

Biotechnological innovations aimed at debittering and quality improvement of table olives

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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.

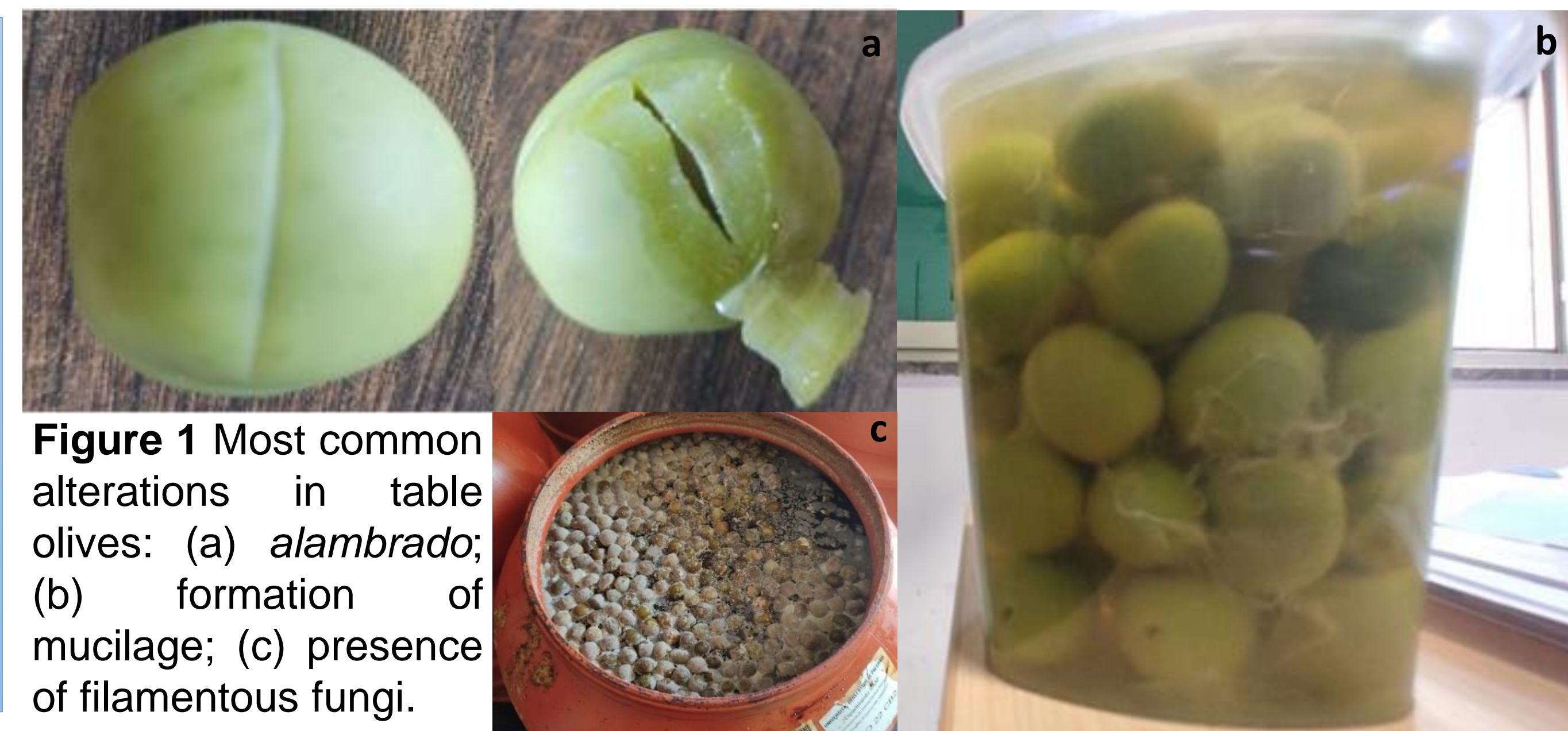


Figure 1 Most common alterations in table olives: (a) *alambardo*; (b) formation of mucilage; (c) presence of filamentous fungi.

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 CO₂ 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.



Figure 2 CO₂ production test.

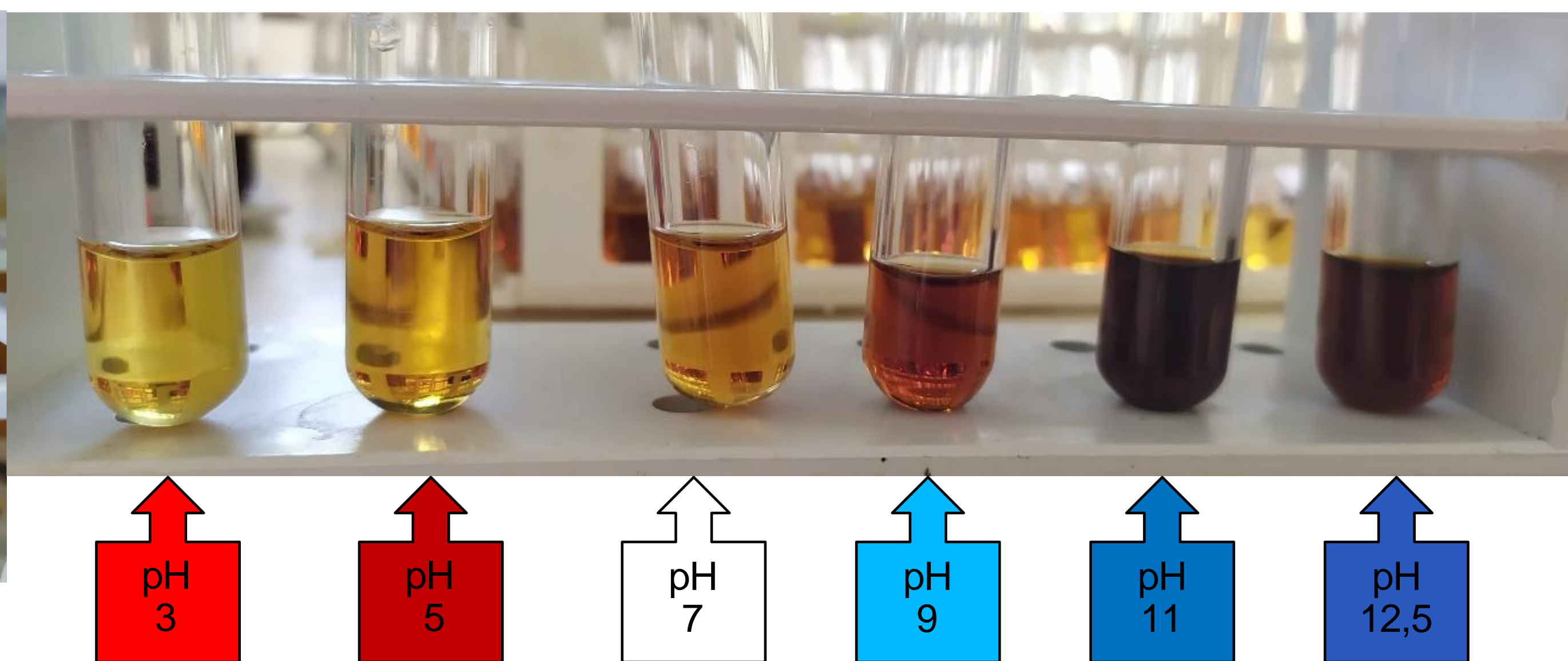
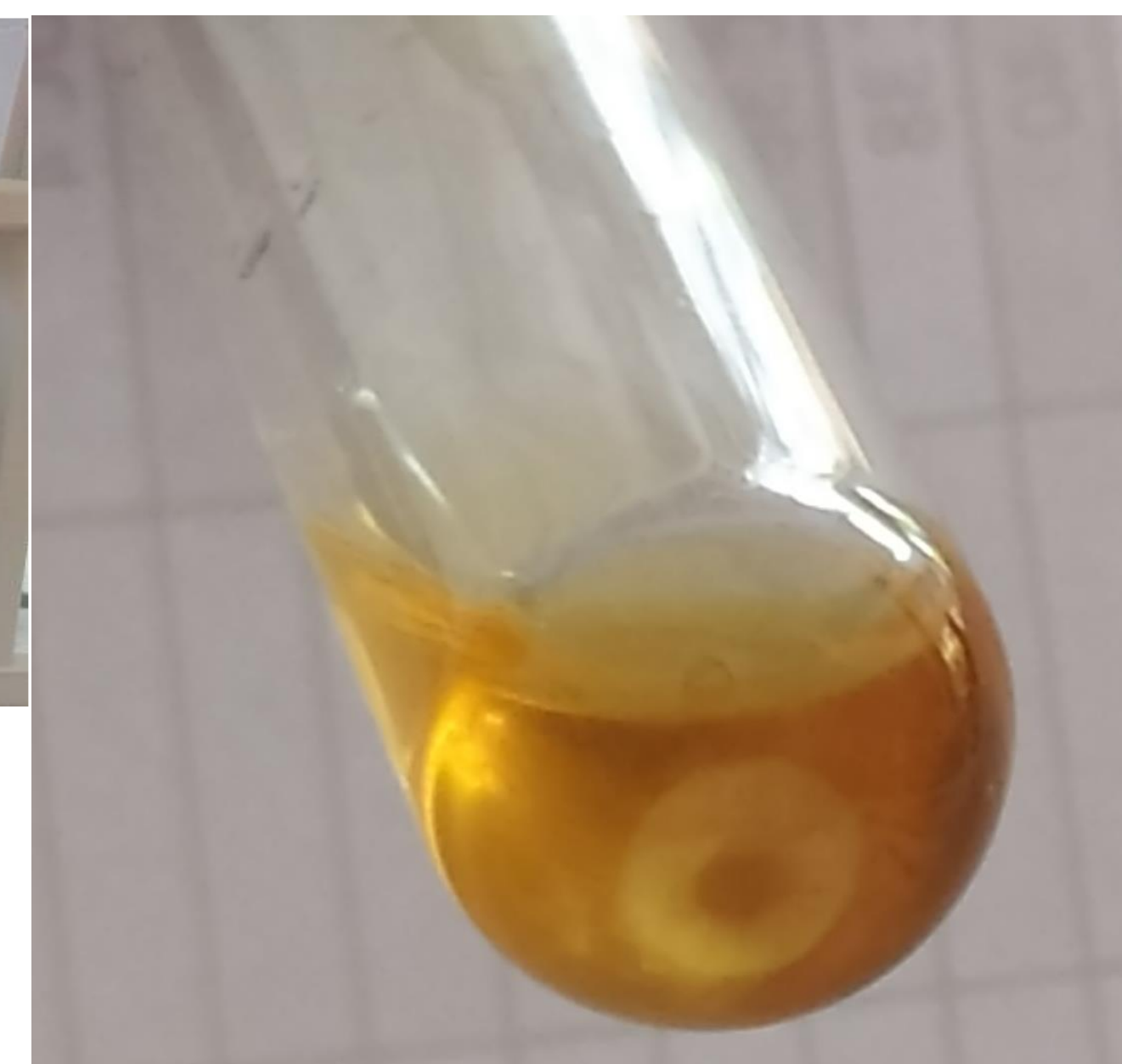


Figure 3 Resistance of yeast strain at different pH.



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.

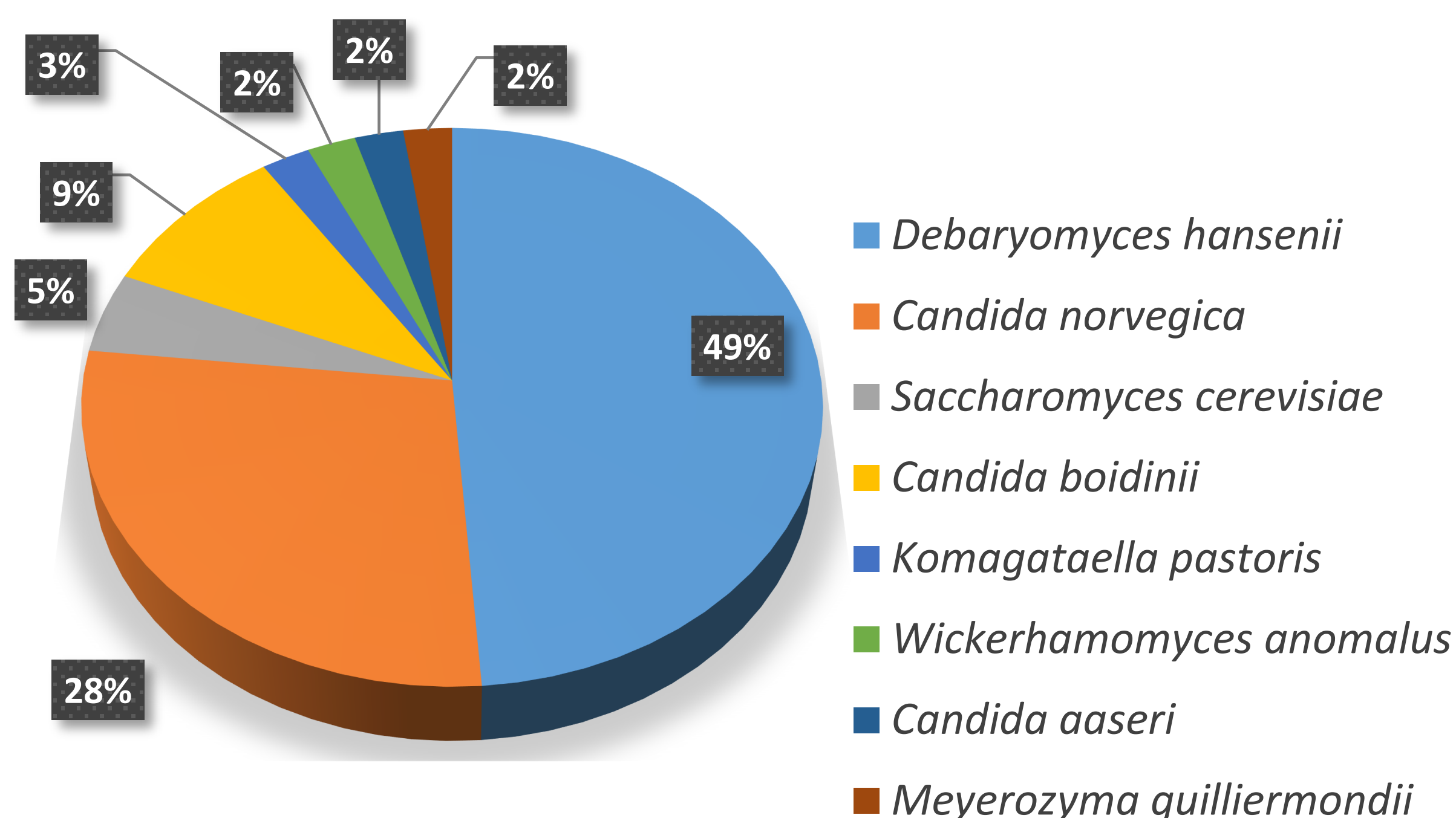


Figure 4 Frequency of yeast species isolated in Nocellara del Belice table olives.

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 [2] Campus, M., Değirmencioğlu, N., & Comunian, R. (2018). Technologies and trends to improve table olive quality and safety. *Frontiers in Microbiology*, 9, 617.
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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
<i>Candida aaseri</i>						
GY113	8	4-6	15-35	+/-	+/-	-
<i>Candida boidinii</i>						
GY109	6	5-9	10-25	+	-	-
LC1	10	3-10	5-25	+	-	-
LC2	10	5-9	5-25	+	-	-
LC5	10	5-9	5-25	+	-	-
<i>Candida norvegica</i>						
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	+/-	-	-
<i>Debaryomyces hansenii</i>						
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	+	-	-