fermentation

MDPI

Article

Bioprospecting Fungi in Mediterranean Fermentations: Functional Insights and Antibacterial Potential

David Willian Bertan [1], Eliana Setsuko Kamimura [1] and Célia Quintas [2,3,*]

[1] Faculdade de Zootecnia e Engenharia de Alimentos, FZEA, Universidade de São Paulo, USP, Av. Duque de Caxias Norte, 225, Pirassununga 13635-900, SP, Brazil; elianask@usp.br (E.S.K.)
[2] Instituto Superior de Engenharia, Universidade do Algarve, Campus da Penha, 8005-139 Faro, Portugal
[3] MED–Mediterranean Institute for Agriculture, Environment and Development & CHANGE–Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
* Correspondence: cquintas@ualg.pt

Abstract

Table olives, particularly traditionally fermented cracked-style green olives, rely on natural microbial activity without chemical debittering, with fungi playing key roles; in contrast, arbutus berry fermentation remains less characterized in terms of microbial functionality. This study investigated the enzymatic and antibacterial potential of fungal isolates from both systems. A total of 84 isolates belonging to *Aureobasidium*, *Candida*, *Cryptococcus*, *Saccharomyces*, *Pichia*, *Issatchenkia*, *Torulaspora*, and *Sporobolomyces* were screened for hydrolytic enzymes (pectinases, amylases, cellulases, xylanases, lipases, proteases, tannases, and β-glucosidases) using selective media, and for antibacterial activity against major foodborne pathogens. Isolates from arbutus fermentation showed no relevant enzymatic or antibacterial ability. In contrast, several isolates from olive fermentation exhibited significant functional traits. *Aureobasidium pullulans* demonstrated broad enzymatic capacity, producing amylases, esterases, and tannases, along with lipid hydrolysis, but also expressed cellulase, pectinase, and protease abilities. *Cryptococcus* spp. displayed interesting profiles, with low cellulolytic and pectinolytic capacity and higher phenolase, esterase, and lipase capacities. Antibacterial activity was observed exclusively against Gram-positive bacteria, particularly *Staphylococcus aureus* and *Listeria monocytogenes*, mainly among *Candida membranifaciens*, *Cryptococcus* spp., and *A. pullulans*. Overall, table olive fermentation isolates showed promising biotechnological potential for food preservation and quality enhancement, whereas arbutus isolates appeared to have limited functional relevance.

Keywords: table olives; arbutus berry; anti-*Staphylococcus aureus*; anti-*Listeria monocytogenes*; *Aureobasidium pullulans*; *Cryptococcus* spp.; *Candida membranifaciens*

Academic Editor: Spiros Paramithiotis

Received: 8 April 2026
Revised: 30 April 2026
Accepted: 8 May 2026
Published: 13 May 2026

1. Introduction

Ensuring food preservation remains a major challenge for the food industry, especially given the need to inhibit the growth of spoilage and pathogenic microorganisms while preserving product quality and safety throughout their shelf life [1,2]. Conventional strategies typically rely on thermal processing and low concentrations of chemical preservatives. However, rising microbial resistance to these agents, growing consumer demand for minimally processed foods, stricter regulations on the use of preservatives, and the sensory and nutritional losses caused by heat treatments have all driven the search for safer, more natural alternatives [3,4].

In this context, biopreservation has emerged as a promising strategy that uses microorganisms or their metabolites to inhibit undesirable microbiota. This approach is particularly relevant in foods with reduced levels of salt, sugar, fat, or acid, where traditional preservation hurdles are less effective. Among bioprotective agents, lactic acid bacteria (LAB) have been extensively studied due to their ability to produce organic acids, hydrogen peroxide, and bacteriocins, as well as to compete for nutrients, thereby inhibiting pathogenic and spoilage microorganisms. These mechanisms often act synergistically, creating an environment unfavorable to undesirable microbes while preserving sensory and nutritional attributes [1,5].

In contrast, yeasts have received less attention for their antibacterial properties against pathogenic bacteria, even though their antagonistic effects against other yeasts and fungi have been known since the 1960s [6]. These effects are largely mediated by killer toxins, proteinaceous or glycoprotein compounds secreted by specific yeast strains that inhibit or kill susceptible yeasts, molds, and even bacteria, while being harmless to the producing organism due to intrinsic immunity mechanisms. Due to their biochemical diversity and broad spectrum of activity, killer toxins have attracted growing interest across multiple fields, from food preservation and fermentation control to medical and biotechnological applications [7–10].

Despite these promising attributes, the antibacterial potential of yeasts remains limited to specific strains and conditions, highlighting an important gap in current knowledge. Classic studies such as Goerges et al. [11] and Waema, Maneesri, and Masniyon [8] show that only a subset of food-derived yeasts inhibit pathogens, including *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. Gil-Rodríguez and Garcia-Gutierrez [12] reported that only 8.7% of 103 wine yeasts exhibited antibacterial activity. Merchán et al. [13] highlighted that among 54 cheese isolates, only 16 showed inhibition, with no effect on *Listeria monocytogenes* and positive results confined to species, such as *Pichia fermentans*. Collectively, these studies suggest that antibacterial activity is highly strain-dependent and influenced by environmental conditions, reinforcing the need for targeted screening of yeast biodiversity.

Table olives, especially the traditionally fermented cracked-style green olives in Portugal, are produced without chemical debittering and depend on microbial activity, with fungi playing key roles. These microorganisms contribute to flavor development by producing volatile compounds (e.g., alcohols, aldehydes, esters), promote the growth of LAB by degrading phenolic compounds, and enhance microbial stability [14,15]. Certain yeast species have also demonstrated technological potential, including *Yarrowia lipolytica*, which can inhibit spoilage molds [16], and *Wickerhamomyces anomalus* and *Kluyveromyces lactis*, which combine β-glucosidase activity with tolerance to high salt concentrations, contributing to both debittering and fermentation control [17,18].

Moreover, the functional relevance of wild yeasts in olive fermentation has been increasingly recognized. Selected strains of *K. lactis* and *W. anomalus* not only contribute to debittering and microbial control but also enrich the volatilome of fermented olives by producing desirable aromatic compounds such as esters, higher alcohols, and aldehydes, without compromising phenolic content [18]. Moreover, *W. anomalus* exhibits strong biofilm-forming capacity on olive surfaces, a trait associated with stable colonization and sustained metabolic activity during fermentation [19]. These findings underscore the relevance of strain-specific metabolic traits in shaping both microbiological safety and sensory profiles of fermented foods.

Beyond table olive fermentation, similar functional diversity has been reported in other natural fermentation systems. Indigenous *Saccharomyces* and non-*Saccharomyces* yeasts isolated from natural grape fermentations have been shown to exhibit high enzymatic activity

(including esterases and β-glucosidases), as well as robust ethanol production and aromatic compound synthesis, reinforcing their value in natural or guided fermentations [20]. This supports the view that yeast communities from traditional fermentations represent reservoirs of biotechnologically valuable traits.

In parallel, the fermentation of arbutus berry (*Arbutus unedo* L.) represents an artisanal process driven by indigenous microbiota, yet still poorly characterized. The arbutus berry, fruit of the strawberry tree, is native to the Mediterranean and especially common in Portugal's Algarve region. The fruit is fleshy, red when ripe, and rich in sugars and phenolics, making it suitable for fermentation. In Portugal, arbutus berries are traditionally used in the artisanal production of arbutus brandy, which has been recognized by the European Union since 1989. This fermentation is associated with high variability in product quality, partly due to the lack of controlled starter cultures and the presence of diverse microbial populations during early stages [21]. Although fungi diversity in this system has been described, including *Aureobasidium*, *Candida*, *Cryptococcus*, *Hanseniaspora*, *Lachancea*, *Issatchenkia*, *Metschnikowia*, *Pichia*, *Saccharomyces*, and *Torulaspora* [21], their functional roles—particularly regarding antimicrobial and enzymatic capacities—remain largely unexplored.

Considering the diversity of fungi present in fermented foods and their metabolic potential, this study aimed to perform an exploratory screening of the enzymatic and antibacterial potential of fungal isolates from table olive and arbutus berry fermentations. The study focused on identifying hydrolytic capacities of biotechnological relevance, including proteolytic, lipolytic, esterase, xylanolytic, pectinolytic, and phenolase activities, and assessing the inhibitory capacity of these isolates against *Listeria innocua* (*L. innocua*) and some major foodborne pathogens, such as *Escherichia coli* (*E. coli*), *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), *Cronobacter sakazakii* (*C. sakazakii*), *Listeria monocytogenes* (*L. monocytogenes*), and *Staphylococcus aureus* (*S. aureus*). A comprehensive understanding of their physiological profiles may support the selection of strains with potential applications in food preservation, quality enhancement, and consumer health promotion.

2. Materials and Methods

2.1. Microorganisms and Maintenance

A total of 84 fungal strains were included in this study, comprising 37 fungi isolated from olive fermentations [22] and 47 from arbutus berry fermentations [21]. The isolates from olive fermentations were taxonomically distributed as follows: *Pichia guilliermondii* (*Meyerozyma guilliermondii*) (n = 2), *Cryptococcus* sp. (n = 5), *Cryptococcus carnescens* (n = 4), *Aureobasidium pullulans* (n = 7), *Candida fermentati* (n = 1), *Candida membranifaciens* (n = 17), and *Sporobolomyces odoratus* (n = 1). And the isolates from arbutus berry fermentations were: *Pichia membranifaciens* (n = 9), *Saccharomyces cerevisiae* (n = 33), *Issatchenkia orientalis* (*Pichia kudriavzevii*) (n = 4), and *Torulaspora delbrueckii* (n = 1).

In addition, seven bacterial strains were used as target pathogens in antibacterial assays: *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *L. monocytogenes* (UAlg Collection), *S.* Typhimurium (ATCC 14028), *S. aureus* (ATCC 25923), *C. sakazakii* (ATCC BA894), and *L. innocua* (CECT 910). Fungi were routinely cultured on yeast malt agar (YM) (Himedia, Mumbai, India) at 25 °C for 48 h, while bacteria were grown on tryptic soy agar (TSA) (Scharlau, Barcelona, Spain) at 37 °C for 24 h. Fresh cultures (48 h for fungi and 24 h for bacteria) were used for all experiments.

2.2. Antibacterial Activity

The antibacterial activity of fungi against pathogenic bacteria was assessed using a modified version of the method described by Santo et al. [21]. Bacterial suspensions were

prepared from 24-h cultures incubated at 37 °C and adjusted in Ringer's solution (Oxoid, Basingstoke, UK) to a turbidity equivalent to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. These suspensions were then evenly spread onto tryptic soy agar (TSA) (Scharlau) plates supplemented with 1% glucose (Himedia) and 1% yeast extract (Biokar Diagnostics, Adana, Turkey), and containing total NaCl (Merck, Darmstadt, Germany) concentrations of 0.8%, 4%, and 6%, using sterile swabs.

Onto the freshly inoculated bacterial lawn, portions of fungal biomass obtained from 48-h cultures grown at 25 °C were transferred using a sterile inoculation loop (Figure 1). The plates were incubated in two stages: initially for 24 h at 25 °C to support the growth of both microorganisms, followed by incubation at 20 °C to favor the production of antibacterial compounds by the fungi. The antibacterial activity results were evaluated after 24, 48, and 120 h of incubation. All assays were performed in triplicate.

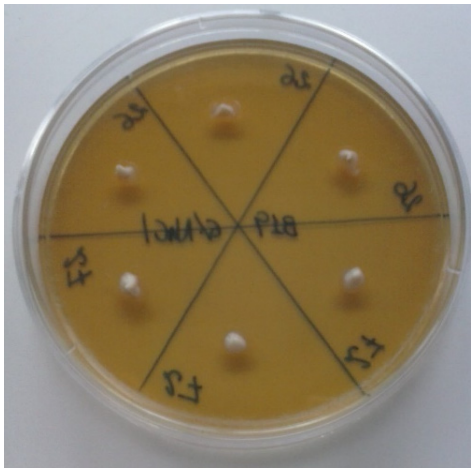

Figure 1. Example of the antibacterial assay procedure: fungi biomass inoculated over a bacterial lawn.

Antibacterial activity was classified using predefined qualitative inhibition thresholds as part of an exploratory screening approach intended to categorize isolates according to inhibitory potential rather than to quantitatively compare halo diameters among strains. To ensure reproducibility, only inhibitory effects consistently detected in all three independent replicates were recorded as positive. The influence of salt concentration (0.8%, 4%, 6%) and incubation time on the qualitative antibacterial activities of fungi strains isolated from olive and arbutus berry fermentations was monitored throughout the assay period.

2.3. Enzymatic Capacity

The extracellular enzymatic capacities of each fungal isolate used in this study were determined according to the specific enzymatic capacity under investigation. The pH of all culture media was adjusted to 5.5 ± 0.25. Each culture medium was radially streak-inoculated with fungal from 48-h cultures incubated at 25 °C, and the plates were then incubated at 25 °C for 6 days.

2.3.1. Amylase Capacity

- Starch Medium: It contained 0.2% (*w*/*v*) soluble starch (Riedel-de Haën, Seelze, Germany), 2% (*w*/*v*) agar (JMVP, Sintra, Portugal), and 0.67% (*w*/*v*) Yeast Nitrogen Base (YNB) (Remel, Lenexa, KS, USA). Starch and agar were autoclaved; YNB was filter-sterilized and aseptically added before pouring. After 6 days of incubation, the medium was flooded with Lugol's iodine solution (Riedel-de Haën) (1% (*w*/*v*) iodine and 2% (*w*/*v*) potassium iodide) for 1 min and then drained. Starch degradation was

indicated by the formation of a yellowish halo around the colonies, while the intact starch in the medium turned dark blue [23].

2.3.2. β-glucosidase Capacity

- Arbutin Medium: It contained 0.5% arbutin (Sigma-Aldrich, Steinheim, Germany), 2% (*w*/*v*) agar, 0.67% (*w*/*v*) YNB, and 2% (*w*/*v*) of a 1% (*w*/*v*) ferric ammonium citrate solution (Fluka, St Louis, MO, USA). YNB and the citrate solution were filter-sterilized and added after autoclaving. Strains capable of hydrolyzing the substrate produced a dark brown coloration around their colonies [24].

2.3.3. Cellulase Capacity

- Cellulose Medium: It contained 0.5% (*w*/*v*) carboxymethylcellulose (CMC) (Fluka), 2% (*w*/*v*) agar, and 0.67% (*w*/*v*) YNB. CMC and agar were autoclaved; YNB was filter-sterilized and added aseptically. After 6 days of incubation, the medium surface was soaked with sterile deionized water for 1 min, drained, and then flooded with a 0.03% (*w*/*v*) Congo Red (Sigma-Aldrich) solution for 15 min. After removing the dye, the medium was flooded with 1 M NaCl solution for 15 min and drained again. Hydrolysis zones appeared as pale-yellow halos, while the presence of non-degraded CMC was indicated by the red coloration of the medium [25].

2.3.4. Pectinase Capacity

- Pectin Medium: It contained 0.5% (*w*/*v*) pectin (Fluka), 2% (*w*/*v*) agar, and 0.67% (*w*/*v*) YNB. Pectin and agar were autoclaved; YNB was filter-sterilized and added aseptically. After incubation, plates were stained with 0.05% (*w*/*v*) Ruthenium Red (Sigma-Aldrich) at 4 °C for 2 h. The dye was then removed, and the surface rinsed with cold distilled water for 15 min. Decolorization around colonies indicated pectin lyase activity [25].

2.3.5. Xylanase Capacity

- Xylan Medium: It contained 0.5% (*w*/*v*) xylan (Sigma-Aldrich), 1.5% (*w*/*v*) malt extract (Scharlau), and 2% (*w*/*v*) agar. The mixture was autoclaved and poured into Petri dishes. Transparent halos indicated enzymatic degradation of xylan [24].

2.3.6. Phenolase Capacity

- Tannic Acid Medium: It consisted of 1.5% (*w*/*v*) malt extract (Scharlau), 2% (*w*/*v*) agar, and 0.5% (*w*/*v*) tannic acid (Merck). Malt extract and agar were autoclaved; tannic acid was filter-sterilized and added aseptically. Clear halos around colonies indicated hydrolysis [26].
- Oleuropein Medium: It contained 0.5% (*w*/*v*) oleuropein (Sigma-Aldrich) and 0.67% (*w*/*v*) YNB. Both components were filter-sterilized and distributed into sterile tubes. Strains capable of utilizing the substrate for growth cause turbidity in the liquid medium, as YNB alone is not sufficient to support growth [27].

2.3.7. Esterase Capacity

- Tween 80 Medium: It contained 1% (*w*/*v*) peptone (Biokar Diagnostics), 0.5% (*w*/*v*) NaCl, 0.01% (*w*/*v*) $CaCl_2$ (Riedel-de Haën), 2% (*w*/*v*) agar, and 1% (*w*/*v*) Tween 80 (Scharlau). Tween 80 was pre-boiled and added aseptically to the autoclaved medium. Precipitate formation around colonies indicated esterase activity, as fatty acids were released.

2.3.8. Lipase Capacity

- Olive Oil Medium: It consisted of 1% (*w*/*v*) peptone (Biokar Diagnostics), 0.5% (*w*/*v*) NaCl, 0.1% (*w*/*v*) glucose, 2% (*w*/*v*) agar, and 5% (*w*/*v*) low-acidity olive oil. Olive oil was boiled, agitated, and added aseptically after autoclaving. After incubation, plates were flooded with saturated copper sulfate (Merck) solution for 15 min and chilled for 1 h. Dark blue colonies indicated lipid hydrolysis [27].

2.3.9. Protease Capacity

- Milk Agar: It consisted of 4% (*w*/*v*) TSA and 10% (*w*/*v*) skimmed powdered milk. TSA was autoclaved at 121 °C for 15 min; milk was autoclaved at 118 °C for 10 min. Both were aseptically mixed and poured into plates. Transparent halos indicated casein degradation [24].
- Gelatin Medium: It contained 2.3% (*w*/*v*) nutrient agar (Oxoid) and 0.5% (*w*/*v*) gelatin (Condalab, Madrid, Spain). Both solutions were autoclaved separately and mixed aseptically before pouring. After incubation, plates were flooded with saturated ammonium sulfate for 2 min. Transparent zones indicated proteolytic activity [28].

The reagents used in the study were analytical grade and obtained from various commercial sources.

3. Results

3.1. Antibacterial Activity

The results of the antibacterial activity assay of fungal isolates against pathogenic bacteria were evaluated at 24, 48, and 120 h and classified as follows: negative (−), when no inhibition halo was observed; weak (+/−), when the halo diameter was less than 13 mm; moderate (+), when the halo diameter ranged between 13 and 16 mm; and strong (++), when it exceeded 16 mm. Fungal isolates obtained from arbutus berry fermentation exhibited no antibacterial activity against any of the tested pathogenic bacteria. In contrast, fungal isolates from olive fermentation showed inhibitory effects exclusively against Gram-positive bacteria (*L. monocytogenes*, *L. innocua*, and *S. aureus*). Notably, only two out of seven *Aureobasidium pullulans* strains (strains 7 and 20) *inhibited L. monocytogenes* and *L. innocua*, and both exhibited strong (++) activity (Figure 2).

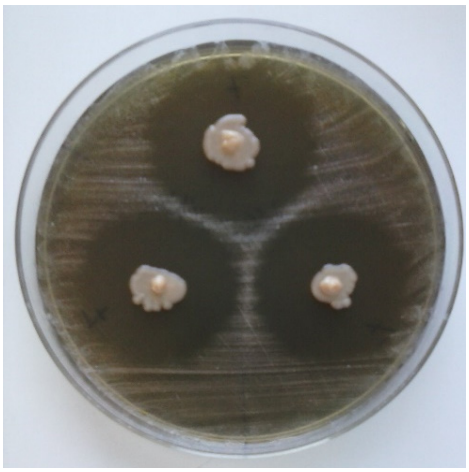

Figure 2. Representative result of the antibacterial assay: strong activity (halo diameter > 16 mm) of *A. pullulans* against *L. monocytogenes* at 6% NaCl.

Anti-*S. aureus* activity was the most frequently observed antibacterial effect among isolates from olive fermentation (Table 1), although none of the strains exhibited strong

inhibition. Specifically, three out of five *Cryptococcus* sp. strains (12, 15, and 16) and 16 out of 17 *Candida membranifaciens* strains (except strain T) inhibited *S. aureus*. A distinct time- and concentration-dependent inhibition pattern (i.e., +, +/−, and −) at 0.8% NaCl was exclusively observed in *A. pullulans* strains (7 and 20), and consistently across all susceptible target bacteria (*S. aureus*, *L. monocytogenes*, and *L. innocua*).

Table 1. Antibacterial activity of isolates from table olive fermentation against *Staphylococcus aureus* *.

Fungi	Strain Code	0.8% NaCl			4% NaCl			6% NaCl		
		24 h	48 h	120 h	24 h	48 h	120 h	24 h	48 h	120 h
Cryptococcus sp.	A	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	B	−	−	−	−	−	−	−	−	−
Candida fermentati	C	−	−	−	−	−	−	−	−	−
Candida membranifaciens	D, G-Q, 22–24, 26–27, 29–31	+	+	+	+	+	+	+	+	+/−
Sporobolomyces odoratus	F	−	−	−	−	−	−	−	−	−
Candida membranifaciens	T	−	−	−	−	−	−	−	−	−
Cryptococcus carnescens	1	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	2	−	−	−	−	−	−	−	−	−
Cryptococcus carnescens	3–4	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	5	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	7	+	+/−	−	+	+	+	+	+	+/−
Cryptococcus sp.	8	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	10	−	−	−	−	−	−	−	−	−
Cryptococcus sp.	12, 15, 16	+	+	+	+	+	+	+	+	+/−
Aureobasidium pullulans	18	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	20	+	+/−	−	+	+	+	+	+	+/−
Cryptococcus carnescens	32	−	−	−	−	−	−	−	−	−
Pichia guilliermondii	34–35	−	−	−	−	−	−	−	−	−

* Symbols denote antibacterial activity as follows: (−) no inhibition halo was observed; (+/−) weak, when the halo diameter was less than 13 mm; (+) moderate, when the halo diameter ranged between 13 and 16 mm < 13 mm.

Regarding the effect of salt concentration and incubation time, the growth of the target bacteria was strongly influenced by NaCl levels. *E. coli* failed to grow in media containing 6% NaCl, whereas *Pseudomonas aeruginosa* exhibited growth only after 48 h at 25 °C in 4% NaCl and was completely inhibited at 6%. Similarly, *S.* Typhimurium, *L. monocytogenes*, and *L. innocua* only showed detectable growth after 48 h in the presence of 6% NaCl, indicating a clear delay or suppression of growth under high-salt conditions.

In terms of antibacterial activity, inhibition of *L. monocytogenes* and *L. innocua* increased with salt concentration, being classified as weak at 0.8% NaCl, moderate at 4%, and strong at 6%. In contrast, against *S. aureus* (Table 1), moderate inhibition was consistently observed across all salt concentrations and incubation times, except at 6% NaCl after 120 h, where a reduction in the inhibition halo was detected.

3.2. Enzymatic Capacity

The results of the enzymatic capacity of isolates obtained from table olive or arbutus berry fermentations were categorized as follows: negative (−), when no substrate utilization was observed; weak (+/−), when substrate degradation by the yeast was minimal; and positive (+), when there was clear evidence of substrate utilization. Yeast isolates from arbutus berry fermentation exhibited enzymatic activity only for the hydrolysis of arbutin and cellulose. Among them, only 2 of 33 *Saccharomyces cerevisiae* strains and 1 of 4 *Issatchenkia orientalis* strains exhibited β-glucosidase activity by hydrolyzing arbutin. Cellulose hydrolysis was observed in just 4 strains of *S. cerevisiae*. However, all detected activities were classified as weak (Figure 3).

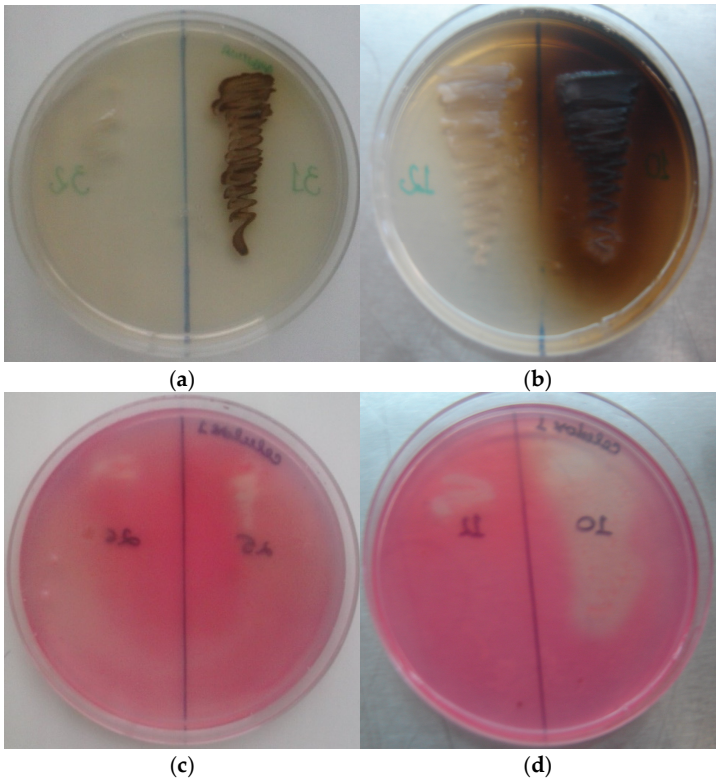

Figure 3. Representative detection of (**a**) β-glucosidase and (**c**) cellulase activities in yeast isolates from arbutus berry fermentation, showing weakly positive reactions (**a**,**c**) compared to strong reactions (**b**,**d**) in arbutin and cellulose media, respectively.

No enzymatic activity was observed in gelatin and xylan media for any of the tested isolates. Additionally, as yeast isolates from arbutus berry fermentation exhibited little to no detectable enzymatic activity overall, Table 2 summarizes only the results obtained for the remaining culture media and for isolates derived from table olive fermentation. Marked variability in enzymatic profiles was observed among the olive-derived isolates. *A. pullulans* strains consistently exhibited the broadest range of capacities, including amylolytic (starch), β-glucosidase (arbutin), cellulolytic, pectinolytic, phenolytic (tannic acid), oleuropeinolytic, and proteolytic (milk) activities, as well as esterase activity (Tween 80) and, in some strains, lipolytic activity in olive oil. In contrast, *C. membranifaciens* strains showed no detectable enzymatic activity in any of the tested media.

Intermediate and more variable profiles were observed for other genera. *Cryptococcus* spp. and *Cryptococcus carnescens* showed limited activity, with some strains displaying oleuropein hydrolysis and lipid-related activities, while cellulolytic activity was weak or absent. *Sporobolomyces odoratus* showed only weak pectinolytic activity, whereas *Candida fermentati* and *Pichia guilliermondii* were characterized by restricted enzymatic profiles, with activity mainly limited to oleuropein hydrolysis. Overall, the results indicate that enzymatic capabilities among the isolates were species- and strain-dependent, with a predominance of activities related to phenolic compound transformation and lipid metabolism, while polysaccharide- and protein-degrading activities were less widespread.

Figure 4 provides an integrated heatmap overview of the antibacterial and enzymatic profiles of the isolates from olive fermentation, enabling comparative visualization of multifunctional traits across taxa. Considerable interspecific variability was observed. *A. pullulans* exhibited the broadest functional profile, combining antibacterial activity against *L. monocytogenes* and *L. innocua* with multiple enzymatic abilities, including amylase, β-glucosidase, esterase, lipase, and pectinase.

Table 2. Enzymatic capacity profiles of isolates from table olive fermentation based on substrate hydrolysis *.

Fungi	Strain Code	Starch	Arbutin	Cellulose	Pectin	Tannic Acid	Oleuropein	Tween 80	Olive Oil	Milk
Cryptococcus sp.	A	−	−	+/−	−	−	+	+	+	−
Aureobasidium pullulans	B	+	+	+	+	+	+	+/−	−	+
Candida fermentati	C	−	−	−	−	−	+	−	−	−
Candida membranifaciens	D, G-T, 22–24, 26–27, 29–31	−	−	−	−	−	−	−	−	−
Sporobolomyces odoratus	F	−	−	−	+/−	−	−	−	−	−
Cryptococcus carnescens	1	−	−	+	−	−	−	+	+	−
Aureobasidium pullulans	2	+	+	+	+	+	+	+/−	−	+
Cryptococcus carnescens	3–4	−	−	+/−	−	−	+	+	+	−
Aureobasidium pullulans	5	+	+	+	+	+	+	+/−	+	+
Aureobasidium pullulans	7	+	+	+	+	+	+	+/−	+	+
Cryptococcus sp.	8	−	−	−	−	−	+	+	+	−
Aureobasidium pullulans	10	+	+	+	+	+	+	+/−	+	+
Cryptococcus sp.	12, 15, 16	−	−	−	−	−	−	−	−	−
Aureobasidium pullulans	18	+	+	+	+	+	+	+/−	−	+
Aureobasidium pullulans	20	+	+/−	+	+	+	+	+/−	−	+
Cryptococcus carnescens	32	−	−	−	+/−	−	+	+	+	−
Pichia guilliermondii	34–35	−	−	−	−	−	+	−	−	−

* Symbols denote substrate degradation as follows: (−) no activity detected; (+/−) weak activity; (+) clear substrate degradation.

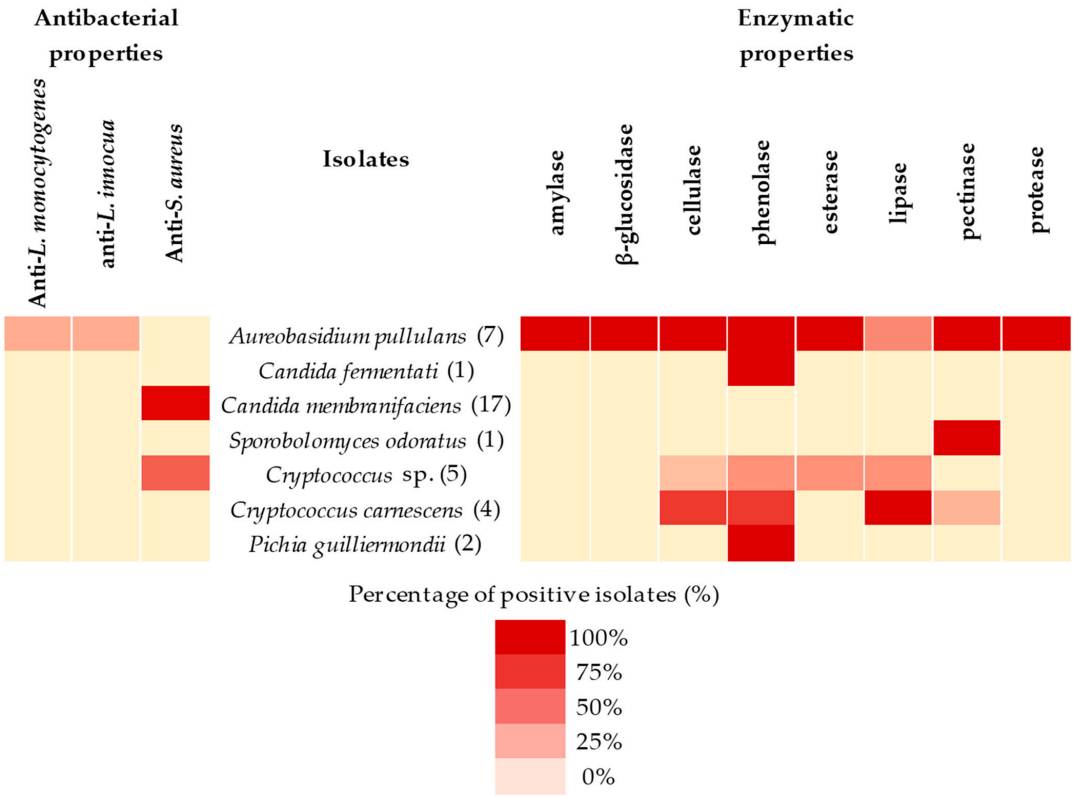

Figure 4. Heatmap summarizing the antibacterial and enzymatic profiles of fungal species isolated from olive fermentation. The number of isolates for each species is given in parentheses. Color intensity represents the proportion (%) of isolates within each species exhibiting positive activity for the corresponding antibacterial or enzymatic trait. Percentages were calculated based on the total number of isolates belonging to each species.

In contrast, *C. membranifaciens* showed high antibacterial activity against *S. aureus* (94% of strains) but no detectable enzymatic activity under the tested conditions. Other species displayed more restricted profiles. *Cryptococcus* spp. and *C. carnescens* exhibited enzymatic activities mainly related to lipid and phenolic compound transformation, with limited or no antibacterial activity. Likewise, *S. odoratus*, *C. fermentati*, and *P. guilliermondii* showed narrow enzymatic profiles and no antibacterial effects.

Additionally, the heatmap highlights the distinct functional specialization patterns among the fungal taxa, suggesting that antibacterial and enzymatic traits were not uniformly distributed across species. While some taxa exhibited predominantly antagonistic behavior (e.g., *C. membranifaciens*), others displayed mainly metabolic and hydrolytic capacities with limited antibacterial effects (e.g., *Cryptococcus* spp. and *C. carnescens*). This functional compartmentalization reinforces the ecological diversity of olive-associated fungi and suggests that different taxa may contribute to fermentation through complementary mechanisms, including pathogen suppression, substrate biotransformation, and the release of aroma precursors.

4. Discussion

4.1. Antibacterial Activity

The antibacterial activity observed in the present study reinforces the recognized multifunctional role of yeasts in table olive fermentations. Previous studies have demonstrated that yeast populations significantly influence microbial community dynamics and volatilome composition during spontaneous fermentations, ultimately affecting product quality and safety [29]. Moreover, wild yeast starters have been shown to modulate phenolic compounds and volatile profiles, contributing to microbial inhibition and fermentation stability [18]. Selected yeast strains have also been identified as effective biopreservative agents that inhibit foodborne pathogens and promote microbial stability during olive fermentation [19]. In this context, the antibacterial activity exhibited by isolates, such as *Aureobasidium pullulans* and *Candida membranifaciens* (Figure 4), supports their potential as natural starter cultures to improve food safety and quality.

Importantly, the antibacterial activity detected in this study was restricted to Gram-positive bacteria, with no inhibition observed against Gram-negative species. This pattern supports a Gram-specific mode of action, as previously described by Bilinski, Innamorato, and Stewart [30], who reported that Gram-positive bacteria are generally more susceptible to yeast antagonism than Gram-negative organisms. This differential susceptibility is commonly attributed to structural differences in the bacterial cell envelope, particularly the presence of an outer membrane in Gram-negative bacteria that can act as a barrier to antibacterial compounds produced by yeasts [31].

The observed inhibitory effects, particularly against *L. monocytogenes* and *S. aureus*, may be partially explained by competitive interactions. Yeasts are known to compete with bacteria for essential nutrients such as nitrogen, iron, and carbon sources, especially under nutrient-limited or stressful environmental conditions. This mechanism has been described as a key antagonistic strategy in microbial ecosystems. For instance, *A. pullulans* exhibited strong inhibition of *Parvularugoglobigerina extensa* on apple fruit, but the inhibition rate decreased significantly when external nitrogen sources were supplied, highlighting the importance of nutrient competition [4]. A similar mechanism may be involved in the present study, particularly under high-salt conditions (e.g., 6% NaCl), where competition for limited resources may be intensified.

Salt concentration plays a central role in shaping both microbial growth and antimicrobial interactions. High concentrations of solutes such as NaCl reduce water activity and impose osmotic stress, thereby limiting microbial growth and metabolic activity [5,32]. In

fermented foods such as table olives, salt also acts as a selective ecological factor, inhibiting salt-sensitive microorganisms, such as Gram-negative bacteria, and favoring the growth of halotolerant yeasts [33]. Traditional Portuguese fermentations, for example, employ NaCl concentrations around 8%, which promote yeast dominance while restricting lactic acid bacteria (LAB) and Gram-negative populations, contributing to product stability and shelf life [32,34]. These ecological conditions help explain the limited or delayed growth of several bacterial strains observed in this study under high-salt conditions.

The increase in inhibition halos against *Listeria* spp. with increasing NaCl concentration is also consistent with previous findings. The study of Llorente et al. [35] reported that salt does not necessarily enhance the production of antimicrobial compounds, but can increase the sensitivity of target microorganisms. Similarly, Hernández et al. [36] observed that the proportion of yeast strains exhibiting antibacterial activity increased at intermediate salt concentrations (5–8% NaCl), although activity declined at higher levels (10% NaCl), except for *Debaryomyces*. Larger inhibition zones at higher salt concentrations may therefore reflect increased susceptibility of the target bacteria rather than enhanced antibacterial performance of the yeast strains. This interpretation is supported by the consistent inhibition patterns observed for *L. monocytogenes* and *L. innocua* across the different NaCl concentrations evaluated (0.8%, 4%, and 6%).

Environmental factors such as pH may also influence antibacterial activity. Since pH was not controlled in the present assays, interactions between salt concentration and pH may have affected the stability or diffusion of antibacterial compounds [36,37]. This may explain the reduction in inhibition halos observed against *S. aureus* after prolonged incubation (120 h) at 6% NaCl (Table 1). Similar inconsistencies have been reported by Llorente et al. [35], suggesting that antibacterial responses may vary with environmental conditions.

The temporal stability of antibacterial activity is another relevant factor. Musmanno, Di Maggio, and Coratza [38] and Ullivarri et al. [39] demonstrated that only yeasts with strong killer phenotypes, isolated from natural wine fermentations, maintain consistent antimicrobial activity over time. In their study, inhibition halos remained stable for extended periods, regardless of salt concentration or target organism. In contrast, the present results suggest that antibacterial activity may be both time- and condition-dependent, as observed for *A. pullulans* strains 7 and 20, which lost activity at low salt concentration (0.8% NaCl) after 48 h but remained active under higher salt conditions.

From an ecological perspective, the antibacterial activity of fungi must also be interpreted within the context of the natural microbiota of plant-based fermentations. The natural microbiota of plant-based substrates includes filamentous fungi and yeasts—especially *Candida*, *Saccharomyces*, *Hansenula*, *Pichia*, and *Rhodotorula*, as well as bacteria, such as *Pseudomonas*, *Flavobacterium*, *Bacillus* (aerobes), and *Enterobacter*, *E. coli*, *Klebsiella* (facultative anaerobes), in addition to lactic acid bacteria (LAB) from the genera *Lactobacillus*, *Pediococcus*, *Streptococcus*, and *Enterococcus*. The fermentation of green olives typically begins with a dominance of Gram-negative enterobacteria, followed by the growth of LAB and yeasts, which progressively suppress undesirable microorganisms [40]. Therefore, the antimicrobial activity of yeasts, together with LAB metabolism, may contribute to the natural stabilization of the fermentation process.

Overall, the absence of antibacterial activity in yeasts isolated from arbutus berry fermentation, in the experimental conditions tested, contrasts with the clear inhibitory effects observed in table olive-derived isolates. Furthermore, the activity detected was restricted to Gram-positive bacteria, with no inhibition of Gram-negative species. This suggests that yeasts alone may not be responsible for controlling Gram-negative pathogens during olive fermentation, a role more likely associated with LAB. Nevertheless, the antibacterial potential demonstrated by specific fungal strains, particularly *A. pullulans* and *C.*

membranifaciens, underscores their relevance for biotechnological applications, particularly in the development of natural biopreservative strategies.

4.2. Enzymatic Capacity

The enzymatic profiles observed in the present study further reinforce the multifunctional role of yeasts in table olive fermentations. The intrinsic composition of olives, typically comprising 59–62% water, 6–8% carbohydrates, 1–2% protein, and 26–27% lipids [40], provides an important framework for interpreting these results. The relatively low carbohydrate content may partly explain the limited occurrence of amylolytic activity among the isolates, with only *A. pullulans* exhibiting starch degradation in the present study (Figure 4). In contrast, the high lipid content is consistent with the more frequent detection of lipolytic and esterase activities, suggesting that yeast metabolism is aligned with the predominant substrates available in the olive matrix.

A key finding was the limited occurrence of polysaccharide-degrading enzymes, which may be considered advantageous for maintaining olive tissue integrity. Cellulose and hemicellulose are the main structural polysaccharides of plant cell walls, with xylan representing the predominant hemicellulosic fraction; therefore, cellulase and xylanase activities are considered undesirable in this context, as they can cause tissue softening [25,41,42]. The low frequency of cellulase—restricted to *A. pullulans* and some strains of *Cryptococcus* spp. and *C. carnescens* (Figure 4)—and the absence of xylanase activity suggest a reduced capacity for structural degradation of plant cell walls. This pattern is consistent with previous observations that yeast strains from olive brines tend to exhibit lower polysaccharolytic activity compared to those isolated from fresh plant materials [43]. From a technological perspective, this indicates a selective adaptation of table olive-associated yeasts toward metabolic functions that do not compromise fruit texture, which is essential for product quality [19].

In contrast, the detection of β-glucosidase activity exclusively in *A. pullulans*, according to a study of Strauss et al. [25], highlights a potentially important functional trait among carbohydrate-active enzymes. This enzyme plays a central role in the hydrolysis of oleuropein, contributing to debittering and improving the sensory acceptability of olives. Moreover, β-glucosidase is involved in the breakdown of cellobiose and short-chain oligosaccharides, linking phenolic metabolism with carbohydrate utilization [24,42]. The restricted distribution of this activity suggests a species-dependent metabolic specialization that may be exploited for targeted starter culture selection.

Pectinolytic activity, although not widespread, was detected in selected isolates, including *A. pullulans*, *C. carnescens*, and *S. odoratus*. This result contrasts with studies reporting the absence or low expression of pectinases in olive-associated yeasts [34] but aligns with findings that certain species may retain the ability to degrade pectic substances [43]. This variability suggests that pectinolytic potential is highly strain-dependent and may represent a critical factor in determining whether a yeast contributes positively (e.g., facilitating metabolite diffusion) or negatively (e.g., promoting tissue softening) to the fermentation process.

The detection of lipolytic activity is generally considered beneficial in olive fermentations due to their role in releasing free fatty acids, such as propanol and 2-butanol, which serve as precursors for volatile aroma compounds [24,34,43]. Therefore, the presence of lipase capacity in selected isolates supports their potential contribution to flavor development.

Proteolytic activity was limited, being observed only in *A. pullulans* in milk medium, while no gelatin degradation was detected. This restricted protease expression may be advantageous, as excessive proteolysis has been associated with texture degradation and reduced product quality [24]. Together with the low polysaccharolytic activity, this finding

suggests that the majority of the isolates are better suited for preserving the structural integrity of olives during fermentation.

The ability to degrade phenolic compounds, including oleuropein and tannins, was more widely distributed among the isolates. This trait is particularly relevant, as oleuropein is responsible for the characteristic bitterness of olives and may inhibit the development of lactic acid bacteria [34,40]. Thus, yeast-mediated phenolic degradation not only improves sensory properties but may also facilitate LAB growth, contributing to the overall progression and stability of the fermentation process.

Various studies have demonstrated that wild yeast populations significantly influence phenolic composition and volatilome development, directly impacting sensory quality and microbial stability [18,29]. In this context, the enzymatic activities detected here, particularly those related to phenolic transformation and lipid metabolism, support the contribution of yeasts to both biochemical modification of the substrate and ecological balance during fermentation.

Importantly, the enzymatic variability observed among strains within a species highlights the need for strain-level selection rather than species-level generalization. This agrees with previous studies showing significant intra-species diversity in enzymatic expression among yeasts from fermented products [25]. Such variability underscores the importance of screening individual isolates when developing functional starter cultures.

Fungal activity during table olive processing can have both positive and negative impacts. Benefits include the production of volatile compounds, sensory-enhancing metabolites, antioxidants, vitamins, amino acids, purines, support for LAB growth, antibacterial activity, and phenolic biodegradation. Drawbacks include CO_2 gas formation, which can lead to packaging rupture, tissue softening, brine turbidity, and the production of off-flavors [44]. Thus, β-glucosidase, phenolase, esterase, and lipase activities are considered desirable for olive fermentation due to their roles in flavor development and debittering. In contrast, undesirable enzymatic activities include those targeting structural polysaccharides (cellulase, pectinase, xylanase) and proteins, as they can compromise fruit texture. Amylase activity is not considered relevant to this type of fermentation [42]. Overall, the enzymatic profile identified in this study reflects a balance between desirable and undesirable activities. This balance is critical for successful olive fermentation, particularly in natural processes that rely on endogenous microbiota. Nevertheless, all enzymatic properties observed here have strong biotechnological potential.

Finally, when combined with the antibacterial properties previously discussed, the enzymatic traits of isolates such as *A. pullulans* and *C. carnescens* highlight their potential as multifunctional starter cultures. These yeasts can simultaneously contribute to microbial control, phenolic transformation, and aroma development, reinforcing their relevance for biotechnological applications in fermented foods [14,15,45].

5. Conclusions

This study highlighted the distinct functional potential of fungal isolates from traditional fermentations of table olives and arbutus berries. While yeasts from arbutus berry fermentation did not exhibit significant antibacterial or enzymatic activity, those isolated from olive fermentation demonstrated relevant biofunctional properties. Notably, strains of *Aureobasidium pullulans*, *Cryptococcus* spp., and *Candida membranifaciens* showed inhibitory effects against Gram-positive pathogens, along with enzymatic profiles favorable for fermentation processes and food preservation.

The multifunctionality of these yeasts, particularly the combination of antibacterial and enzymatic activities, underscores their biotechnological potential for biopreservation and the enhancement of fermented food quality. However, the presence of enzymes that

may compromise product integrity, such as cellulases and proteases, emphasizes the need for careful evaluation before practical application.

Future studies should validate the performance of the most promising multifunctional isolates, particularly *Aureobasidium pullulans*, in model olive fermentation systems to confirm their technological behavior under real fermentation conditions. Such validation will be essential to assess strain competitiveness, persistence, metabolic activity, and overall impact on fermentation dynamics and final product quality.

Overall, this study contributes to understanding fungal functional diversity in natural fermentations, reinforcing the importance of selecting specific strains to optimize industrial processes and promote natural alternatives for food preservation that align with current safety and quality demands.

Author Contributions: Conceptualization, C.Q.; methodology, C.Q. and D.W.B.; validation, C.Q. and D.W.B.; formal analysis, D.W.B.; investigation, D.W.B.; resources, C.Q. and E.S.K.; data curation, C.Q. and D.W.B.; writing—original draft preparation, D.W.B.; writing—review and editing, C.Q. and E.S.K.; visualization, D.W.B.; supervision, C.Q. and E.S.K.; project administration, C.Q. and E.S.K.; funding acquisition, E.S.K., D.W.B. and C.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the AUSPIN (USP Innovation Agency) International Exchange Scholarship-Entrepreneurship Scholarship, a support program designed for USP undergraduate students who wish to develop entrepreneurship and innovation projects abroad (D.W.B.). This research was funded by the R&D unit MED—Mediterranean Institute for Agriculture, Environment and Development (https://doi.org/10.54499/UID/05183/2025) and the Associate Laboratory CHANGE—Global Change and Sustainability Institute (https://doi.org/10.54499/LA/P/0121/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors would like to thank Neusa Maria da Silva Ferro Rodrigues and Sílvia Alexandra Madeira for their valuable technical assistance and support in the laboratory.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Garín-Murguialday, N.; Virto, R.; Pagán, R.; Espina, L. Bioprotective cultures as a clean label strategy for food preservation: From concept to market. *Food Res. Int.* **2026**, *230*, 118605. [CrossRef]
2. Glicerina, V.; Siroli, L.; Canali, G.; Chinnici, F.; Capelli, F.; Lanciotti, R.; Colombo, V.; Romani, S. Efficacy of biodegradable, antimicrobial packaging on safety and quality parameters maintenance of a pear juice and rice milk-based smoothie product. *Food Control* **2021**, *128*, 108170. [CrossRef]
3. Chen, W.; Ma, H.; Gariepy, H.; Raghavan, V.; Shen, C. From synergy to superiority: Harnessing ultrasound and cold plasma for next-generation food safety and quality enhancement. *Trends Food Sci. Technol.* **2026**, *172*, 105664. [CrossRef]
4. Ma, Y.; Huang, S.; Yang, G.; Peng, S.; Qi, R.; Dong, B.; Qiao, S.; Zeng, X.; Yu, H. Biosynthesis of a novel antimicrobial brevicidine as potential food biopreservative from *Brevibacillus laterosporus* 811 and its anti-infection effect against *Escherichia coli* and *Salmonella*. *Food Biosci.* **2025**, *69*, 106937. [CrossRef]
5. Malakar, P.K.; Liu, J.; Wu, Q.; Zhang, Z.; Zhao, Y. Response of Foodborne Pathogens to Osmotic Stress. In *Stress Responses of Foodborne Pathogens*; Ding, T., Liao, X., Feng, J., Eds.; Springer: Cham, Switzerland, 2022; pp. 125–140. [CrossRef]
6. Sawant, A.D.; Abdelal, A.T.; Ahearn, D.G. Anti-*Candida albicans* activity of *Pichia anomala* as determined by a growth rate reduction assay. *Appl. Environ. Microbiol.* **1988**, *54*, 1099–1103. [CrossRef] [PubMed]
7. Reis, P.J.M.; Tavares, T.G.; Rocha, J.M.; Malcata, F.X.; Macedo, A.C. Cobrançosa Table Olives: Characterization of Processing Method and Lactic Acid Bacteria Profile throughout Spontaneous Fermentation. *Appl. Sci.* **2022**, *12*, 9738. [CrossRef]
8. Waema, S.; Maneesri, J.; Masniyom, P. Isolation and identification of killer yeast from fermented vegetables. *Asian J. Food Agro-Ind.* **2009**, *2*, 126–134.

9. Villalba, M.L.; Mazzucco, M.B.; Lopes, C.A.; Ganga, M.A.; Sangorrín, M.P. Purification and characterization of *Saccharomyces eubayanus* killer toxin: Biocontrol effectiveness against wine spoilage yeasts. *Int. J. Food Microbiol.* **2020**, *331*, 108714. [CrossRef] [PubMed]
10. Younis, G.; Awad, A.; Dawod, R.E.; Yousef, N.E. Antimicrobial activity of yeasts against some pathogenic bacteria. *Vet. World* **2017**, *10*, 979–983. [CrossRef]
11. Goerges, S.; Aigner, U.; Silakowski, B.; Scherer, S. Inhibition of *Listeria monocytogenes* by food-borne yeasts. *Appl. Environ. Microbiol.* **2006**, *72*, 313–318. [CrossRef]
12. Gil-Rodríguez, A.M.; Garcia-Gutierrez, E. Antimicrobial mechanisms and applications of yeasts. *Adv. Appl. Microbiol.* **2021**, *114*, 37–72. [CrossRef] [PubMed]
13. Merchán, A.V.; Benito, M.J.; Galván, A.I.; Herrera, S.R.M.S. Identification and selection of yeast with functional properties for future application in soft paste cheese. *LWT* **2020**, *124*, 109173. [CrossRef]
14. Campus, M.; Değirmencioğlu, N.; Comunian, R. Technologies and trends to improve table olive quality and safety. *Front. Microbiol.* **2018**, *9*, 617. [CrossRef] [PubMed]
15. Mateus, T.; Santo, D.; Saúde, C.; Pires-Cabral, P.; Quintas, C. The effect of NaCl reduction in the microbiological quality of cracked green table olives of the Maçanilha Algarvia cultivar. *Int. J. Food Microbiol.* **2016**, *218*, 57–65. [CrossRef]
16. Caridi, A.; Panebianco, F.; Scibetta, S.; Schena, L. Selection of yeasts for their anti-mold activity and prospective use in table olive fermentation. *J. Food Process. Preserv.* **2019**, *43*, e14259. [CrossRef]
17. Lucena-Padrós, H.; Caballero-Guerrero, B.; Maldonado-Barragán, A.; Ruiz-Barba, J.L. Microbial diversity and dynamics of Spanish-style green table-olive fermentations in large manufacturing companies through culture-dependent techniques. *Food Microbiol.* **2014**, *42*, 154–165. [CrossRef]
18. Ruiz-Barba, J.L.; Cortés-Delgado, A.; Sánchez, A.H.; López-López, A.; Montaño, A. Impact of selected wild yeast starters on the volatilome and phenolic contents of Gordal, Manzanilla and Hojiblanca naturally fermented green olives. *LWT* **2024**, *195*, 115811. [CrossRef]
19. López-García, E.; Benítez-Cabello, A.; Martín-Arranz, V.; Garrido-Fernández, A.; Jiménez-Díaz, R.; Arroyo-López, F.N. Optimisation of working parameters for lactic acid bacteria and yeast recovery from table olive biofilms, preserving fruit integrity and reducing chloroplast recovery. *LWT* **2022**, *166*, 113787. [CrossRef]
20. Fazio, N.A.; Pino, A.; Foti, P.; Esteve-Zarzoso, B.; Randazzo, C.L.; Torija, M.J.; Caggia, C. Screening and characterization of indigenous *Saccharomyces cerevisiae* and non-*Saccharomyces* yeasts isolated from Sicilian vineyards. *Food Biosci.* **2024**, *62*, 105282. [CrossRef]
21. Santo, D.M.C.E.; Galego, L.; Gonçalves, T.; Quintas, C. Yeast diversity in the Mediterranean strawberry tree (*Arbutus unedo* L.) fruits' fermentations. *Food Res. Int.* **2012**, *47*, 45–50. [CrossRef]
22. Rodrigues, N.M.S.F. *Estudo da Evolução da Microbiota em Fermentações Naturais de Azeitona Verde do Algarve*; Universidade do Algarve: Faro, Portugal, 2011.
23. Buzzini, P.; Martini, A. Extracellular enzymatic activity profiles in yeast and yeast-like strains isolated from tropical environments. *J. Appl. Microbiol.* **2002**, *93*, 1020–1025. [CrossRef]
24. Bautista-Gallego, J.; Rodríguez-Gómez, F.; Barrio, E.; Querol, A.; Garrido-Fernández, A.; Arroyo-López, F.N. Exploring the yeast biodiversity of green table olive industrial fermentations for technological applications. *Int. J. Food Microbiol.* **2011**, *147*, 89–96. [CrossRef] [PubMed]
25. Strauss, M.L.; Jolly, N.P.; Lambrechts, M.G.; van Rensburg, P. Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. *J. Appl. Microbiol.* **2001**, *91*, 182–190. [CrossRef]
26. Paranthaman, R.; Vidyalakshmi, R.; Murugesh, S.; Singaravadivel, K. Optimization of Various Culture Media for Tannase Production in Submerged Fermentation by Aspergillus flavus. *Adv. Biol. Res.* **2009**, *3*, 34–39.
27. Marquina, D.; Peres, C.; Caldas, F.V.; Marques, J.F.; Peinado, J.M.; Spencer-Martins, I. Characterization of the yeast population in olive brines. *Lett. Appl. Microbiol.* **1992**, *14*, 279–283. [CrossRef]
28. Vermelho, A.B.; Meirelles, M.N.L.; Lopes, A.; Petinate, S.D.G.; Chaia, A.A.; Branquinha, M.H. Detection of extracellular proteases from microorganisms on agar plates. *Mem. Inst. Oswaldo Cruz* **1996**, *91*, 755–760. [CrossRef]
29. Ruiz-Barba, J.L.; Sánchez, A.H.; López-López, A.; Cortés-Delgado, A.; Montaño, A. Microbial community and volatilome changes in brines along the spontaneous fermentation of Spanish-style and natural-style green table olives (Manzanilla cultivar). *Food Microbiol.* **2023**, *113*, 104286. [CrossRef] [PubMed]
30. Bilinski, C.A.; Innamorato, G.; Stewart, G.G. Identification and characterization of antimicrobial activity in two yeast genera. *Appl. Environ. Microbiol.* **1985**, *50*, 1330–1332. [CrossRef]
31. Filip, S.; Đurović, S.D.; Blagojević, S.; Delić, O.; Skhvediani, A.E.; Vasin, A.V.; Micić, D.M. Investigation of technological, chemical, biological aspects, and in-silico pharmacokinetic and docking predictions of winter savory (*Satureja montana* L.) essential oil. *Process Biochem.* **2026**, *161*, 188–203. [CrossRef]

32. Bonatsou, S.; Iliopoulos, V.; Mallouchos, A.; Gogou, E.; Oikonomopoulou, V.; Krokida, M.; Taoukis, P.; Panagou, E.Z. Effect of osmotic dehydration of olives as pre-fermentation treatment and partial substitution of sodium chloride by monosodium glutamate in the fermentation profile of Kalamata natural black olives. *Food Microbiol.* **2017**, *63*, 72–83. [CrossRef] [PubMed]
33. Medina-Pradas, E.; Arroyo-López, F.N. Presence of toxic microbial metabolites in table olives. *Front. Microbiol.* **2015**, *6*, 873. [CrossRef] [PubMed]
34. Silva, T.; Reto, M.; Sol, M.; Peito, A.; Peres, C.M.; Peres, C.; Xavier Malcata, F. Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior. *Food Sci. Technol.* **2011**, *44*, 1349–1354. [CrossRef]
35. Llorente, P.; Domingo, M.; Santos, A.; Peinado, J.; Spencer-Martins, I. Effect of salt on the killer phenotype of yeasts from olive brines. *Appl. Environ. Microbiol.* **1997**, *63*, 1165–1167. [CrossRef]
36. Hernández, A.; Martín, A.; Córdoba, M.G.; Benito, M.J.; Aranda, E.; Pérez-Nevado, F. Determination of killer activity in yeasts isolated from the elaboration of seasoned green table olives. *Int. J. Food Microbiol.* **2008**, *121*, 178–188. [CrossRef] [PubMed]
37. Yoon, J.H.; Han, A.; Lee, S.Y. Salt can antagonize the lethal effect of weak organic acids against *Escherichia coli* O157:H7 inoculated in laboratory culture media and acidic/acidified foods. *Food Res. Int.* **2025**, *212*, 116387. [CrossRef]
38. Musmanno, R.A.; Di Maggio, T.; Coratza, G. Studies on strong and weak killer phenotypes of wine yeasts: Production, activity of toxin in must, and its effect in mixed culture fermentation. *J. Appl. Microbiol.* **1999**, *87*, 932–938. [CrossRef]
39. Ullivarri, M.F.; Mendoza, L.M.; Raya, R.R.; Farías, M.E. Killer phenotype of indigenous yeasts isolated from Argentinian wine cellars and their potential starter cultures for winemaking. *Biotechnol. Lett.* **2011**, *33*, 2177–2183. [CrossRef]
40. Saúde, C.; Barros, T.; Mateus, T.; Quintas, C.; Pires-Cabral, P. Effect of chloride salts on the sensory and nutritional properties of cracked table olives of the Maçanilha Algarvia cultivar. *Food Biosci.* **2017**, *19*, 73–79. [CrossRef]
41. Fundador, N.G.V.; Enomoto-Rogers, Y.; Takemura, A.; Iwata, T. Synthesis and characterization of xylan esters. *Polymer* **2012**, *53*, 3885–3893. [CrossRef]
42. Tofalo, R.; Perpetuini, G.; Schirone, M.; Suzzi, G.; Corsetti, A. Yeast biota associated to naturally fermented table olives from different Italian cultivars. *Int. J. Food Microbiol.* **2013**, *161*, 203–208. [CrossRef]
43. Hernández, A.; Martín, A.; Aranda, E.; Pérez-Nevado, F.; Córdoba, M.G. Identification and characterization of yeast isolated from the elaboration of seasoned green table olives. *Food Microbiol.* **2007**, *24*, 346–351. [CrossRef] [PubMed]
44. Alves, M.; Esteves, E.; Quintas, C. Effect of preservatives and acidifying agents on the shelf life of packed cracked green table olives from Maçanilha cultivar. *Food Packag. Shelf Life* **2015**, *5*, 32–40. [CrossRef]
45. Guantario, B.; Zinno, P.; Schifano, E.; Roselli, M.; Perozzi, G.; Palleschi, C.; Uccelletti, D.; Devirgiliis, C. In vitro and in vivo selection of potentially probiotic Lactobacilli from Nocellara del Belice table olives. *Front. Microbiol.* **2018**, *9*, 595. [CrossRef] [PubMed]