When selecting psychrophilic strains for table olive production, tolerance to salinity and varying pH levels is crucial. Additionally, the ability to synthesize bioactive compounds that hinder undesirable microorganisms is considered (Siedler et al.,2019). LAB are extensively studied for their inhibitory effects against Gram-positive and Gram-negative bacteria, as well as yeasts and filamentous fungi (Ağagündüz et al., 2022; Simões et al., 2022). Inoculating *Lactiplantibacillus plantarum* strains into table olives improves quality of table olives by reducing microbiological risks. In particular, a reduction in the incidence of gas pockets was observed in packaged olives (Ghabbour et al.,2016). While LAB dominate, certain yeast strains also exhibit bioprotective properties, albeit limited research and application (Campus et al., 2018).
The utilization of these bioprotection technologies in the table olive supply chain remains limited at the industrial level. This project aims to thoroughly investigate these technologies by identifying LAB and/or yeast species suitable for pilot-scale testing and subsequent industrial level in the processing of the most widespread olive varieties in Sicily such as 'Nocellara del Belice', 'Giarraffa', and 'Nocellara Etnea', using common processing methods.

2. PhD Thesis Objectives and Milestones

The objectives of this dissertation research project can be achieved through the following activities:
1) **Quantitative analysis of microbial populations**: conduct cultural analyses on samples from various industrial olive productions, considering factors such as olive variety, processing type, and production area. Collect isolates of yeasts and LAB;
2) **Phenotypic and genotypic characterization of microorganisms:** characterize LAB and yeast isolates obtained from olive or brine samples during microbiological monitoring. This characterization will help identify species associated with production and assess biodiversity across different strains;
3**) Technological selection of LAB and yeast strains:** perform *in vitro* tests to assess the technological properties of strains. Evaluate pH resistance, salinity tolerance, temperature, resistance to acetic acid and lactic acid, CO_2 production, and enzymatic activities (pectinolytic, xylanolytic, lipasic, and betaglucosidasic). Also, test resistance common

preservatives used in the table olive industry;

4) **Performance of the selected strains and their impact on the aromatic and sensory characteristics of the final product:** inoculate the most effective LAB and/or yeast strains in pilot-scale productions. Assess their bioprotective activity in limiting spoilage and potentially pathogenic microorganisms during industrial-scale productions. Evaluate aromatic components (volatile organic compounds) and sensory profiles compared to controls following the company protocol. This confirms treatment effects under real conditions.

5) **Writing of the doctoral thesis, scientific papers and oral and/or poster communications at national and international scientific congresses:** the results of the research will be published in national and international scientific journals and presented as oral or poster presentation at national and international congresses.

Table 1

Activity		Months																							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	**Quantitive analysis of microbial population**																								
	Monitoring of microbial population																								
2	**Phenotypic and genotypic characterization of microorganism**																								
	Isolation of LAB and yeasts strain																								
	Phenotypic characterization																								
	Genotypic charaterization																								
3	**Technology selection of LAB and yeasts strains**																								
	Resistance test at low temperatures and different pH and salinity values																								
	Resistance test at different food preservatives																								
4	**Performance of selected strains and their impact of sensory profile of final product**																								
	Monitoring of sperimental production																								
	Evaluation of the aroma component																								
	Evaluation of shelf life improvement																								
5	**Writing thesis and scientific papers**																								

3. Selected References

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State of the Art

Table olives are a widely consumed fermented product in the Mediterranean region. However, table olives undergo alterations during processing [1], often of microbial origin (**Fig. 1**). Recently, lactic acid bacteria (LAB) in table olives have been used as a starter for the rapid acidification of brine. Despite the low pH values and appropriate salt content, the proliferation of undesirable microorganisms remains a concern [2]. In such cases, the implementation of a strategy involving the use of yeasts and LAB with bio-protective action can prove effective to limit the development of spoilage and/or pathogenic microorganisms.

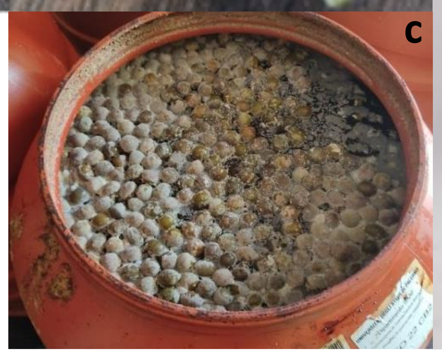


Figure 1 Most common alterations in table olives: (a) *alambrado*; (b) formation of mucilage; (c) presence of filamentous fungi.

Phd thesis objectives

The aim of this project is to select strains of LAB and yeasts with bioprotective activity in order to improve the safety and quality of table olive production. Initially, microbiological analyses will be carried out on samples from different industrial olive productions in order to collect yeast and LAB isolates. All the isolates collected will then be subjected to phenotypic and genotypic characterisation. The strains are then subjected to *in vitro* tests to evaluate CO_2 production (**Fig.2**), pH resistance (**Fig. 3**), salinity tolerance, temperature, resistance to acetic and lactic acid, and enzymatic activities (pectinolytic, xylanolytic, lipasic and betaglucosidasic). Finally, the strains with the best characteristics will be inoculated first in pilot-scale production to assess their bioprotective activity in limiting spoilage and potentially pathogenic microorganisms, and then in industrial-scale production.

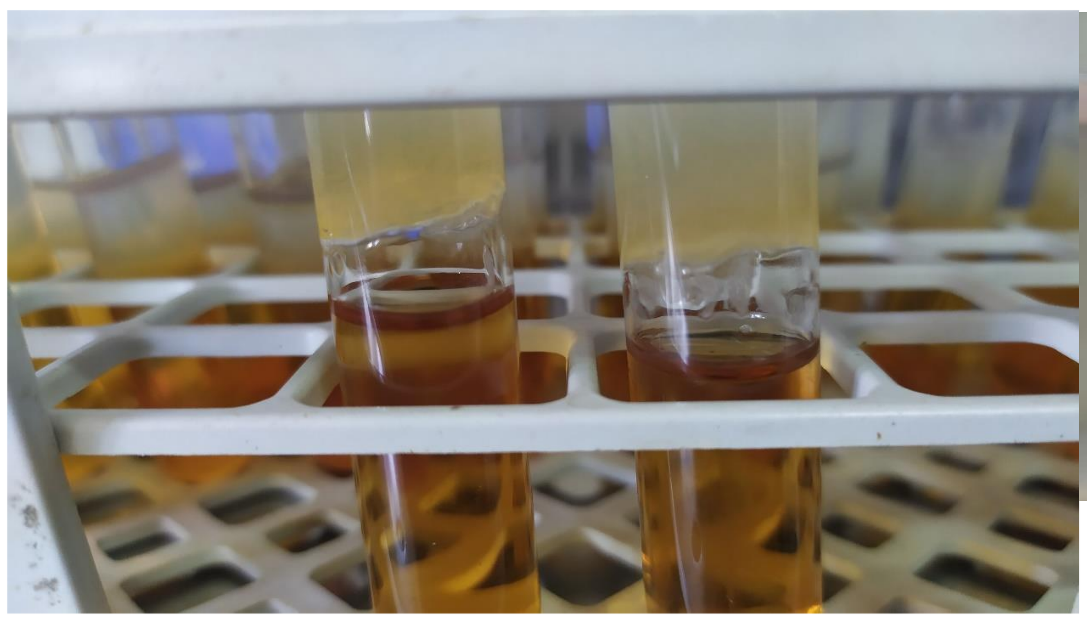
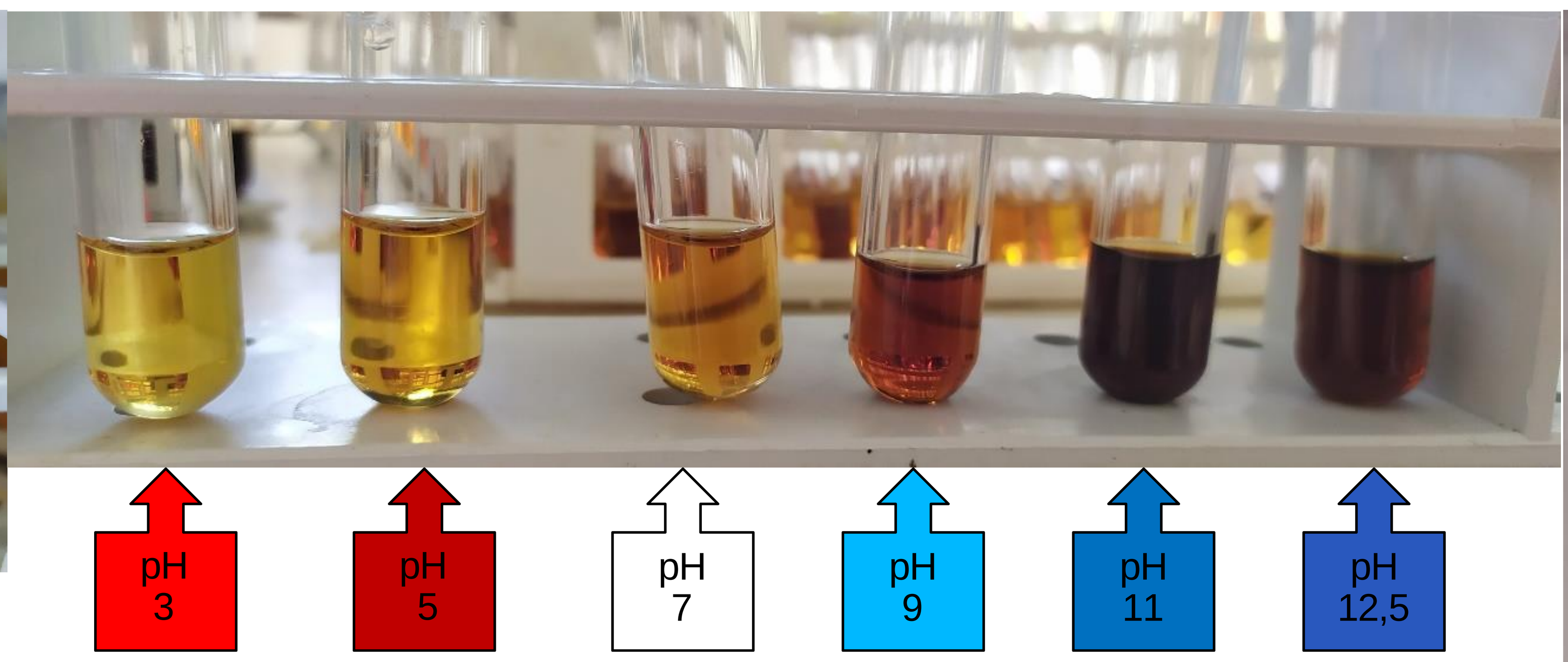


Figura 2 CO_2 production test.



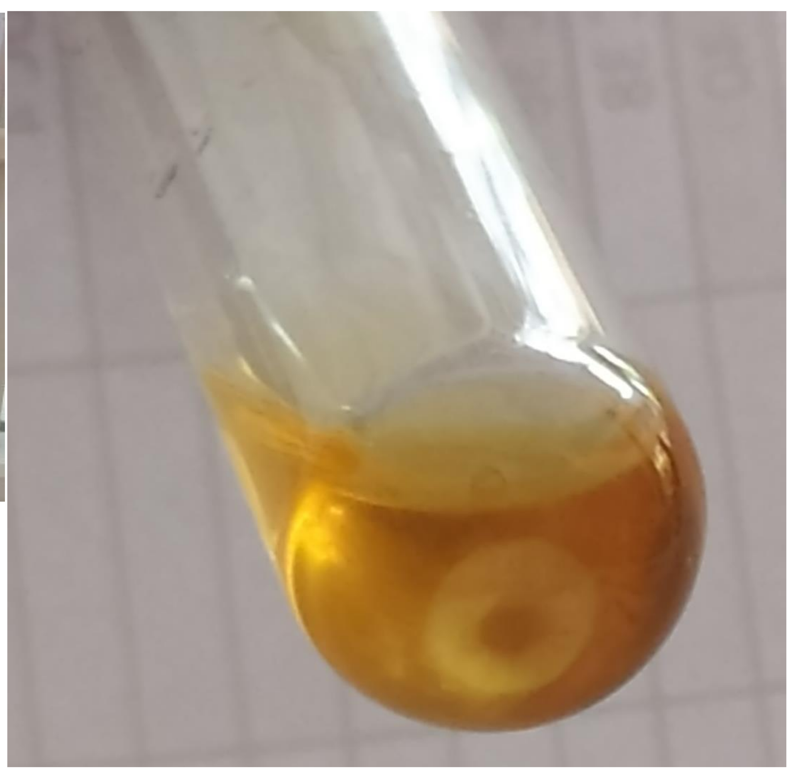


Figure 3 Resistance of yeast strain at different pH.

First results

As shown in **Fig. 4**, the most frequently isolated yeast species were: *Debaryomyces hansenii*, *Candida norvegica*, *Candida boidinii* and *Saccharomyces cerevisiae*. From the results of the technology screening, the best performing strains were selected as shown in **Tab. 1**. The best strains showed an ability to resist high NaCl values (10% w/v), a wide pH (3-11) and temperature range (5-35°C), lactic and acetic acid (2.0 g/L). The strains that showed the best performance in the preliminary screening, also showed remarkable resistance to the main preservatives used in table olive production. The strains, *Candida boidinii* LC1 and *Candida norvegica* OC10, were selected as potential protective yeasts for use in experimental table olive productions due to both their lipase activity and the absence of pectinolytic activity.

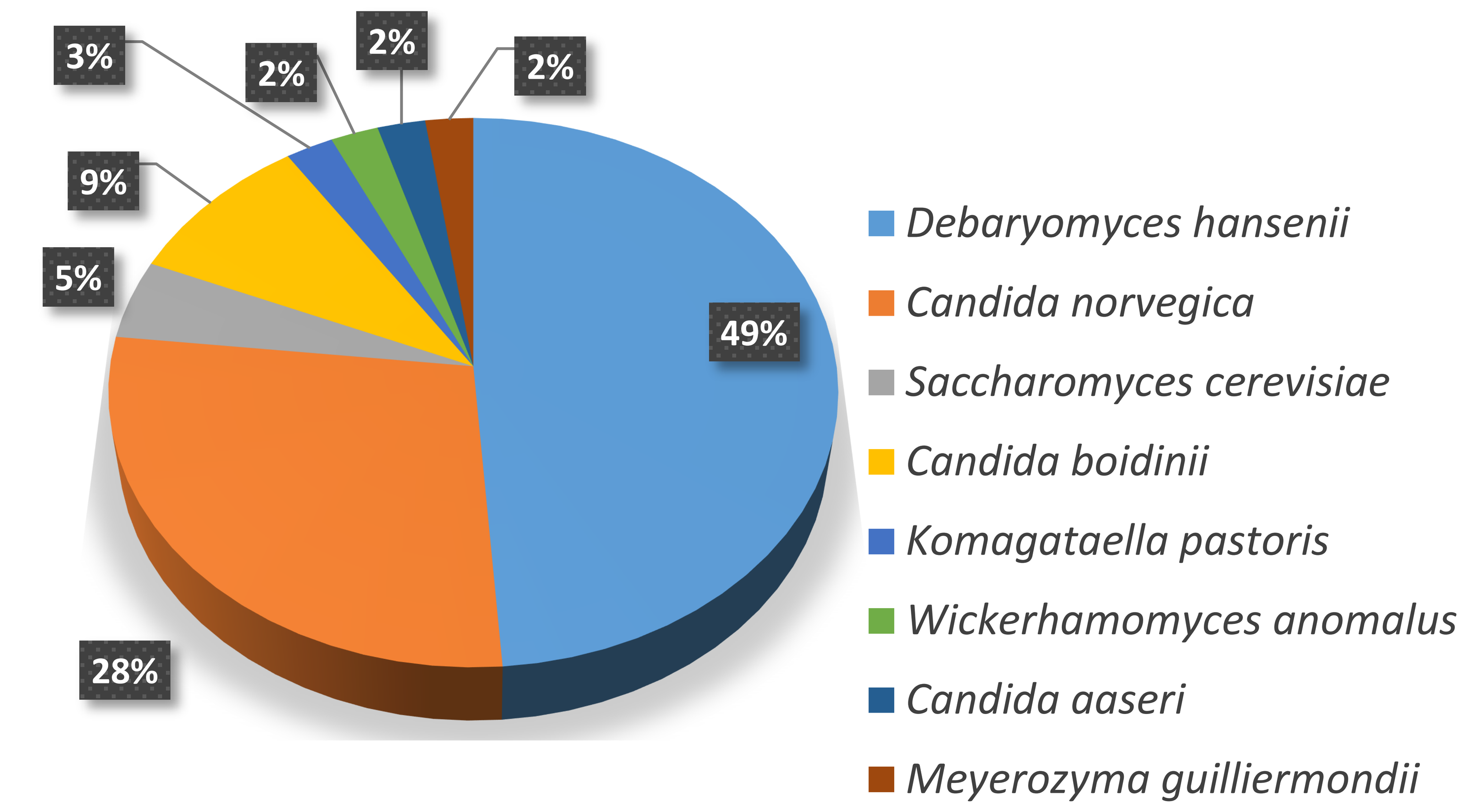


Figure 4 Frequency of yeast species isolated in Nocellara del Belice table olives.

Table 1 Technological proprieties of yeasts strains.

Strains	NaCl (% w/v)	pH (min–max)	Temperature (min–max; °C)	Lactic acid (2.0 g/L)	Acetic acid (2.0 g/L)	CO_2 production
Candida aaseri						
GY113	8	4–6	15–35	+/–	+/–	–
Candida boidinii						
GY109	6	5–9	10–25	+	–	–
LC1	10	3–10	5–25	+	–	–
LC2	10	5–9	5–25	+	–	–
LC5	10	5–9	5–25	+	–	–
Candida norvegica						
LC15	10	5–10	5–35	+	–	–
LC16	10	5–9	5–25	+	–	–
LC21	10	5–9	5–25	+	–	–
LC25	10	5–10	5–25	+	–	–
LC28	10	5–9	5–25	+	–	–
LC31	10	5–9	5–25	+	–	–
LC32	10	5–10	5–15	+	–	–
OC10	10	3–10	5–35	+	–	–
OC16	10	5–10	5–15	+	–	–
OC20	10	5–10	5–25	+	–	–
OC72	10	5–10	5–15	+/–	–	–
OC89	10	5–9	5–10	+/–	–	–
Debaryomyces hansenii						
GY19	10	4–6	10–25	+	–	–
GY55	10	4–6	10–25	+	–	–
GY57	10	4–6	10–15	+	–	–
GY60	10	4–6	10–25	+	–	–
GY74	10	4–6	10–15	+	–	–
GY102	10	4–6	10–25	+	–	–
GY110	10	4–6	10–25	+	–	–
GY117	10	4–6	10–25	+	–	–

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