applied sciences

MDPI

Article

Interactions among Relevant Non-*Saccharomyces*, *Saccharomyces*, and Lactic Acid Bacteria Species of the Wine Microbial Consortium: Towards Advances in Antagonistic Phenomena and Biocontrol Potential

Marianna De Gioia [1], Pasquale Russo [2], Nicola De Simone [1], Francesco Grieco [3,*], Giuseppe Spano [1], Vittorio Capozzi [4] and Mariagiovanna Fragasso [1,*]

[1] Department of Agriculture Food Natural Science Engineering, University of Foggia, Via Napoli 25, 71122 Foggia, Italy
[2] Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Luigi Mangiagalli 25, 20133 Milano, Italy
[3] Institute of Sciences of Food Production, National Research Council, Via Prov.le, Lecce-Monteroni, 73100 Lecce, Italy
[4] Institute of Sciences of Food Production, National Research Council (CNR), c/o CS-DAT, Via Michele Protano, 71121 Foggia, Italy
* Correspondence: francesco.grieco@ispa.cnr.it (F.G.); mariagiovanna.fragasso@gmail.com (M.F.)

check for updates

Citation: De Gioia, M.; Russo, P.; De Simone, N.; Grieco, F.; Spano, G.; Capozzi, V.; Fragasso, M. Interactions among Relevant Non-*Saccharomyces*, *Saccharomyces*, and Lactic Acid Bacteria Species of the Wine Microbial Consortium: Towards Advances in Antagonistic Phenomena and Biocontrol Potential. *Appl. Sci.* **2022**, *12*, 12760. https://doi.org/10.3390/app122412760

Academic Editors: Lorena Butinar, Guillaume Antalick, Melita Sternad Lemut and Christian Philipp

Received: 16 November 2022
Accepted: 9 December 2022
Published: 12 December 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: The topic of microbial interactions is of notable relevance in oenology, being connected with their impact on microbial biodiversity and wine quality. The interactions among different couples of microorganisms, in particular yeasts and lactic acid bacteria representative of the must/wine microbial consortium, have been tested in this study. This interaction's screening has been implemented by means of plate assays, using culture medium, grape juice, and wine agar as substrates. Different antagonistic phenomena have been detected, belonging to the following interaction categories: yeast-yeast, yeast-bacteria, bacteria-yeast, and bacteria-bacteria. In general, the inhibitory activity has been observed in all three media agar used as substrates, resulting in more frequent on culture medium, followed by grape juice and, finally, wine. Specifically, the work is one of the first reports demonstrating the reciprocal interactions between non-*Saccharomyces* yeasts (NSY) and malolactic bacteria. The findings shed new light on the co-inoculation of the yeast starter culture with malolactic bacteria, as well as the biocontrol potential of Lactic Acid Bacteria (LAB) strains. Highlighted microbial interactions are relevant for the management of alcoholic fermentation, malolactic fermentation, and the development of distinctive aroma profiles, control of spoilage yeasts, and the selection of tailored mixed starter cultures. In addition, the plate assay method could be a fast, cheap, and suitable method to exclude negative interactions among *Saccharomyces* spp., NSY, and malolactic bacteria during trials from regional spontaneous fermentations with the aim to select tailored mixed starter cultures.

Keywords: grape must; wine; yeast; *Saccharomyces cerevisiae*; non-*Saccharomyces*; malolactic bacteria; lactic acid bacteria; inhibitory activity

1. Introduction

As reviewed by Liu et al. [1] the Wine Microbial Consortium (WMC) is mainly composed of different microorganisms belonging to *Saccharomyces* spp., Non-*Saccharomyces* Yeasts (NSY), Lactic Acid Bacteria (LAB) Species, Acetic Acid Bacteria (AAB) species, *Bacillus* spp., and filamentous fungi, all microbes with diverse origins, occurrences, and paths of diffusion from the field to the winery [2]. In particular, *Saccharomyces* spp., NSY, and LAB are considered pro-technological microbes in oenology and play a pivotal role in the production of high-quality wines [3]. To a different extent, microbial diversity within these

three classes contributes to shaping sensory characteristics and the safety of wine [4,5]. In fact, *Saccharomyces* spp., NSY, and LAB are present in spontaneous wine microbial consortia and used as starter cultures in oenology [6,7]. *Saccharomyces* spp. play the key role in Alcoholic Fermentation (AF), determining wine production and shaping wine quality crucially [8,9]. The positive effect of non-*Saccharomyces* yeasts can be dual. On the one side, NSY can be relevant for specific pro-technological applications [10–14]. On the other, they produce a variety of volatile compounds and extracellular enzymes with an important impact on the sensorial profile of wine [15,16]. These yeasts prevail at the beginning of the vinification process and are quickly replaced by *S. cerevisiae*, which completes AF, in reason of the different tolerance to peculiar wine stressors, such as ethanol and SO_2. Concerning the role of LAB in the microbial wine-associated ecosystem, specific malolactic bacteria concerns modulate both microbial stability and sensorial quality of wine [17,18]. They consume residual nutrients and synthesize inhibitory compounds, contrasting the growth of undesired microbes [19]. LAB can produce volatile secondary metabolites with a positive impact on wine's chemical composition and aroma [4,20].

Irrelevant, positive, and negative interactions can influence the growth and/or the metabolic activity of the species composing the WMC, affecting the evolution of microbial resources during fermentation and wine quality [1,21]. The WMC interactions include direct (cell-cell contact, quorum sensing, predation, parasitism, symbiosis, and inhibition) and indirect (neutralism, mutualism, commensalism, amensalism, and competition) interactions [22]. Several studies delve into the principal interactions among the main categories belonging to the wine microbial consortium: yeast-yeast (*Saccharomyces* spp.-*Saccharomyces* spp. [22,23]; *Saccharomyces* spp.-NSY [24,25]; NSY-NSY [26,27]), yeast-bacteria (NSY-LAB [28–30]; *Saccharomyces* spp.-LAB [31]), bacteria-yeast (LAB-NSY [32,33]; LAB-*Saccharomyces* spp. [34]), bacteria-bacteria (LAB-LAB [35]).

The heterogeneity, oenological significance, and the temporary succession of the different microbial categories contribute to must/wine microbiota, making this system an interesting model to study microbial interactions. In the last years, different methods and substrates have been used to test the relationships among microorganisms of enological interest. Generally, their evaluation is performed on plates [36–38], through co-/sequential inoculation in culture medium [39,40], synthetic [41], and commercial [36,42,43] grape juice/must or wine. In this context, the present work aims to verify, using an integrated plate assays methodological approach, the occurrence and the extent of interactions among all the possible combinations of non-*Saccharomyces*, *Saccharomyces*, and lactic acid bacteria isolated from spontaneous fermentations, used as oenological starter cultures, or from public collections. Interactions among yeasts and bacteria affect the wine quality, but only limited information on these phenomena is reported in the scientific literature [44]. Here, plate assays have been selected as a low-cost and fast method to evaluate the interactions for a large number of strains, with the novelty of introducing the use of plates made by including must and wine in order to achieve, despite the limitations of plate screening, a progressive approach with respect to oenological conditions.

2. Materials and Methods

2.1. Microorganisms

Forty-five microbial strains belonging to 15 different species of enological interest have been used in this work (Table 1).

In particular, bacterial species included three strains of *Oenococcus oeni*, 5 *Lactiplantibacillus plantarum* strains, one *Pediococcus* spp., and one strain for each of the following species: *Levilactobacillus brevis*, *Pediococcus parvulus*, *Lentilactobacillus hilgardii*. The yeast species included *Saccharomyces cerevisiae* (7 strains), *Torulaspora delbrueckii* (3 strains), *Hanseniaspora uvarum* (2 strains), *Metschnikowia pulcherrima* (10 strains), *Pichia fermentans* (3 strains), *Brettanomyces bruxellensis* (5 strains), *Hanseniaspora guillermondii* (1 strain), *Issatchenkia terricola* (1 strain), and *Starmerella bacillaris* (1 strain). The microorganisms submitted to this analysis derived from public collections, isolated from wine commercial

starter cultures, or they have been isolated from spontaneous oenological fermentations (e.g., [42,45–51]) (Table 1). All the microbial strains have been stored at −80 °C in MRS or YPD medium and added with 30% of glycerol for bacteria and yeasts, respectively.

Table 1. List of microorganisms of enological interest used in this work.

Species	Strain Code	Matrix/Source
Saccharomyces cerevisiae	superlievito alcoligens	CSC
S. cerevisiae	elegance	CSC
S. cerevisiae ex bayanus	EC1118	CSC
S. cerevisiae	*E4*	OSF
S. cerevisiae	*I6*	OSF
S. cerevisiae	SUPRARED HG	CSC
S. cerevisiae	T2	CSC
Hanseniaspora uvarum	1444	CECT
H. uvarum	B05B29	OSF
Hanseniaspora guilliermondii	M105A31	OSF
Torulaspora delbrueckii	11199	CECT
T. delbrueckii	B05B12	OSF
T. delbrueckii	291	CSC
Pichia fermentans	M105A3	OSF
P. fermentans	B05A36	OSF
P. fermentans	B05A29	OSF
Issatchenkia terricola	B05B8	OSF
Starmerella bacillaris	B05B6	OSF
Metschnikowia pulcherrima	B0512B3	OSF
M. pulcherrima	B0512B24	OSF
M. pulcherrima	B0512B25	OSF
M. pulcherrima	B0512B26	OSF
M. pulcherrima	B0512B15	OSF
M. pulcherrima	B05B2P	OSF
M. pulcherrima	B05A36	OSF
M. pulcherrima	M105A51	OSF
M. pulcherrima	B0522	OSF
M. pulcherrima	346	CSC
Brettanomyces bruxellensis	2	OSF
B. bruxellensis	4	OSF
B. bruxellensis	5	OSF
B. bruxellensis	6	OSF
B. bruxellensis	7	OSF
Lactiplantibacillus plantarum	Lp90	OSF
L. plantarum	44	OSF
L. plantarum	V22	CSC
L. plantarum	38 CDS	OSF
L. plantarum	T1	OSF
Levilactobacillus brevis	9809	IOEB
Lentilactobacillus hilgardii	4786	CECT
Pediococcus parvulus	126	OSF
Pediococcus spp.	32	OSF
Oenococcus oeni	OT3	OSF
O. oeni	6	OSF
O. oeni	OT4	OSF

OSF, oenological spontaneous fermentation, UNIFG collection; CSC, commercial starter culture; CECT, Colección Española de Cultivos Tipo; IOEB, Bacteria collection of the "Faculté d'Oenologie de Bordeaux".

2.2. Culture Medium and Growth Conditions

Yeast cultures were grown in YPD broth: 10 g/L of yeast extract (Oxoid, Basingstoke, UK), 20 g/L of bacteriological peptone (Oxoid), and 20 g/L of dextrose (Oxoid). The growth of non-*Saccharomyces* yeasts was also evaluated in WL nutrient broth (Wallerstein Laboratory, Oxoid) [52]. Malolactic bacteria were grown in MRS broth (Oxoid), or MRS supplemented with 10 g/L of L-malic acid (Sigma Aldrich, St Louis, MO, USA) and adjusted to pH 5.5, with 1 M NaOH (Sigma Aldrich), for *O. oeni* strains. The growth of all microorganisms was carried out at 30 °C for 48 h, apart from *O. oeni* strains that were incubated in a jar (anaerobic conditions) for a week.

Considering the media for the plate screening, for yeast-yeast interactions YPD medium has been used for both layers; for bacteria-bacteria interactions, MRS medium has been employed for both layers; while, for bacteria-yeast and yeast-bacteria interactions, MRS and YPD media have been used for the bottom and/or top layer, respectively. The concentration of microbial suspensions was assessed by spectrophotometric (turbidimetric) analysis.

2.3. Interaction Plate Screening: Double-Layer Agar Diffusion Assay

The interactions between couples of microorganisms have been evaluated using (i) YPD and MRS culture medium, (ii) commercial red grape juice (VitaFit; Emig GmbH, Rellingen, Germany), and (iii) commercial red and white wine (alcohol concentration 10.5% (*w/v*); pH 3.5) as substrate.

The first methodological approach to test microbial interactions has been a double-layer agar diffusion assay, proposed by Comitini et al. [53]. A layer of culture medium agar (10 mL) was poured into Petri dishes. After solidification, 5 µL of six different strains overnight cultures were seeded to form rings of inoculum.

Plates have been incubated at room temperature (25–30 °C) for 24 h or, in the case of *O. oeni* strains, in the jar for a week. After that time, a top layer of culture medium soft agar (10 mL; 1% of agar), containing $6 \cdot \times 10^5$ CFU/mL of another strain, was poured onto the bottom layer and, finally, the Petri dishes incubated for about 72 h at room temperature.

2.4. Interaction Plate Screening: Agar Diffusion Assay

To simulate the real situation, the evolution of must/wine microbial consortium and the interactions between microorganisms have been tested in grape juice and wine. This attempt has been implemented through agar diffusion assay. This method has been suggested by Mehlomakulu et al. [38] and, unlike the above-described approach, was performed on a single layer. Grape juice has been added with 1% of yeast extract only to test the interactions involving bacteria (to "simulate" the conditions occurring at the end of AF) and adjusted to pH 4.5 with 1 M NaOH; this medium has been heated up to reach 55 °C and kept at this temperature. 1:100 *v/v* of each strain overnight culture have been inoculated into 7.5 mL of the modified grape juice. Hence, 2.5 mL of 4% agar (kept at 55 °C) was mixed with the inoculated medium and poured into sterile Petri dishes. Finally, 5–10 µL of different strains overnight cultures were spotted on the surface of the solidified agar plates (each strain must have the possibility to grow in correspondence with its own spot). The plates were incubated at room temperature until a well-developed lawn of the strain inoculated into grape juice was observed (a week in the case of *O. oeni* strains). Grape juice was selected as a commercial matrix capable of mimicking the physicochemical conditions of grape must.

As mentioned above, agar diffusion assay has also been performed using wine as substrate, added with 1% yeast extract for all the interactions to test. In this case, the different bacterial strains were pre-stressed in wine and incubated at 30 °C for a week in order to adapt them to the medium promoting their growth: 8 mL of wine (without the addition of yeast extract and pH change) were added to 4 mL of the overnight culture. Furthermore, interactions between all microbial couples were assessed in the red wine while those detected with the first and this method (i.e., in red wine) have also been carried

out in white wine in order to evaluate the maintenance of the phenotype as the oenological context varies.

2.5. Interaction Plate Screening: Results Interpretation

It is important to point out that, for both the methodological approaches used in this work, strains seeded through the spots are the ones that eventually interact with the inoculated strain. The presence of interaction has been verified by observing the growth around the spots, which could determine two situations. The lack of growth was displayed by a clear area surrounding the spot, the so-called halo of inhibition, with a diameter proportional to the extent of inhibition itself. On the contrary, a major growth around the spot identifies a positive interaction between the two strains under examination, indicating that the development of the spotted one stimulates the development of the inoculated strain. Otherwise, none of the previous situations occurred if the microbial couple tested does not interact.

3. Results and Discussion

Four categories of microbial interactions, functional to the discussion, can be identified among those tested in this work: yeast-yeast, yeast-bacteria, bacteria-yeast, and bacteria-bacteria. As a whole, negative and neutral interactions have been detected, while no examples of positive interactions have been reported. This is in contrast to the literature in the field, which identifies different behaviors attributable to mutualism/synergism and commensalism reported in matrices of oenological interest [1,54] (e.g., between *S. cerevisiae* and *L. plantarum* in grape juices [55], *Kloeckera apiculata*/*S. cerevisiae* in grape juice integrated with yeast extract [56]). The findings highlight that plate screening did not seem adequate to detect positive interactions between microorganisms in the wine sector.

Inhibitions representative of all the possible typologies of antagonisms have been observed, including all tested microbial categories (i.e., *Saccharomyces*/non-*Saccharomyces* yeasts and lactic acid bacteria). According to the extent of their antagonistic behaviors, the different microorganisms used in this study have been classified as strains of mild (±), middle (+), or strong (++) inhibitory activity showing halos of inhibition, surrounding the respective spots, with a diameter lower than 3 mm, ranging from 3–6 mm or more than 6 mm, respectively. Evidence has been detected in all three media agar used as substrates, resulting in a more frequent on culture medium, followed by grape juice and, finally, wine. In particular, referring to the exerted (Figure 1a) and suffered (Figure 1b) inhibitions, non-*Saccharomyces* yeasts and LAB showed activity on culture medium and grape juice while *S. cerevisiae* strains on culture medium and—in only a few cases—wine. These results confirmed, in the oenological field, that in vitro antagonisms do not necessarily correlate with the same behavior in in situ studies [57]. Highlighted trends indicate a selective maintenance of the antagonistic character shifting from tests on a culture medium to evaluation on the edible matrix. Intriguingly, in the case of the yeasts, this selectivity seemed to correspond to the phase of dominance during winemaking: grape juice for non-*Saccharomyces* and wine for *S. cerevisiae* [58,59].

Therefore, selected strains belonging to all the microbial categories tested in this plate screening [non-*Saccharomyces*, *S. cerevisiae*, and Lactic Acid Bacteria (LAB)] have interacted on culture medium agar (examples in Figure S1).

Evidence highlights only a certain connection with the clear succession of microbial dominance (i.e., non-*Saccharomyces* in grape must and in early Alcoholic fermentation (AF), *Saccharomyces* in the middle/late AF, LAB during malolactic fermentation) [30], underlining how the dominance phenomenon is only one of the complex WMC interactions.

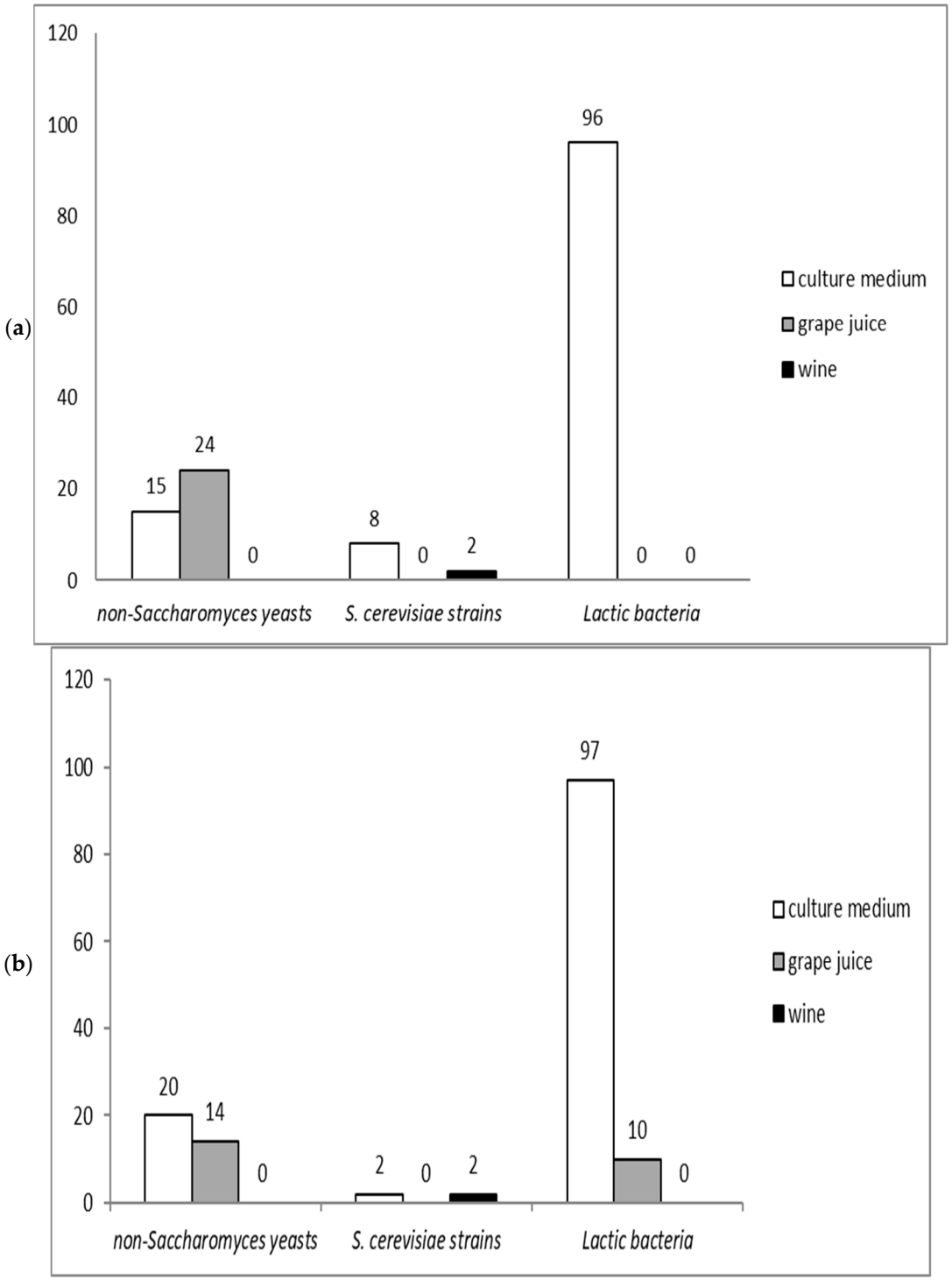

Figure 1. Frequency of inhibitory activity concerning the inhibiting (**a**) and inhibited (**b**) species, according to the different media tested.

3.1. Non-Saccharomyces as Inhibiting Species

Delving into the results found for each of the couple of tested microorganisms, Table 2 shows the inhibitory activity of tested non-*Saccharomyces* strains against all the studied yeast and bacterial strains.

Table 2. Inhibitory activity of non-*Saccharomyces* yeasts according to the diameter of halo of inhibition: "±" = lower than 3 mm, "+" = ranging from 3–6 mm and "++" = more than 6 mm.

Inhibiting Species	Inhibited Species	Inhibitory Activity
M. pulcherrima 346	*S. cerevisiae* I6	± [C]
	L. plantarum V22	± [C]
	L. brevis IOEB	± [C]
	L. plantarum 38 CDS	± [C]
	Pediococcus spp.	± [C]
	O. oeni OT3	± [C]
T. delbrueckii 291	*L. plantarum* Lp90	++ [C]
	L. plantarum 44	++ [C]
	L. brevis IOEB	++ [C]
	P. parvulus 126	++ [C]
	L. hilgardii CECT 4786	++ [C]
	L. plantarum 38 CDS	++ [C]
	Pediococcus spp.	± [C]
P. fermentans M105A30	*Pediococcus* spp.	± [C]
P. fermentans B05A36	*Pediococcus* spp.	± [C]
H. guilliermondii M105A31	*P. fermentans* M105A30	± [J]
H. uvarum B05B29	*P. fermentans* M105A30	± [J]
P. fermentans B05A29	*T. delbrueckii* 291	± [J]
	T. delbrueckii CECT 11199	± [J]
	B. bruxellensis 2	± [J]
	B. bruxellensis 6	± [J]
M. pulcherrima B0512B3	*P. fermentans* M105A30	± [J]
M. pulcherrima B0512B24	*P. fermentans* M105A30	± [J]
M. pulcherrima B0512B25	*P. fermentans* M105A30	± [J]
M. pulcherrima B0512B26	*P. fermentans* M105A30	± [J]
M. pulcherrima B0512B15	*P. fermentans* M105A30	± [J]
M. pulcherrima B05B2P	*P. fermentans* M105A30	± [J]
M. pulcherrima B05A36	*P. fermentans* M105A30	± [J]
M. pulcherrima B0522	*P. fermentans* M105A30	± [J]
B. bruxellensis 2	*Pediococcus* spp.	++ [J]
	O. oeni 6	++ [J]
B. bruxellensis 4	*Pediococcus* spp.	± [J]
	O. oeni 6	+ [J]
B. bruxellensis 5	*Pediococcus* spp.	± [J]
	O. oeni 6	+ [J]
B. bruxellensis 6	*Pediococcus* spp.	± [J]
	O. oeni 6	+ [J]
B. bruxellensis 7	*Pediococcus* spp.	± [J]
	O. oeni 6	+ [J]

The superscripts indicate the medium onto which the results have been observed: "[C]" = culture medium and "[J]" = grape juice.

In literature, several studies deepened the antimicrobial potential of *M. pulcherrima* (e.g., [27,60]). Interestingly, *M. pulcherrima* 346, an isolate from commercial starter culture [10], inhibited, on culture medium, bacterial strains representative of spontaneous malolactic consortia (*L. plantarum*, *L. brevis*, *Pedicoccus* spp., and *O. oeni*) and the autochthonous *S. cerevisiae* strain I6 [42]. This is consistent with what has been found in previous studies that demonstrated the antimicrobial activity of *M. pulcherrima* strains against *S. cerevisiae*, *O. oeni,* and other lactobacilli of wine interest [37,61–63]. Furthermore, these findings could contribute to explaining the modulatory effects on the spontaneous malolactic consortium observed when *M. pulcherrima*-based commercial starter culture was used in winemaking [30]. On the contrary, the other *M. pulcherrima* strains exert their antagonistic influence only toward *P. fermentans* M105A30, in agreement with the evidence observed by Oro et al. [27]. Surprisingly, we found no antimicrobial action on spoilage yeasts *Brettanomyces/Dekkera* [62]. Taken together, these results proved the strain-dependent character of *M. pulcherrima* antimicrobial activity. This is probably connected to the intraspecific diversity in terms of the molecular basis responsible for the antagonistic phenotype [64]. The inhibitory activity of *M. pulcherrima* is addressable to the production of low molecular weight and heat-sensitive metabolites, principally pulcherriminic acid [63]. The acid forms an insoluble red pigment pulcherrimin in the presence of iron (III) ions, with subsequent precipitation. This iron sequestration which depletes the medium of iron, making it unavailable to the other microorganisms' mechanism appeared to be strain-dependent [64]. Additionally, the inhibition of *S. cerevisiae* by *M. pulcherrima* could also come from the competition for nutrients: in the sequential inoculation, the consumption of the nutrients by the non-*Saccharomyces* yeast at the beginning of AF could prevent the following growth of *S. cerevisiae* [62].

In this study, *T. delbrueckii* 291, a commercial strain, showed inhibitory activity toward some bacterial strains on culture medium agar. This might contribute to explaining a negative influence found after inoculation of commercial *T. delbrueckii* on spontaneous malolactic consortium [30] strains, which is perfectly in accordance with the evidence reported by Nardi et al. [65] that successfully tested the combination of *T. delbrueckii* and *O. oeni* strains in red Barbera wine. Together with results reported for *M. pulcherrima* 346, these findings added a piece to the intricate puzzle of the possible interaction among non-*Saccharomyces* and malolactic bacteria in oenology [13,28,65–69].

Concerning the other non-*Saccharomyces* yeasts, some inhibitions on culture medium and grape juice testify to the occurrence of other phenomena of interest. In particular, *P. fermentans* M105A30 was found largely inhibited. *P. fermentans* B05A29 displayed a certain negative activity against *T. delbrueckii* and *B. bruxellensis* (some *Pichia* strains have been found to produce a killer toxin called zymocins [70,71]). Finally, all the strains of *B. bruxellensis* tested in this study inhibited *Pediococcus* spp. and *O. oeni* 6 on grape juice agar.

3.2. Saccharomyces as Inhibiting Species

Table 3 reported the inhibitory spectrum of tested *Saccharomyces* strains with respect to all the studied yeast and bacterial strains.

In this study, the inhibitory activity of *S. cerevisiae* strains was detected on both culture medium and wine agar. With regard to the first substrate, *S. cerevisiae* superlievito alcoligens inhibited all the bacterial strains, with the exception of *O. oeni* strains and *L. plantarum* T1. These findings confirm the potential of selected *S. cerevisiae* strains against spoilage LAB in wine [72]. In particular, it was proven the existence of a *S. cerevisiae* peptidic fraction, with a molecular weight lower than 10 kDa, that inhibits the growth of *L. hilgardii* [63]. This antagonism could find application also in the reduction of biogenic amines produced by specific *L. hilgardii* strains in wine [72–74]. The trends against *L. plantarum* strains, but not counter to *O. oeni* strains, could contribute to explaining the impact of inoculation of the *S. cerevisiae* selected strain on the spontaneous malolactic consortium [30]. This is an aspect of particular interest if we consider the rising attention delved into *L. plantarum* as

a new species of interest in malolactic control [75]. In general, the evidence confirms the continuous interest in the study of yeast-bacteria compatibility in winemaking [28,69,76].

Table 3. Inhibitory activity of *S. cerevisiae* strains according to the diameter of halo of inhibition: "±" = lower than 3 mm, "+" = ranging from 3–6 mm and "++" = more than 6 mm.

Inhibiting Species	Inhibited Species	Inhibitory Activity
S. cerevisiae T2	*S. cerevisiae* superlievito alcoligens	± ^{W}
	S. cerevisiae I6	± ^{C,W}
S. cerevisiae superlievito alcoligens	*L. plantarum* Lp90	++ ^{C}
	L. plantarum 44	++ ^{C}
	L. plantarum V22	++ ^{C}
	L. brevis IOEB	++ ^{C}
	P. parvulus 126	++ ^{C}
	L. hilgardii CECT 4786	++ ^{C}
	L. plantarum 38 CDS	++ ^{C}

The superscripts indicate the medium onto which the results have been observed: "^{C}" = culture medium and "^{W}" = wine.

In this study, it has been detected inhibitory activity of *S. cerevisiae* T2 toward two other strains of *S. cerevisiae*: superlievito alcoligens (on wine agar) and on the autochthonous strain I6 (on culture medium and wine agar). This second inhibition represents the only case in which the same inhibition occurred on more than one substrate by means of interactions plate assay in the present study. In the last years, intra-specific inhibitions between different strains of *S. cerevisiae* have been reported, also with contrasting results in terms of prevalence among wild and commercial *S. cerevisiae* strains [77–79].

3.3. LAB as Inhibiting Species

Interestingly, in this study, the LAB inhibitory activity was detected only on culture medium agar (Table 4).

Table 4. Inhibitory activity of lactic bacteria species according to the diameter of halo of inhibition: "±" = lower than 3 mm, "+" = ranging from 3–6 mm and "++" = more than 6 mm.

Inhibiting Species	Inhibited Species	Inhibitory Activity
L. plantarum Lp90	*L. plantarum* 44	± ^{C}
	L. plantarum V22	± ^{C}
	L. brevis IOEB	± ^{C}
	P. parvulus 126	± ^{C}
	L. hilgardii CECT 4786	± ^{C}
	L. plantarum 38 CDS	+ ^{C}
	Pediococcus spp.	± ^{C}
L. plantarum 44	*L. plantarum* 44	± ^{C}
	L. plantarum V22	± ^{C}
	L. brevis IOEB	± ^{C}
	P. parvulus 126	± ^{C}
	L. hilgardii CECT 4786	± ^{C}
	L. plantarum 38 CDS	+ ^{C}
	Pediococcus spp.	± ^{C}

Table 4. *Cont.*

Inhibiting Species	Inhibited Species	Inhibitory Activity
L. plantarum V22	*L. plantarum* Lp90	+ [C]
	L. plantarum 44	± [C]
	L. plantarum V22	± [C]
	L. brevis IOEB	± [C]
	P. parvulus 126	± [C]
	L. hilgardii CECT 4786	± [C]
	L. plantarum 38 CDS	+ [C]
	Pediococcus spp.	± [C]
L. brevis IOEB	*L. plantarum* Lp90	+ [C]
	L. plantarum 44	± [C]
	L. plantarum V22	± [C]
	L. brevis IOEB	± [C]
	P. parvulus 126	± [C]
	L. hilgardii CECT 4786	± [C]
	L. plantarum 38 CDS	+ [C]
	Pediococcus spp.	± [C]
P. parvulus 126	*L. plantarum* Lp90	+ [C]
	L. plantarum 44	± [C]
	L. plantarum V22	± [C]
	L. brevis IOEB	± [C]
	P. parvulus 126	± [C]
	L. hilgardii CECT 4786	± [C]
	L. plantarum 38 CDS	+ [C]
	Pediococcus spp.	± [C]
L. hilgardii CECT 4786	*L. plantarum* 44	± [C]
	L. plantarum V22	± [C]
	L. brevis IOEB	± [C]
	P. parvulus 126	± [C]
	L. hilgardii CECT 4786	± [C]
	L. plantarum 38 CDS	+ [C]
	Pediococcus spp.	± [C]
L. plantarum 38 CDS	*L. plantarum* 44	± [C]
	L. plantarum V22	± [C]
	L. brevis IOEB	± [C]
	P. parvulus 126	± [C]
	L. hilgardii CECT 4786	± [C]
	L. plantarum 38 CDS	+ [C]
	Pediococcus spp.	± [C]
L. plantarum T1	*L. plantarum* Lp90	++ [C]
	L. plantarum 44	++ [C]
	L. brevis IOEB	++ [C]

Table 4. *Cont.*

Inhibiting Species	Inhibited Species	Inhibitory Activity
	P. parvulus 126	++ ^{C}
	L. plantarum 38 CDS	++ ^{C}
	Pediococcus spp.	+ ^{C}
Pediococcus spp.	*O. oeni* OT3	+ ^{C}
	L. plantarum Lp90	++ ^{C}
	L. plantarum 44	+ ^{C}
	L. plantarum V22	++ ^{C}
	L. brevis IOEB	± ^{C}
	P. parvulus 126	+ ^{C}
	L. hilgardii CECT 4786	± ^{C}
	L. plantarum 38 CDS	++ ^{C}
	M. pulcherrima B0512B3	± ^{C}
	M. pulcherrima B0512B25	± ^{C}
	P. fermentans M105A30	++ ^{C}
	P. fermentans B05A36	++ ^{C}
	T. delbrueckii 291	++ ^{C}
O. oeni OT3	*O. oeni* OT3	+ ^{C}
	L. plantarum Lp90	++ ^{C}
	L. plantarum 44	+ ^{C}
	L. plantarum V22	++ ^{C}
	L. brevis IOEB	± ^{C}
	P. parvulus 126	± ^{C}
	L. hilgardii CECT 4786	± ^{C}
	L. plantarum 38 CDS	++ ^{C}
	M. pulcherrima B0512B3	± ^{C}
	M. pulcherrima B0512B25	± ^{C}
	M. pulcherrima B0512B26	± ^{C}
	M. pulcherrima B0522	± ^{C}
	P. fermentans M105A30	+ ^{C}
	P. fermentans B05A36	++ ^{C}
	T. delbrueckii 291	+ ^{C}
O. oeni 6	*O. oeni* OT3	± ^{C}
O. oeni OT4	*O. oeni* OT3	± ^{C}
	M. pulcherrima B0512B3	± ^{C}
	P. fermentans M105A30	++ ^{C}
	P. fermentans B05A36	± ^{C}
	T. delbrueckii 291	++ ^{C}
	B. bruxellensis 2	+ ^{C}
	B. bruxellensis 4	± ^{C}
	B. bruxellensis 6	± ^{C}
	B. bruxellensis 7	± ^{C}

The superscript indicates the medium onto which the results have been observed: "C" = culture medium.

Excluding a few strains, auto-inhibition was observed among different bacterial species/strains, that are probably addressable to nutrient depletion and acidification [80–82]. In this study, *Pediococcus* spp. inhibited *O. oeni* OT3. Discordant results concern the couple *L. hilgardii*—*O. oeni*, since Rodriguez et al. [83] observed that the growth of *O. oeni* could be inhibited by means of H_2O_2 produced by *L. hilgardii* while, according to Aredes Fernandez et al. [84], the inhibition of *O. oeni* in co-culture with *L. hilgardii* seems to be due to competition for arginine, a stimulating agent for the growth of *O. oeni*, and to the consumption of peptides by *L. hilgardii*.

Interesting results of this study are referred to the different inhibitions between some LAB and non-*Saccharomyces* yeasts. Specifically, *Pediococcus* spp., *O. oeni* OT3, and *O. oeni* OT4 inhibited some strains of *M. pulcherrima*, *P. fermentans* B05A36, *P. fermentans* M105A30, and *T. delbrueckii* 291. Furthermore, *O. oeni* OT4 has shown inhibitory activity also toward four strains of *B. bruxellensis*. These last inhibitions are attractive due to the possible application in the biocontrol of this bacterium to inhibit one of the main spoilage agents in wine [32,33].

4. Conclusions

An improved understanding of the interactions among must/wine-associated microorganisms could provide a useful tool to avoid fermentations that are stuck or sluggish, optimize wine quality/safety, and minimize the production of those compounds that depreciate wine quality. From this point of view, the study (i) characterized the in vitro potential of microbial resources that might be exploited for biocontrol activities on grape/wine and (ii) provided original information that can contribute to explaining a range of microbial interactions in the oenological trials. However, this reservoir of microbial antagonisms was drastically reduced when tested on the real matrices (must and wine). As said, it was possible to observe a considerable number of inhibitions exerted by non-*Saccharomyces* strains (on grape juice) and a few inhibitions by *S. cerevisiae* (on wine). This is consistent with the broader challenge in dealing with the exploitation of microbial controlling traits directly in situ in the food industry [85,86]. It is important to underline that the interactions studied in this paper are related to the growth/no growth of microorganisms, with research activities that are propaedeutic to but not considering the metabolic interaction, which is one of the current trends in wine microbiological studies (see [87–90]). Highlighted microbial interactions are very important for the sustainable control of spoilage yeasts and the management of alcoholic fermentation, malolactic fermentation, and the development of a distinctive aroma profile [71]. In addition, the plate assay method could be a fast, cheap, and suitable method to exclude negative interactions among *Saccharomyces* spp., NSY, and malolactic bacteria during trials from regional spontaneous fermentations with the aim to select tailored mixed starter cultures [42,91].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app122412760/s1, Figure S1: inhibitions tests on agarised grape juice.

Author Contributions: Conceptualisation, M.D.G., P.R., M.F., F.G., G.S. and V.C.; Methodology, M.D.G., P.R., N.D.S., F.G., M.F., G.S. and V.C.; Investigation, M.D.G., P.R., N.D.S. and M.F.; Resources, G.S. and V.C.; Data curation, M.D.G., P.R., M.F. and V.C.; Writing—original draft preparation, M.D.G., M.F., P.R. and V.C.; Writing—review and editing, N.D.S., F.G. and G.S.; Supervision, P.R., M.F., F.G., G.S. and V.C.; Project administration, P.R., F.G., G.S. and V.C.; Funding acquisition, G.S., F.G., and V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by the Apulia Region Projects: "Innovazione nella tradizione: tecnologie innovative per esaltare le qualità dei vini autoctoni spumante della murgia barese—INVISPUBA" and "Spumantizzazione e frizzantatura per il rilancio della vitivinicoltura dell'areale Centro Nord della regione Puglia—SPUMAPULIA" (P.S.R. Puglia 2014/2020-Misura 16.2).

Acknowledgments: The authors acknowledge Massimo Franchi, Domenico Genchi, Pasquale Del Vecchio and Giuseppe Panzarini of the Institute of Sciences of Food Production—CNR for the skilled technical support provided during the realizations of this work.

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

References

1. Liu, Y.; Rousseaux, S.; Tourdot-Maréchal, R.; Sadoudi, M.; Gougeon, R.; Schmitt-Kopplin, P.; Alexandre, H. Wine Microbiome: A Dynamic World of Microbial Interactions. *Crit. Rev. Food Sci. Nutr.* **2017**, *57*, 856–873. [CrossRef] [PubMed]
2. Camilo, S.; Chandra, M.; Branco, P.; Malfeito-Ferreira, M. Wine Microbial Consortium: Seasonal Sources and Vectors Linking Vineyard and Winery Environments. *Fermentation* **2022**, *8*, 324. [CrossRef]
3. Berbegal, C.; Spano, G.; Tristezza, M.; Grieco, F.; Capozzi, V. Microbial Resources and Innovation in the Wine Production Sector. *South Afr. J. Enol. Vitic.* **2017**, *38*, 156–166. [CrossRef]
4. Belda, I.; Ruiz, J.; Esteban-Fernández, A.; Navascués, E.; Marquina, D.; Santos, A.; Moreno-Arribas, M.V. Microbial Contribution to Wine Aroma and Its Intended Use for Wine Quality Improvement. *Molecules* **2017**, *22*, 189. [CrossRef]
5. Russo, P.; Capozzi, V.; Spano, G.; Corbo, M.R.; Sinigaglia, M.; Bevilacqua, A. Metabolites of Microbial Origin with an Impact on Health: Ochratoxin A and Biogenic Amines. *Front. Microbiol.* **2016**, *7*, 482. [CrossRef]
6. Roudil, L.; Russo, P.; Berbegal, C.; Albertin, W.; Spano, G.; Capozzi, V. Non-Saccharomyces Commercial Starter Cultures: Scientific Trends, Recent Patents and Innovation in the Wine Sector. *FNA* **2019**, *10*, 27–39. [CrossRef]
7. Petruzzi, L.; Capozzi, V.; Berbegal, C.; Corbo, M.R.; Bevilacqua, A.; Spano, G.; Sinigaglia, M. Microbial Resources and Enological Significance: Opportunities and Benefits. *Front. Microbiol.* **2017**, *8*, 995. [CrossRef]
8. Bindon, K.A.; Kassara, S.; Solomon, M.; Bartel, C.; Smith, P.A.; Barker, A.; Curtin, C. Commercial *Saccharomyces cerevisiae* Yeast Strains Significantly Impact Shiraz Tannin and Polysaccharide Composition with Implications for Wine Colour and Astringency. *Biomolecules* **2019**, *9*, 466. [CrossRef]
9. Tufariello, M.; Maiorano, G.; Rampino, P.; Spano, G.; Grieco, F.; Perrotta, C.; Capozzi, V.; Grieco, F. Selection of an Autochthonous Yeast Starter Culture for Industrial Production of Primitivo "Gioia Del Colle" PDO/DOC in Apulia (Southern Italy). *LWT* **2019**, *99*, 188–196. [CrossRef]
10. Morata, A.; Loira, I.; Escott, C.; del Fresno, J.M.; Bañuelos, M.A.; Suárez-Lepe, J.A. Applications of *Metschnikowia Pulcherrima* in Wine Biotechnology. *Fermentation* **2019**, *5*, 63. [CrossRef]
11. Morata, A.; Escott, C.; Bañuelos, M.A.; Loira, I.; del Fresno, J.M.; González, C.; Suárez-Lepe, J.A. Contribution of Non-Saccharomyces Yeasts to Wine Freshness. A Review. *Biomolecules* **2020**, *10*, 34. [CrossRef] [PubMed]
12. Russo, P.; Tufariello, M.; Renna, R.; Tristezza, M.; Taurino, M.; Palombi, L.; Capozzi, V.; Rizzello, C.G.; Grieco, F. New Insights into the Oenological Significance of *Candida zemplinina*: Impact of Selected Autochthonous Strains on the Volatile Profile of Apulian Wines. *Microorganisms* **2020**, *8*, 628. [CrossRef] [PubMed]
13. Tufariello, M.; Capozzi, V.; Spano, G.; Cantele, G.; Venerito, P.; Mita, G.; Grieco, F. Effect of Co-Inoculation of *Candida Zemplinina*, *Saccharomyces Cerevisiae* and *Lactobacillus plantarum* for the Industrial Production of Negroamaro Wine in Apulia (Southern Italy). *Microorganisms* **2020**, *8*, 726. [CrossRef] [PubMed]
14. Du Plessis, H.; Du Toit, M.; Nieuwoudt, H.; Van der Rijst, M.; Hoff, J.; Jolly, N. Modulation of Wine Flavor Using *Hanseniaspora Uvarum* in Combination with Different *Saccharomyces cerevisiae*, Lactic Acid Bacteria Strains and Malolactic Fermentation Strategies. *Fermentation* **2019**, *5*, 64. [CrossRef]
15. Escribano, R.; González-Arenzana, L.; Garijo, P.; Berlanas, C.; López-Alfaro, I.; López, R.; Gutiérrez, A.R.; Santamaría, P. Screening of Enzymatic Activities within Different Enological Non-Saccharomyces Yeasts. *J. Food Sci. Technol.* **2017**, *54*, 1555–1564. [CrossRef]
16. Berbegal, C.; Khomenko, I.; Russo, P.; Spano, G.; Fragasso, M.; Biasioli, F.; Capozzi, V. PTR-ToF-MS for the Online Monitoring of Alcoholic Fermentation in Wine: Assessment of VOCs Variability Associated with Different Combinations of Saccharomyces/Non-Saccharomyces as a Case-Study. *Fermentation* **2020**, *6*, 55. [CrossRef]
17. Bartowsky, E.J. *Oenococcus oeni* and Malolactic Fermentation—Moving into the Molecular Arena. *Aust. J. Grape Wine Res.* **2005**, *11*, 174–187. [CrossRef]
18. Campbell-Sills, H.; Khoury, M.E.; Gammacurta, M.; Miot-Sertier, C.; Dutilh, L.; Vestner, J.; Capozzi, V.; Sherman, D.; Hubert, C.; Claisse, O.; et al. Two Different *Oenococcus oeni* Lineages Are Associated to Either Red or White Wines in Burgundy: Genomics and Metabolomics Insights. *OENO One* **2017**, *51*, 309. [CrossRef]
19. Lasik, M. The Application of Malolactic Fermentation Process to Create Good-Quality Grape Wine Produced in Cool-Climate Countries: A Review. *Eur. Food Res. Technol.* **2013**, *237*, 843–850. [CrossRef]
20. Campbell-Sills, H.; Capozzi, V.; Romano, A.; Cappellin, L.; Spano, G.; Breniaux, M.; Lucas, P.; Biasioli, F. Advances in Wine Analysis by PTR-ToF-MS: Optimization of the Method and Discrimination of Wines from Different Geographical Origins and Fermented with Different Malolactic Starters. *Int. J. Mass Spectrom.* **2016**, *397–398*, 42–51. [CrossRef]
21. Bordet, F.; Joran, A.; Klein, G.; Roullier-Gall, C.; Alexandre, H. Yeast–Yeast Interactions: Mechanisms, Methodologies and Impact on Composition. *Microorganisms* **2020**, *8*, 600. [CrossRef] [PubMed]
22. Alonso-del-Real, J.; Lairón-Peris, M.; Barrio, E.; Querol, A. Effect of Temperature on the Prevalence of *Saccharomyces Non Cerevisiae* Species against a *S. Cerevisiae* Wine Strain in Wine Fermentation: Competition, Physiological Fitness, and Influence in Final Wine Composition. *Front. Microbiol.* **2017**, *8*, 150. [CrossRef]
23. Alonso-del-Real, J.; Pérez-Torrado, R.; Querol, A.; Barrio, E. Dominance of Wine *Saccharomyces Cerevisiae* Strains over *S. Kudriavzevii* in Industrial Fermentation Competitions Is Related to an Acceleration of Nutrient Uptake and Utilization. *Environ. Microbiol.* **2019**, *21*, 1627–1644. [CrossRef] [PubMed]

24. Bagheri, B.; Bauer, F.F.; Setati, M.E. The Impact of Saccharomyces Cerevisiae on a Wine Yeast Consortium in Natural and Inoculated Fermentations. *Front. Microbiol.* **2017**, *8*, 1988. [CrossRef]
25. Branco, P.; Francisco, D.; Chambon, C.; Hébraud, M.; Arneborg, N.; Almeida, M.G.; Caldeira, J.; Albergaria, H. Identification of Novel GAPDH-Derived Antimicrobial Peptides Secreted by Saccharomyces Cerevisiae and Involved in Wine Microbial Interactions. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 843–853. [CrossRef] [PubMed]
26. Comitini, F.; De Ingeniis, J.; Pepe, L.; Mannazzu, I.; Ciani, M. *Pichia Anomala* and *Kluyveromyces Wickerhamii* Killer Toxins as New Tools against Dekkera/Brettanomyces Spoilage Yeasts. *FEMS Microbiol. Lett.* **2004**, *238*, 235–240. [CrossRef] [PubMed]
27. Oro, L.; Ciani, M.; Comitini, F. Antimicrobial Activity of *Metschnikowia pulcherrima* on Wine Yeasts. *J. Appl. Microbiol.* **2014**, *116*, 1209–1217. [CrossRef]
28. Balmaseda, A.; Bordons, A.; Reguant, C.; Bautista-Gallego, J. Non-Saccharomyces in Wine: Effect Upon *Oenococcus oeni* and Malolactic Fermentation. *Front. Microbiol.* **2018**, *9*, 534. [CrossRef]
29. Ferrando, N.; Araque, I.; Ortís, A.; Thornes, G.; Bautista-Gallego, J.; Bordons, A.; Reguant, C. Evaluating the Effect of Using Non-Saccharomyces on *Oenococcus Oeni* and Wine Malolactic Fermentation. *Food Res. Int.* **2020**, *138*, 109779. [CrossRef]
30. Berbegal, C.; Borruso, L.; Fragasso, M.; Tufariello, M.; Russo, P.; Brusetti, L.; Spano, G.; Capozzi, V. A Metagenomic-Based Approach for the Characterization of Bacterial Diversity Associated with Spontaneous Malolactic Fermentations in Wine. *Int. J. Mol. Sci.* **2019**, *20*, 3980. [CrossRef]
31. Englezos, V.; Torchio, F.; Vagnoli, P.; Krieger-Weber, S.; Rantsiou, K.; Cocolin, L. Impact of *Saccharomyces Cerevisiae* Strain Selection on Malolactic Fermentation by *Lactobacillus Plantarum* and *Oenococcus Oeni*. *Am. J. Enol. Vitic.* **2020**, *71*, 157–165. [CrossRef]
32. Berbegal, C.; Garofalo, C.; Russo, P.; Pati, S.; Capozzi, V.; Spano, G. Use of Autochthonous Yeasts and Bacteria in Order to Control *Brettanomyces bruxellensis* in Wine. *Fermentation* **2017**, *3*, 65. [CrossRef]
33. Berbegal, C.; Spano, G.; Fragasso, M.; Grieco, F.; Russo, P.; Capozzi, V. Starter Cultures as Biocontrol Strategy to Prevent *Brettanomyces bruxellensis* Proliferation in Wine. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 569–576. [CrossRef] [PubMed]
34. Huang, Y.-C.; Edwards, C.G.; Peterson, J.C.; Haag, K.M. Relationship Between Sluggish Fermentations and the Antagonism of Yeast by Lactic Acid Bacteria. *Am. J. Enol. Vitic.* **1996**, *47*, 1–10.
35. Yurdugül, S.; Bozoglu, F. Studies on an Inhibitor Produced by Lactic Acid Bacteria of Wines on the Control of Malolactic Fermentation. *Eur. Food Res. Technol.* **2002**, *215*, 38–41. [CrossRef]
36. Comitini, F.; Ciani, M. The Inhibitory Activity of Wine Yeast Starters on Malolactic Bacteria. *Ann. Microbiol.* **2007**, *57*, 61. [CrossRef]
37. Sipiczki, M. Overwintering of Vineyard Yeasts: Survival of Interacting Yeast Communities in Grapes Mummified on Vines. *Front. Microbiol.* **2016**, *7*, 212. [CrossRef]
38. Mehlomakulu, N.N.; Setati, M.E.; Divol, B. Characterization of Novel Killer Toxins Secreted by Wine-Related Non-Saccharomyces Yeasts and Their Action on *Brettanomyces Spp. Int. J. Food Microbiol.* **2014**, *188*, 83–91. [CrossRef]
39. Pinto, L.; Malfeito-Ferreira, M.; Quintieri, L.; Silva, A.C.; Baruzzi, F. Growth and Metabolite Production of a Grape Sour Rot Yeast-Bacterium Consortium on Different Carbon Sources. *Int. J. Food Microbiol.* **2019**, *296*, 65–74. [CrossRef]
40. Kemsawasd, V.; Branco, P.; Almeida, M.G.; Caldeira, J.; Albergaria, H.; Arneborg, N. Cell-to-Cell Contact and Antimicrobial Peptides Play a Combined Role in the Death of *Lachanchea Thermotolerans* during Mixed-Culture Alcoholic Fermentation with *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* **2015**, *362*, fnv103. [CrossRef]
41. Kapetanakou, A.E.; Kollias, J.N.; Drosinos, E.H.; Skandamis, P.N. Inhibition of A. Carbonarius Growth and Reduction of Ochratoxin A by Bacteria and Yeast Composites of Technological Importance in Culture Media and Beverages. *Int. J. Food Microbiol.* **2012**, *152*, 91–99. [CrossRef] [PubMed]
42. Garofalo, C.; Khoury, M.E.; Lucas, P.; Bely, M.; Russo, P.; Spano, G.; Capozzi, V. Autochthonous Starter Cultures and Indigenous Grape Variety for Regional Wine Production. *J. Appl. Microbiol.* **2015**, *118*, 1395–1408. [CrossRef] [PubMed]
43. Berbegal, C.; Peña, N.; Russo, P.; Grieco, F.; Pardo, I.; Ferrer, S.; Spano, G.; Capozzi, V. Technological Properties of *Lactobacillus Plantarum* Strains Isolated from Grape Must Fermentation. *Food Microbiol.* **2016**, *57*, 187–194. [CrossRef] [PubMed]
44. Englezos, V.; Jolly, N.P.; Di Gianvito, P.; Rantsiou, K.; Cocolin, L. Microbial Interactions in Winemaking: Ecological Aspects and Effect on Wine Quality. *Trends Food Sci. Technol.* **2022**, *127*, 99–113. [CrossRef]
45. Di Toro, M.R.; Capozzi, V.; Beneduce, L.; Alexandre, H.; Tristezza, M.; Durante, M.; Tufariello, M.; Grieco, F.; Spano, G. Intraspecific Biodiversity and 'Spoilage Potential' of *Brettanomyces bruxellensis* in Apulian Wines. *LWT Food Sci. Technol.* **2015**, *60*, 102–108. [CrossRef]
46. Lamontanara, A.; Caggianiello, G.; Orrù, L.; Capozzi, V.; Michelotti, V.; Bayjanov, J.R.; Renckens, B.; van Hijum, S.A.F.T.; Cattivelli, L.; Spano, G. Draft Genome Sequence of *Lactobacillus plantarum* Lp90 Isolated from Wine. *Genome Announc.* **2015**, *3*, e00097-15. [CrossRef]
47. Lamontanara, A.; Orrù, L.; Cattivelli, L.; Russo, P.; Spano, G.; Capozzi, V. Genome Sequence of *Oenococcus oeni* OM27, the First Fully Assembled Genome of a Strain Isolated from an Italian Wine. *Genome Announc.* **2014**, *2*, e00658-14. [CrossRef]
48. Capozzi, V.; Russo, P.; Lamontanara, A.; Orrù, L.; Cattivelli, L.; Spano, G. Genome Sequences of Five *Oenococcus oeni* Strains Isolated from Nero Di Troia Wine from the Same Terroir in Apulia, Southern Italy. *Genome Announc.* **2014**, *2*, e01077-14. [CrossRef]
49. Garofalo, C.; Russo, P.; Beneduce, L.; Massa, S.; Spano, G.; Capozzi, V. Non-Saccharomyces Biodiversity in Wine and the 'Microbial Terroir': A Survey on Nero Di Troia Wine from the Apulian Region, Italy. *Ann. Microbiol.* **2016**, *66*, 143–150. [CrossRef]
50. Garofalo, C.; Tristezza, M.; Grieco, F.; Spano, G.; Capozzi, V. From Grape Berries to Wine: Population Dynamics of Cultivable Yeasts Associated to "Nero Di Troia" Autochthonous Grape Cultivar. *World J. Microbiol. Biotechnol.* **2016**, *32*, 59. [CrossRef]

51. Garofalo, C.; Berbegal, C.; Grieco, F.; Tufariello, M.; Spano, G.; Capozzi, V. Selection of Indigenous Yeast Strains for the Production of Sparkling Wines from Native Apulian Grape Varieties. *Int. J. Food Microbiol.* **2018**, *285*, 7–17. [CrossRef] [PubMed]
52. Capozzi, V.; Berbegal, C.; Tufariello, M.; Grieco, F.; Spano, G.; Grieco, F. Impact of co-inoculation of *Saccharomyces cerevisiae*, *Hanseniaspora uvarum* and *Oenococcus oeni* autochthonous strains in controlled multi starter grape must fermentations. *LWT* **2019**, *109*, 241–249. [CrossRef]
53. Comitini, F.; Ferretti, R.; Clementi, F.; Mannazzu, I.; Ciani, M. Interactions between *Saccharomyces cerevisiae* and Malolactic Bacteria: Preliminary Characterization of a Yeast Proteinaceous Compound (s) Active against *Oenococcus oeni*. *J. Appl. Microbiol.* **2005**, *99*, 105–111. [CrossRef] [PubMed]
54. Ivey, M.; Massel, M.; Phister, T.G. Microbial Interactions in Food Fermentations. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 141–162. [CrossRef]
55. Ponomarova, O.; Gabrielli, N.; Sévin, D.C.; Mülleder, M.; Zirngibl, K.; Bulyha, K.; Andrejev, S.; Kafkia, E.; Typas, A.; Sauer, U.; et al. Yeast Creates a Niche for Symbiotic Lactic Acid Bacteria through Nitrogen Overflow. *Cell Syst.* **2017**, *5*, 345–357.e6. [CrossRef]
56. Mendoza, L.M.; de Nadra, M.C.M.; Farías, M.E. Kinetics and Metabolic Behavior of a Composite Culture of *Kloeckera Apiculata* and *Saccharomyces cerevisiae* Wine Related Strains. *Biotechnol. Lett.* **2007**, *29*, 1057–1063. [CrossRef]
57. Köhl, J.; Kolnaar, R.; Ravensberg, W.J. Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy. *Front. Plant Sci.* **2019**, *10*, 845. [CrossRef]
58. Vejarano, R. Non-Saccharomyces in Winemaking: Source of Mannoproteins, Nitrogen, Enzymes, and Antimicrobial Compounds. *Fermentation* **2020**, *6*, 76. [CrossRef]
59. Berbegal, C.; Fragasso, M.; Russo, P.; Bimbo, F.; Grieco, F.; Spano, G.; Capozzi, V. Climate Changes and Food Quality: The Potential of Microbial Activities as Mitigating Strategies in the Wine Sector. *Fermentation* **2019**, *5*, 85. [CrossRef]
60. Jolly, N.P.; Varela, C.; Pretorius, I.S. Not Your Ordinary Yeast: Non-Saccharomyces Yeasts in Wine Production Uncovered. *FEMS Yeast Res.* **2014**, *14*, 215–237. [CrossRef]
61. Türkel, S.; Ener, B. Isolation and Characterization of New *Metschnikowia Pulcherrima* Strains as Producers of the Antimicrobial Pigment Pulcherrimin. *Z. Naturforsch. C J. Biosci.* **2009**, *64*, 405–410. [CrossRef] [PubMed]
62. Medina, K.; Boido, E.; Dellacassa, E.; Carrau, F. Growth of Non-Saccharomyces Yeasts Affects Nutrient Availability for *Saccharomyces Cerevisiae* during Wine Fermentation. *Int. J. Food Microbiol.* **2012**, *157*, 245–250. [CrossRef] [PubMed]
63. Mendoza, L.M.; de Nadra, M.C.M.; Farías, M.E. Antagonistic Interaction between Yeasts and Lactic Acid Bacteria of Oenological Relevance: Partial Characterization of Inhibitory Compounds Produced by Yeasts. *Food Res. Int.* **2010**, *43*, 1990–1998. [CrossRef]
64. Melvydas, V.; Svediene, J.; Skridlaite, G.; Vaiciuniene, J.; Garjonyte, R. In Vitro Inhibition of *Saccharomyces cerevisiae* Growth by *Metschnikowia Spp*. Triggered by Fast Removal of Iron via Two Ways. *Braz. J. Microbiol.* **2020**, *51*, 1953–1964. [CrossRef]
65. Nardi, T.; Panero, L.; Petrozziello, M.; Guaita, M.; Tsolakis, C.; Cassino, C.; Vagnoli, P.; Bosso, A. Managing Wine Quality Using *Torulaspora Delbrueckii* and *Oenococcus Oeni* Starters in Mixed Fermentations of a Red Barbera Wine. *Eur. Food Res. Technol.* **2019**, *245*, 293–307. [CrossRef]
66. Du Plessis, H.W.; du Toit, M.; Hoff, J.W.; Hart, R.S.; Ndimba, B.K.; Jolly, N.P. Characterisation of Non-Saccharomyces Yeasts Using Different Methodologies and Evaluation of Their Compatibility with Malolactic Fermentation. *South Afr. J. Enol. Vitic.* **2017**, *38*, 46–63. [CrossRef]
67. Russo, P.; Englezos, V.; Capozzi, V.; Pollon, M.; Río Segade, S.; Rantsiou, K.; Spano, G.; Cocolin, L. Effect of Mixed Fermentations with *Starmerella Bacillaris* and *Saccharomyces cerevisiae* on Management of Malolactic Fermentation. *Food Res. Int.* **2020**, *134*, 109246. [CrossRef]
68. Nardi, T. Microbial Resources as a Tool for Enhancing Sustainability in Winemaking. *Microorganisms* **2020**, *8*, 507. [CrossRef]
69. Bartle, L.; Sumby, K.; Sundstrom, J.; Jiranek, V. The Microbial Challenge of Winemaking: Yeast-Bacteria Compatibility. *FEMS Yeast Res.* **2019**, *19*, foz040. [CrossRef]
70. Capozzi, V.; Garofalo, C.; Chiriatti, M.A.; Grieco, F.; Spano, G. Microbial Terroir and Food Innovation: The Case of Yeast Biodiversity in Wine. *Microbiol. Res.* **2015**, *181*, 75–83. [CrossRef]
71. Belda, I.; Ruiz, J.; Alonso, A.; Marquina, D.; Santos, A. The Biology of Pichia Membranifaciens Killer Toxins. *Toxins* **2017**, *9*, 112. [CrossRef] [PubMed]
72. De Ullivarri, M.F.; Mendoza, L.M.; Raya, R.R. Killer Yeasts as Biocontrol Agents of Spoilage Yeasts and Bacteria Isolated from Wine. *BIO Web Conf.* **2014**, *3*, 02001. [CrossRef]
73. Russo, P.; Fragasso, M.; Berbegal, C.; Grieco, F.; Spano, G.; Capozzi, V. Microorganisms Able to Produce Biogenic Amines and Factors Affecting Their Activity. In *Biogenic Amines in Food*; Royal Society of Chemistry Publishing: Cambridge, UK, 2019; pp. 18–40.
74. Barbieri, F.; Montanari, C.; Gardini, F.; Tabanelli, G. Biogenic Amine Production by Lactic Acid Bacteria: A Review. *Foods* **2019**, *8*, 17. [CrossRef]
75. Krieger-Weber, S.; Heras, J.M.; Suarez, C. *Lactobacillus plantarum*, a New Biological Tool to Control Malolactic Fermentation: A Review and an Outlook. *Beverages* **2020**, *6*, 23. [CrossRef]
76. Alexandre, H.; Costello, P.J.; Remize, F.; Guzzo, J.; Guilloux-Benatier, M. *Saccharomyces cerevisiae–Oenococcus oeni* Interactions in Wine: Current Knowledge and Perspectives. *Int. J. Food Microbiol.* **2004**, *93*, 141–154. [CrossRef]

77. Aponte, M.; Romano, R.; Villano, C.; Blaiotta, G. Dominance of *S. cerevisiae* Commercial Starter Strains during Greco Di Tufo and Aglianico Wine Fermentations and Evaluation of Oenological Performances of Some Indigenous/Residential Strains. *Foods* **2020**, *9*, 1549. [CrossRef] [PubMed]
78. Perrone, B.; Giacosa, S.; Rolle, L.; Cocolin, L.; Rantsiou, K. Investigation of the Dominance Behavior of *Saccharomyces cerevisiae* Strains during Wine Fermentation. *Int. J. Food Microbiol.* **2013**, *165*, 156–162. [CrossRef]
79. Barrajón, N.; Arévalo-Villena, M.; Úbeda, J.; Briones, A. Enological Properties in Wild and Commercial *Saccharomyces cerevisiae* Yeasts: Relationship with Competition during Alcoholic Fermentation. *World J. Microbiol. Biotechnol.* **2011**, *27*, 2703–2710. [CrossRef]
80. Papadimitriou, K.; Alegría, Á.; Bron, P.A.; de Angelis, M.; Gobbetti, M.; Kleerebezem, M.; Lemos, J.A.; Linares, D.M.; Ross, P.; Stanton, C.; et al. Stress Physiology of Lactic Acid Bacteria. *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 837–890. [CrossRef] [PubMed]
81. Leroy, F.; De Vuyst, L. Growth of the Bacteriocin-Producing *Lactobacillus sakei* Strain CTC 494 in MRS Broth Is Strongly Reduced Due to Nutrient Exhaustion: A Nutrient Depletion Model for the Growth of Lactic Acid Bacteria. *Appl. Environ. Microbiol.* **2001**, *67*, 4407–4413. [CrossRef]
82. Arena, M.P.; Capozzi, V.; Spano, G.; Fiocco, D. The Potential of Lactic Acid Bacteria to Colonize Biotic and Abiotic Surfaces and the Investigation of Their Interactions and Mechanisms. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 2641–2657. [CrossRef] [PubMed]
83. Rodriguez, A.V.; de Nadra, M.C.M. Production of Hydrogen Peroxide by *Lactobacillus hilgardii* and Its Effect on *Leuconostoc oenos* Growth. *Curr. Microbiol.* **1995**, *30*, 23–25. [CrossRef]
84. Aredes Fernández, P.A.; Farías, M.E.; de Nadra, M.C.M. Interaction between *Oenococcus oeni* and *Lactobacillus hilgardii* Isolated from Wine. Modification of Available Nitrogen and Biogenic Amine Production. *Biotechnol. Lett.* **2010**, *32*, 1095–1102. [CrossRef] [PubMed]
85. Castellano, P.; Pérez Ibarreche, M.; Blanco Massani, M.; Fontana, C.; Vignolo, G.M. Strategies for Pathogen Biocontrol Using Lactic Acid Bacteria and Their Metabolites: A Focus on Meat Ecosystems and Industrial Environments. *Microorganisms* **2017**, *5*, 38. [CrossRef]
86. Kuchen, B.; Vazquez, F.; Maturano, Y.P.; Scaglia, G.J.E.; Pera, L.M.; Vallejo, M.D. Toward Application of Biocontrol to Inhibit Wine Spoilage Yeasts: The Use of Statistical Designs for Screening and Optimisation. *OENO One* **2021**, *55*, 75–96. [CrossRef]
87. Petitgonnet, C.; Klein, G.L.; Roullier-Gall, C.; Schmitt-Kopplin, P.; Quintanilla-Casas, B.; Vichi, S.; Julien-David, D.; Alexandre, H. Influence of Cell-Cell Contact between *L. thermotolerans* and *S. cerevisiae* on Yeast Interactions and the Exo-Metabolome. *Food Microbiol.* **2019**, *83*, 122–133. [CrossRef]
88. Roullier-Gall, C.; David, V.; Hemmler, D.; Schmitt-Kopplin, P.; Alexandre, H. Exploring Yeast Interactions through Metabolic Profiling. *Sci. Rep.* **2020**, *10*, 6073. [CrossRef]
89. Ciani, M.; Comitini, F. Yeast Interactions in Multi-Starter Wine Fermentation. *Curr. Opin. Food Sci.* **2015**, *1*, 1–6. [CrossRef]
90. Marzano, M.; Fosso, B.; Manzari, C.; Grieco, F.; Intranuovo, M.; Cozzi, G.; Mulè, G.; Scioscia, G.; Valiente, G.; Tullo, A.; et al. Complexity and dynamics of the winemaking bacterial communities in berries, musts, and wines from Apulian grape cultivars through time and space. *PLoS ONE* **2016**, *11*, e0157383. [CrossRef]
91. Morata, A.; Arroyo, T.; Bañuelos, M.A.; Blanco, P.; Briones, A.; Cantoral, J.M.; Castrillo, D.; Cordero-Bueso, G.; Del Fresno, J.M.; Escott, C. Wine Yeast Selection in the Iberian Peninsula: Saccharomyces and Non-Saccharomyces as Drivers of Innovation in Spanish and Portuguese Wine Industries. *Crit. Rev. Food Sci. Nutr.* **2022**, 1–29. [CrossRef]