Postharvest Biology and Technology 86 (2013) 456–462

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Biocontrol of fungi isolated from sour rot infected table grapes by *Saccharomyces* and other yeast species

M.C. Nally[a,*], V.M. Pesce[a], Y.P. Maturano[a], M.E. Toro[a], M. Combina[b], L.I. Castellanos de Figueroa[c,d], F. Vazquez[a]

[a] IBT, Instituto de Biotecnología, Facultad de Ingeniería, Universidad Nacional de San Juan, Av. Libertador San Martín 1109 oeste, 5400 San Juan, Argentina
[b] INTA, Luján de Cuyo, Centro de Estudios Enológicos, Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (INTA), San Martín 3853, 5507 Luján de Cuyo, Mendoza, Argentina
[c] PROIMI, Planta Piloto de Procesos Industriales Microbiológicos, Av. Belgrano y Pasaje Caseros, 4000 Tucumán, Argentina
[d] FBQyF – UNT, Facultad de Bioquímica, Química y Farmacia, Ayacucho 455, 4000 Tucumán, Argentina

ARTICLE INFO

Article history:
Received 19 November 2012
Accepted 15 July 2013

Keywords:
Biocontrol
Table grape
Sour rot
Fungi
Yeasts
Pathogenicity

ABSTRACT

Sour rot is an important disease of grapes caused by an etiologic complex of microorganisms in which filamentous fungi play a key role. Yeasts are used for biocontrol of pathogenic filamentous fungi on fruits. The major objective of this study was to assess *in vivo* on detached berries the effect of viticultural yeasts on phytopathogenic fungi involved in grape sour rot. Yeasts that were found to be effective *in vivo* against the fungi were assayed for their possible pathogenicity in humans: growth at 42 °C, pseudohyphal formation, adhesion, and phospholipase and protease activity. A total of 234 yeasts belonging to 14 genera were assayed against the following pathogens: *Aspergillus caelatus*, *Aspergillus carbonarius*, *Aspergillus terreus*, *Aspergillus versicolor*, *Fusarium oxysporum*, *Penicillium comune*, *Rhizopus stolonifer* and *Ulocladium* sp. Forty-three (16 *Saccharomyces* and 27 non-*Saccharomyces*) showed antagonistic properties against some of the fungi assayed in grapes at 25 °C. Yeast isolates determined as biocontrol agents under *in vivo* conditions were isolated from fermenting musts (35), viticultural soils (6) and grape berries (2). Twenty biocontrol agents did not show phenotypical characteristics associated with pathogenicity in humans.
© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Vitis vinifera L., or commonly grape berry, is a nonclimacteric fruit with a relatively low rate of physiological activity. The province of San Juan is the main producer of table grapes in Argentina. These grapes are exported to different countries such as Holland, Russia, Brazil and Germany among others (SENASA, 2012). Maintenance of postharvest quality of table grapes is becoming increasingly significant as the supply of high quality commodities constantly exceeds demand. A significant quantity of table grapes is lost at various stages between pre-harvest and consumption. Grape quality can be affected by a wide range of rots, and "sour rot" is an emergent grape disease affecting late ripening cultivars with tightly-packed and dense bunches close to the harvest, causing heavy crop losses (Barata et al., 2012). This disease is associated with a complex of microorganisms including yeasts, bacteria, and filamentous fungi such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Cladosporium* (Barata, 2011; Hewstone et al., 2007). *Peniciliium* and *Cladosporium* were isolated from table grapes conserved in frigorific chamber (Donoso and Latorre, 2006). However, the etiologic origin and sequence of appearance of each pathogen has not yet been fully elucidated. Some researchers suggest that filamentous fungi are primarily responsible for this disease and yeasts and bacteria act as secondary pathogens (Latorre et al., 2002; Mateluna Estay, 2006). In contrast, Oriolani et al. (2007) reported that filamentous fungi act as secondary pathogens, being primary yeasts and bacteria.

In Argentina, the incidence of sour rot has increased in table and wine grapes (Oriolani et al., 2009; INTA, 2012). In the summer of 2009, for example, 20,000 t of table grapes were affected by sour rot in San Juan, Argentina (personal communication by Beatriz Pugliese). Control of this disease is mainly dependent on the use of chemical fungicides. Fenhexamid, iprodione, cyprodinil, copper hydroxide and captan are chemical fungicides that are generally used in vineyards (Hashim-Buckey et al., 2008). However, the use of synthetic fungicides is not allowed in organic and biodynamic agriculture (Guzzon et al., 2011), and in conventional agriculture there are increasing regulatory restrictions on the use of chemical fungicides (Mlikota Gabler and Smilanick, 2001; Palou et al., 2008). The development of biological control tools as an alternative to the use of chemical fungicides would help the table grape industry become more environmental friendly

* Corresponding author. Tel.: +54 0264 4211700; fax: +54 0264 4213672.
E-mail address: cristinanally@yahoo.com.ar (M.C. Nally).

0925-5214/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.postharvbio.2013.07.022

and, at the same time, reduce yield losses due to sour rot. Control may be by antagonistic microorganisms, even natural plant or animal derived compounds (Chanchaichaovivat et al., 2007), or by plant immunity with particular emphasis on immunity to bacterial and fungal infection (Gust et al., 2010). The use of microorganisms and particularly yeasts occurring naturally on the surface of fruits has been efficacious as biological control agents. Several yeasts have been studied for their use against fungal fruit pathogens (Droby et al., 1993; Mercier and Wilson, 1994; Suzzi et al., 1995; El-Ghaouth et al., 1998, 2000; Zahavi et al., 2000; Rabosto et al., 2006; Bleve et al., 2006; Nally et al., 2012). The products of modern yeast biotechnology impinge on many commercially important sectors including foods, beverages, chemicals, pharmaceuticals, industrial enzymes, and agriculture and environmental bioremediation. Although most yeasts are beneficial to human life (Banerjee, 2009), there are numerous cases of clinical infections caused by *Candida* and other yeasts like *Saccharomyes*, *Rhodotorula*, *Pichia*, *Kluyveromyces*, *Hanseniaspora*, and *Yarrowia*, particularly in immunocompromised individuals (García-Martos et al., 1999; Lherm et al., 2002; Diekema et al., 2005; de Llanos Frutos, 2007). Thus it is important to study the possible pathogenicity of biocontrol yeasts in humans and animals. In a previous study, the antagonistic activity of 234 viticultural yeasts against a *Botrytis cinerea* strain (gray rot) (Nally et al., 2012) were studied. However, activity of these yeasts against filamentous fungi causing sour rot was not assayed. Therefore, the current study analyzed viticulture yeasts as biocontrol agents *in vivo* against filamentous fungi causing sour rot. The pathogenicity of bioactive strains was tested *in vitro*.

2. Materials and methods

2.1. Microorganisms

2.1.1. Yeasts

Two hundred and thirty-four yeasts belonging to 14 genera were assayed for bio-fungicidal activity. All strains had been isolated from different viticultural environments. Nineteen strains were isolated from Redglobe table grapes (Zonda, San Juan), 8 from vineyard soil (Caucete, San Juan) and 207 from fermenting musts of different grape varieties from San Juan, Argentina. None of the yeasts increased the level of berry rot. Identification of the strains yielded 140 *Saccharomyces* and 94 non-*Saccharomyces* (Nally et al., 2012).

2.1.1.1. Yeast inoculum. A loop of pure isolated yeast was transferred to a 250 mL Erlenmeyer flask containing 100 mL of YEPD (10 g/L Yeast Extract, 20 g/L Peptone, 20 g/L Dextrose, pH 4.5) and agitated on a rotary shaker for 12 h. Yeast cells were pelleted by centrifugation, re-suspended in sterile distilled water and centrifuged again. The resulting pellets were re-suspended in sterile distilled water and the yeast concentration was adjusted to 10^6 cfu/mL using a Neubauer chamber (El-Ghaouth et al., 1998; Nally et al., 2012). This preparation was used throughout the study.

2.1.2. Fungi

2.1.2.1. Isolations. Phytopathogenic fungi were isolated from sour rot-damaged grapes, collected in the Zonda district near the city of San Juan, Argentina. Each sample consisted of, at least, one infected berry, which was placed in a plastic sterile bag and transferred as quickly as possible to the laboratory. Ten grams of damaged berries were aseptically removed from the respective bunches, diluted twofold with water in 250 mL Erlenmeyer flasks, which were vigorously vortexed for 2 min. Decimal dilutions were obtained with water and spread on plates (in duplicate). Exactly 0.1 mL from each dilution was inoculated on Czapeck-Agar (20 g/L sucrose, 2 g/L $NaNO_3$, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4$, 20 g/L agar). Fungi were purified by monospore culture and maintained on Czapeck-Agar. Incubation was carried out at 25 °C for 10 d. Colonies were differentiated according to their morphology and representative types were selected (Nally et al., 2012).

Phytopathogenicity assays were performed by inoculating 20 μL of a fungus spore suspension (10^4 spores/mL) in grape berry wounds (3 mm diameter and 3 mm deep) that had been surface sterilized by washing first with 0.5% (v/v) sodium hypochlorite for 5 min and then three times with sterile distilled water. Grapes (18 for each replicate) were incubated in a dark wet chamber (80% RH) for 5 d at room temperature, and then the surface injury diameter of the plant tissue as well as the disease incidence were determined. These experiments were carried out in triplicate and phytopathogenic isolates selected (Nally et al., 2012). The pathogenicity of fungi was maintained by inoculating grape berries every 6 months (Utkhede et al., 2001).

2.1.2.2. Fungus identification. Fungi were identified by morphological characteristics (Pitt and Hocking, 1997) and molecular techniques based on PCR-RFLP of the 5.8S-ITS region (Diguta et al., 2011).

2.1.2.3. Fungus inoculum. Spores from 10-d-old cultures grown at 20–25 °C were collected in sterile water containing 0.1% (v/v) Tween 20. The suspension was filtered through a double layer of lens cleaning tissue (Whatman 105) to remove mycelial fragments and then centrifuged at 11,000 × *g* (2 min, 4 °C). The supernatant was decanted and the spore pellet re-suspended in 0.01% (v/v) Tween 20 to remove nutrients from the medium. This procedure was repeated twice. Spores were re-suspended in sterile water and their concentration was adjusted by dilution to10^4 fungal spores per milliliter (Neubauer chamber) (Commenil et al., 1999; Nally et al., 2012).

Redglobe grapes (*V. vinifera* cv. Redglobe) were harvested during the commercial ripening period from a local vineyard (Zonda district, San Juan) and immediately transferred to the laboratory. Homogeneous bunches were selected according to size, shape, color, weight and absence of injuries (Martínez- Romero et al., 2007). Before each assay, berries were washed with sodium hypochlorite solution (1% active chlorine), rinsed with distilled water and left to dry at room temperature (Nally et al., 2012).

2.2. In vivo yeast–pathogen assays

The viticultural yeasts were assayed *in vivo* for biocontrol activity at 25 °C for 5 d. A single wound (3 mm diameter and 3 mm deep) was made at the equator of each fruit using the tip of a sterile dissecting needle. Twenty microliters of each yeast suspension in water (10^6 cfu/mL) were pipetted into each wound. After 2 h, 20 μL of 10^4 fungus spores per milliliter of sterile distilled water were poured into each wound (preventive effect). Treated grapes were air-dried and placed in plastic bags (with wet paper towels to maintain high humidity). At the end of the experiment, the disease incidence on each infected grape was calculated as follows: incidence (%) = (number of decayed wounds/number of total wounds) × 100. A positive control (wounded grapes with 20 μL of fungal spore suspension and 20 μL of sterile distilled water) was included as well as two different negative controls: wounded grapes with 40 μL of sterile distilled water and wounded grapes with 20 μL of yeast suspension and 20 μL of sterile water. Each experiment used 18 berries per replicate and three replicates per treatment in a randomized complete block design. A reduction in disease incidence of 60% or more was considered the selection criterion for antagonistic yeasts. The experiment was repeated twice

to confirm reproducibility of the results (Nally et al., 2012; Taqarort et al., 2008; Bouzerda et al., 2003; Garmendia et al., 2005).

2.3. Phenotypic assays associated with pathogenicity of biocontrol yeasts

Growth at 42 °C, phospholipase and protease enzyme production, invasive growth and pseudohyphal formation assays were carried out to assess yeast pathogenicity (Nally et al., 2012).

A human pathogenic yeast (*Candida albicans* ATCC10231) was used as positive control in all pathogenicity assays. All yeast samples were handled according to biosecurity standards of the World Health Organization (WHO, 2004; Nally et al., 2012) and National Committee for Clinical Laboratory Standards (NCCLS, 1997; Nally et al., 2012). All experiments were carried out in triplicate.

2.4. Statistical analysis

In order to improve the homogeneity of variances (Levene's test) the data of percentages of wounds infected by fungi were arcsine-square-root transformed. Data (% of disease incidence) were submitted to one-way univariate analysis of variance (ANOVA, SPSS release 17.0 for Windows; SPSS Inc., Chicago, IL). The threshold for statistical significance was set at $p < 0.05$ (Nally et al., 2012). In the case of statistical significance, Tukey test was applied to separate the means (Lima et al., 1999).

3. Results

3.1. Fungi isolated from sour rot-damaged grapes

Fifty-four filamentous fungi were isolated from sour rot-damaged grapes (Zonda, San Juan). Eight of them were phytopathogenic fungi in grape wounds incubated at 25 °C, which were identified as *Aspergillus caelatus*, *Aspergillus carbonarius*, *Aspergillus terreus*, *Aspergillus versicolor*, *Fusarium oxysporum*, *Penicillium comune*, *Rhizopus stolonifer* and *Ulocladium* sp.

3.2. In vivo assays

The *in vivo* experiments revealed that from 234 yeasts assayed, 43 isolates (9 *Candida*, 2 *Debaryomyces*; 2 *Dekkera*; 1 *Issatchenkia*, 2 *Kluyveromyces*; 2 *Pichia*, 16 *Saccharomyces*, 1 *Sporobolomyces* and 8 *Torulaspora*) significantly reduced growth (60% or more) of at least one phytopathogenic fungus isolated from sour rot-damaged grapes. The disease incidence of all phytopathogenic fungi assayed on control grapes was 100%. The 43 biocontrol strains were isolated from fermenting musts (35), vineyard soil (6) and table grapes (2) (Tables 1 and 2).

3.3. Antagonistic activity of viticultural yeasts against each phytopathogenic fungus

3.3.1. Aspergillus caelatus

Five yeasts, belonging to both *Saccharomyces* and non-*Saccharomyces* genera significantly reduced this fungus in grape wounds after incubation at 25 °C (Tables 1 and 2). *A. caelatus* was completely inhibited by *Dekkera anomala* BDa184, whereas the 4 remaining biocontrol yeasts, *Saccharomyces chevalieri* BSch25, *S. cerevisiae* BSc119, *Torulaspora delbrueckii* BTd156 and *C. rugosa* BCr182, showed lower but mutually similar antifungal activity *in vivo* (values between 32.5 and 34.8%) ($p < 0.06$) (Tables 1 and 2).

3.3.2. Aspergillus carbonarius

Only *C. sake* BCs198 presented inhibitory activity against *A. carbonarius* (22.8% disease incidence) (Table 2).

3.3.3. Aspergillus terreus

Nineteen isolates (8 *Saccharomyces* and 11 non-*Saccharomyces*, belonging to *Saccharomyces*, *Dekkera*, *Torulaspora*, *Pichia*, *Kluyveromyces*, *Candida* and *Debaryomyces*) significantly reduced the disease incidence in grape wounds (Tables 1 and 2). *S. cerevisiae* BSc110 and *Issatchenkia orientalis* BIo148 reduced the disease incidence to 0%, whereas the remaining biocontrol yeasts showed a reduction in the incidence between 65.8% and 88.5%.

Table 1
Disease incidence by 8 phytopathogenic fungi involved in sour rot after inoculation with ***Saccharomyces*** strains on Redglobe grapes. Disease incidence (% of infected wounds) was obtained by inoculation of each fungus and yeast after 5 d of incubation at 25 °C. Means were obtained from three trials. Pathogenicity factors for humans and animals are mentioned and enumerated in the last column.

Saccharomyces biofungicides	Phytopathogenic fungi (sour rot grape)								Pathogenicity
	A. caelatus	*A. carbonarius*	*A. terreus*	*A. versicolor*	*F. oxysporum*	*P. comune*	*R. stolonifer*	*Ulocladium* sp.	
S. cerevisiae BSc22 (M)	▲	▲	▲	▲	▲	▲	▲	35.3 ± 0.9	1(F)
S. cerevisiae BSc62 (M)	▲	▲	▲	11.5 ± 1.2	▲	▲	▲	▲	2(T,F)
S. cerevisiae BSc109 (M)	▲	▲	22.8 ± 1.3	▲	▲	▲	▲	▲	0
S. cerevisiae BSc110 (M)	▲	▲	0	▲	▲	▲	▲	▲	0
S. cerevisiae BSc114 (M)	▲	▲	▲	▲	▲	▲	21.5 ± 1.67	▲	0
S. cerevisiae BSc115 (M)	▲	▲	33.8 ± 2.1	▲	▲	▲	▲	▲	0
S. cerevisiae BSc119 (M)	31.0 ± 1.1	▲	▲	▲	▲	▲	▲	23.8 ± 1.2	0
S. cerevisiae BSc123 (M)	▲	▲	▲	▲	▲	32.1 ± 1.3	▲	▲	0
S. cerevisiae BSc149 (M)	▲	▲	12.9 ± 0.9	▲	▲	▲	▲	▲	0
S. cerevisiae BSc169 (M)	▲	▲	34.2 ± 1.1	▲	▲	▲	▲	33.8 ± 1.9	1(A)
S. cerevisiae BSc172 (M)	▲	▲	11.6 ± 0.7	▲	▲	▲	▲	▲	1(F)
S. cerevisiae BSc187 (M)	▲	▲	33.2 ± 0.68	▲	▲	▲	▲	▲	1(A)
S. cerevisiae BSc206 (M)	▲	▲	22.4 ± 0.1	▲	▲	▲	▲	▲	1(F)
S. cerevisiae BSc218 (G)	▲	▲	▲	▲	▲	22.1 ± 1.5	▲	▲	2(P,T)
S. chevalieri BSch25 (M)	34.8 ± 0.9	▲	▲	▲	▲	▲	▲	▲	1(F)
S. kluyveri BSk11 (M)	▲	▲	▲	▲	33.7 ± 1.12	▲	22.3 ± 1.1	▲	1(F)
Control (H_2O)	▲	▲	▲	▲	▲	▲	▲	▲	–

References, ▲: 100% incidence disease, A: presence of pathogenicity factor: "Adhesion", P: presence of pathogenicity factor: "Proteases", F: presence of pathogenicity factor: "Phospholipases", H: presence of pathogenicity factor: "Pseudohyphal formation", T: presence pathogenicity factor: "Growth at 42 °C", *M*: yeast isolated from fermenting must, *G*: yeast isolated from grapes, *S:* yeast isolated from viticultural soil.

Table 2
Disease incidence by 8 phytopathogenic fungi involved in sour rot after inoculation with **non-*Saccharomyces*** strains on Redglobe grapes. Disease incidence (% of infected wounds) was obtained by inoculation of each fungus and yeast after 5 d of incubation at 25 °C. Means were obtained from three trials. Pathogenicity factors for humans are mentioned and enumerated in the last column.

Non-Saccharomyces biofungicides	Phytopathogenic fungi (sour rot grape)								Pathogenicity
	A. caelatus	*A. carbonarius*	*A. terreus*	*A. versicolor*	*F. oxysporum*	*P. comune*	*R. stolonifer*	*Ulocladium* sp.	
C. catenulata BCc 180 (S)	▲	▲	▲	▲	▲	▲	0	▲	1(A)
C. catenulata BCc185 (S)	▲	▲	33.9 ± 1.8	11.8 ± 0.7	▲	▲	▲	▲	2(T,H)
C. famata BCf210 (M)	▲	▲	23.9 ± 0.7	▲	▲	▲	▲	▲	0
C. rugosa BCr182 (S)	34.6 ± 1.9	▲	▲	▲	▲	▲	▲	▲	2(T,H)
C. sake BCs54 (M)	▲	▲	▲	12.5 ± 1.1	▲	▲	▲	▲	1(F)
C. sake BCs186 (S)	▲	▲	33.9 ± 1.5	▲	▲	▲	▲	▲	1(T)
C. sake BCs192 (M)	▲	▲	▲	▲	▲	0	▲	▲	1(H)
C. sake BCs198 (M)	▲	22.8 ± 0.9	▲	▲	▲	▲	▲	▲	0
C. versatilis BCv222 (G)	▲	▲	▲	▲	▲	0	▲	▲	0
D. vanrijiae BDv179 (S)	▲	▲	22.6 ± 0.8	▲	▲	▲	▲	▲	2(T,H)
D. vanrijiae BDv197 (M)	▲	▲	22.7 ± 1.1	▲	▲	▲	▲	▲	0
D. anomala BDa143 (M)	▲	▲	22.4 ± 1.6	▲	▲	▲	▲	34.7 ± 1.9	1(H)
D. anomala BDa184 (S)	0	▲	▲	▲	▲	▲	▲	▲	0
I. orientalis BIo148 (M)	▲	▲	0	▲	▲	0	▲	▲	2(T,H)
K. marxianus BKm 128 (M)	▲	▲	22.9 ± 1.3	▲	▲	▲	▲	▲	2(T,H)
K. marxianus BKm 145 (M)	▲	▲	11.5 ± 0.9	▲	▲	▲	▲	▲	2(T,H)
P. membranifaciens BPm6 (M)	▲	▲	33.5 ± 2.1	▲	▲	▲	▲	▲	0
P. membranifaciens BPm113 (M)	▲	▲	▲	▲	▲	▲	23.3 ± 1.8	▲	0
S. roseus BSr157(M)	▲	▲	▲	▲	▲	22.5 ± 2.1	▲	▲	0
T. delbrueckii BTd125 (M)	▲	▲	▲	▲	▲	33.2 ± 2.9	▲	▲	0
T. delbrueckii BTd126 (M)	▲	▲	▲	▲	▲	32.15 ± 2.8	▲	▲	0
T. delbrueckii BTd129 (M)	▲	▲	▲	▲	▲	22.25 ± 1.9	▲	▲	0
T. delbrueckii BTd136 (M)	▲	▲	▲	▲	▲	▲	▲	11.7 ± 1.7	0
T. delbrueckii BTd152 (M)	▲	▲	22.7 ± 2.3	▲	▲	▲	▲	▲	1(A)
T. delbrueckii BTd156 (M)	34.0 ± 2.6	▲	▲	▲	▲	▲	▲	▲	0
T. delbrueckii BTd161 (M)	▲	▲	▲	23.2 ± 2.1	▲	▲	▲	▲	1(H)
T. delbrueckii BTd211 (M)	▲	▲	▲	▲	23.9 ± 1.6	▲	▲	▲	3(F,P,H)
Control (H_20)	▲	▲	▲	▲	▲	▲	▲	▲	–

References, ▲: 100% incidence disease, A: presence of pathogenicity factor: “Adhesion”, P: presence of pathogenicity factor: “Proteases”, F: presence of pathogenicity factor: “Phospholipases”, H: presence of pathogenicity factor: “Pseudohyphal formation”, T: presence of pathogenicity factor: “Growth at 42 °C”, *M:* yeast isolated from fermenting must, *G:* yeast isolated from grapes, *S:* yeast isolated from viticultural soil.

3.3.4. Aspergillus versicolor

Four isolates, belonging to *Saccharomyces*, *Torulaspora* and *Candida* genera, significantly decreased the disease incidence between 76.8% and 88.5% (Tables 1 and 2).

3.3.5. Fusarium oxysporum

Two isolates, *Saccharomyces kluyveri* BSk11 and *T. delbrueckii* BTd211, significantly reduced *F. oxysporum* growth, but antagonistic activity by *T. delbrueckii* BTd211 was significantly higher than that by *S. kluyveri* BSk11 (Tables 1 and 2).

3.3.6. Penicillium comune

Seven non-*Saccharomyces* and 2 *Saccharomyces* isolates showed biofungicidal activity (Tables 1 and 2). The non-*Saccharomyces* biocontrol isolates belonged to different species such as *T. delbrueckii*, *I. orientalis*, *S. roseus*, *C. versatilis* and *C. sake*. The *Saccharomyces* isolates belonged to *S. cerevisiae*. *I. orientalis* BIo148, *C. sake* BCs192 and *C. versatilis* BCv222 completely reduced the disease incidence (0%) (Tables 1 and 2).

3.3.7. Rhizopus stolonifer

Two *Saccharomyces* and 2 non-*Saccharomyces* reduced the disease incidence by this pathogen more than 60%. *Candida catenulata* BCc180 completely reduced the incidence, while the other biocontrol yeasts, *S. kluyveri* BSk11, *Pichia membranifaciens* BPm113 and *S. cerevisiae* BSc114, showed a decrease between 78.5% and 76.7% (Tables 1 and 2).

3.3.8. Ulocladium sp.

The disease incidence by this pathogen was reduced 60% or more by 5 yeasts: 3 *Saccharomyces* and 2 non-*Saccharomyces*. *T. delbrueckii* BTd136 strongly inhibited *Ulocladium* growth (11.7% disease incidence), more than *Saccharomyces* and *Dekkera*. All isolates were isolated from fermenting musts (Tables 1 and 2).

Our results show that 3 *Saccharomyces* (*S. cerevisiae* BSc119, *S. cerevisiae* BSc169 and *S. kluyveri* BSk11) and 3 non-*Saccharomyces* (*C. catenulata* BCc185, *D. anomala* BDa143 and *I. orientalis* BIo148) inhibited two phytopathogenic fungi (a disease incidence of 40% or less) (Tables 1 and 2). One strain, *I. orientalis* Bio148, completely suppressed two fungi (Table 2), and 5 other isolates completely inhibited one fungus: *S. cerevisiae* BSc110 (Table 1), *C. sake* BCs192, *C. versatillis* BCv222, *C. catenulata* BCc180 and *D. anomala* BDa184 (Table 2). The remaining yeasts (36) reduced the disease incidence between 65.0 and 88.2% (Tables 1 and 2).

3.4. Phenotypical assaying for human pathogenicity of biocontrol yeasts

All 43 isolates that *in vivo* reduced the disease incidence to 40% or less were assayed for pathogenicity characteristics.

None of the 43 selected yeasts presented all pathogenicity factors assayed. *T. delbrueckii* BTd211 was the most virulent strain with 3 pathogenicity factors: pseudohyphal formation, and production of phospholipase and protease (Table 2). Eight biocontrol yeasts presented 2 pathogenicity characteristics (2 *Saccharomyces*, 2 *Kluyveromyces*, 1 *Issatchenkia*, 2 *Candida*, 1 *Debaryomyces*) and 14 isolates showed 1 factor (4 *Candida*, 1 *Dekkera*, 7 *Saccharomyces*, 2 *Torulaspora*) (Tables 1 and 2). The twenty strains (3 *Candida*, 1 *Debaryomyces*, 1 *Dekkera*, 2 *Pichia*, 7 *Saccharomyces*, 1 *Sporobolomyces* and 5 *Torulaspora*) did not present human pathogenicity factors (Tables 1 and 2).

4. Discussion

Sour rot is one of the most important diseases that affect the quality of table and wine grapes (Lisperguer et al., 2003). Involvement of different filamentous fungi in sour rot-damaged grapes has been reported previously. In Chile, Latorre et al. (2002) isolated phytopathogenic filamentous fungi like *Aspergillus*, *Penicillium, Rhizopus*, *Cladosporium* and *Mucor* from sour rot-damaged grapes. In Argentina, Díaz and Krauz, 2002 isolated *Penicillium*, *Ulocladium*, *Cladosporium* and *Rhizopus*. In the present study, the 8 phytopathogenic filamentous fungi isolated from grapes with sour rot belonged to the following 5 genera: *Aspergillus*, *Ulocladium*, *Penicillium*, *Rhizopus* and *Fusarium*.

This is the first study that refers to the *Fusarium* genus as a possible agent of sour rot in grapes. *F. oxysporum* is considered a normal constituent of fungal communities in the rhizosphere (Fravel et al., 2005). This fungus has been reported to be responsible for different diseases such as "vascular wilt" and "Corm rot" in gladiolus species (Shanmugam et al., 2011), "potato dry rot" in potatoes (García Bayona et al., 2011), "Fusarium wilt" in bananas (Akila et al., 2011), and "tomato foot" and "root rot" in tomatoes (Validov et al., 2007).

Several reports have shown the potential use of different biofungicidal yeasts regarding table grapes (Masih et al., 2001; Salmon, 2009; Nally et al., 2012). However, there are no studies that have assessed the biocontrol activity of yeasts against filamentous fungi involved in grapes with sour rot. The present work deals with this problem and it is the first study that has assayed the biofungicidal activity of viticultural yeasts against 8 filamentous fungi involved in sour rot. Forty-three isolates (18.3%), most of which were isolated from fermenting musts, inhibited several sour rot filamentous fungi in grapes (35). The biofungicidal yeasts selected belonged to different genera such as *Candida*, *Debaryomyces*, *Dekkera*, *Issatchenkia*, *Kluyveromyces*, *Pichia*, *Sporobolomyces*, *Torulaspora* and *Saccharomyces* (Tables 1 and 2). *Debaryomyces*, *Dekkera*, *Sporobolomyces* and *Torulaspora* have not been previously reported as grape biofungicides, contrary to the other genera: *Saccharomyces* (Salmon, 2009; Nally et al., 2012), *Candida* (Zahavi et al., 2000; Bleve et al., 2006; Cañamás et al., 2008), *Pichia* (Masih et al., 2001), *Kluyveromyces* (Ponsone et al., 2011) and *Issatchenkia* (Bleve et al., 2006). In the present study, 14 *S. cerevisiae* strains showed antifungal activity against *A. terreus*, *P. comune*, *Ulocladium* sp., *A. versicolor*, *R. stolonifer* and *A. caelatus* when assayed in grapes (Table 1). Several researchers have reported on the biocontrol activity of *S. cerevisiae* against different pathogens: *Penicillium roqueforti* in stored wheat (Petersson and Schnurer, 1995), *Macrophomina phaseolina* and *Fusarium solani* in tomatoes (Attyia and Youssry, 2001), *B. cinerea* in grapes (Salmon, 2009; Nally et al., 2012), *Monilia fruticola* in apples (Zhou et al., 2008) and *Alternaria alternata* in pinus (Payne et al., 2000). However, thus far there are no reports about *S. cerevisiae* as biocontrol agent of filamentous fungi isolated from sour rot-damaged grapes. Despite the high antifungal potential of *S. cerevisiae*, there is presently no commercial biocontrol product containing this species.

In a previous study by Nally et al. (2012) antagonistic activity of 16 *Saccharomyces* and 1 *Schizosaccharomyces* was reported against *B. cinerea* in Redglobe grapes. However, none of these isolates showed antagonistic activity against the filamentous fungi assayed in the present study.

Karabulut and Baykal (2003) showed that *Metchnikowia fructicola* (AgroGreen SHEMER® Ltd) decreased the incidence of *B. cinerea* (45.67%) and *Alternaria* spp. (39.47%), and completely inhibited *A. niger* in Thompson Seedless grapes in Israel. In the present study, 6 biocontrol yeasts, *S. cerevisiae* (2), *S. kluyveri* (1) (Table 1), *C. catenulata* (1), *D. anomala* (1) and *I. orientalis* (1) (Table 2), significantly inhibited two phytopathogenic fungi in grapes stored at 25 °C (disease incidence between 0 and 35.3%).

None of the yeasts selected as biocontrol agents (Tables 1 and 2) produced rot in berries, although some of them, *Candida* (Guerzoni and Marchetti, 1982, 1987; Barata et al., 2008), *Issatchenkia* (Barata et al., 2008) and *Pichia* (Guerzoni and Marchetti, 1987) have been considered to be involved in grape sour rot.

Some fungal properties are frequently associated with pathogenesis, *e.g.* the ability to grow at high temperatures, pseudohyphal formation, and secretion of degradative enzymes such as proteinases and phospholipases to invade host cells (de Llanos et al., 2006). The present study assessed the pathogenicity of 43 viticultural yeasts selected as biocontrol agents in grapes (Tables 1 and 2) (growth at 42 °C, invasive growth, pseudohyphae formation, and production of phospholipases and proteases). Twenty-three biocontrol yeasts, identified as *Candida*, *Dekkera*, *Debaryomyces*, *Issatchenkia*, *Kluyveromyces*, *Torulaspora* and *Saccharomyces* (Tables 1 and 2), showed pathogenicity to humans and animals. Some of these genera have been associated with human infections: *Saccharomyces* (Piarroux et al., 1999; Lherm et al., 2002; Cassone et al., 2003; Enache-Angoulvant and Hennequin, 2005) *Kluyveromyces* (Lutwick et al., 1980; Nielsen et al., 1990; García-Martos et al., 1999) and *Candida* (Krcmery and Barnes, 2002). Proteinase secretion occurs in *Candida* and *S. cerevisiae* species (de Llanos et al., 2006; Nally et al., 2012), and phospholipases have been detected in *C. albicans*, *C. neoformans*, *C. glabrata, S. cerevisiae*, *Malassezia furfur* and *Rhodotorula rubra* (Chen et al., 2000; Kantarcioglu and Yucel, 2002; de Llanos et al., 2006; Nally et al., 2012). Pseudohyphal formation and invasive growth are other pathogenicity factors for humans detected in *Saccharomyces* yeasts (de Llanos et al., 2006; Nally et al., 2012).

5. Conclusions

Viticultural yeasts isolated from fermenting musts, vineyard soil and table grapes were found effective as *in vivo* biocontrol agents against filamentous fungi involved in grape sour rot. The current study established that several *Saccharomyce*s and non-*Saccharomyces* strains inhibited eight fungi involved in sour rot in grapes, although to a different degree. To the best of our knowledge this is the first report that has studied pathogenicity of yeasts belonging to the following species: *C. catenulata*, *C. famata*, *C. rugosa*, *C. sake*, *C. versatilis*, *D. vanrijiae*, *D. anomala*, *K. marxianus*, *P. membranifaciens*, *S. roseus*, *S. chevalieri* and *S. kluyveri*. Results of this research can encourage studies in other pathosystems in which *Aspergillus, Fusarium, Ulocladium, Rhizopus* and *Penicillium* genera are serious pathogens. This is also the first study that mentions the presence of *F. oxysporum* in sour rot-damaged grapes. This work is an initial step concerning the possible application of viticultural yeasts for sour rot disease prevention. It is necessary to evaluate culture conditions of the yeasts at an industrial level, and implement some field tests for commercial use of these selected strains, taking into account that sour rot develops at pre-harvest and postharvest conditions. In future studies it would be important to evaluate the antifungal activity of selected biocontrol yeasts in pure and mixed cultures, against phytopathogenic bacteria and yeasts isolated from sour rot-damaged grapes.

References

Akila, R., Rajendran, L., Harish, S., Saveetha, K., Raguchander, T., Samiyappan, R., 2011. Combined application of botanical formulations and biocontrol agents for the management of *Fusarium oxysporum* f. sp. cubense (Foc) causing Fusarium wilt in banana. Biol. Control 57, 175–183.

Attyia, S.H., Youssry, A.A., 2001. Application of *Saccharomyces cerevisiae* as a biocontrol agent against some diseases of *Solanaceae* caused by *Macrophomina phaseolina* and *Fusarium solani*. Egypt. J. Biol. 3, 79–87.

Banerjee, U., 2009. Oportunistic pathogenic yeasts. In: Satyanarayana, T., Kunze, G. (Eds.), Yeast Biotechnology: Diversity and Applications. Springer Science Business Media BV, New York, ISBN 978-1-4020-8291-7, p. 215.

Barata, A., Gonzalez, S., Malfeito Ferreira, M., Querol, A., Loureiro, V., 2008. Sour rot-damaged grapes are sources of wine spoilage yeasts. FEMS Yeast Res. 8, 1008–1017.

Barata, A., 2011. Microbial ecology of sour rotten grapes and their influence on chemical and sensorial wine quality. Tese apresentada para obtenção do grau de Doutor em Engenharia Alimentar. Instituto Superior de Agronomía, Universidad Técnica de Lisboa, Portugal, pp. 6.

Barata, A., Malfeito-Ferreira, M., Loureiro, V., 2012. The microbial ecology of wine grape berries. Int. J. Food Microbiol. 3, 243–259.

Bleve, G., Grieco, F., Cozzi, G., Logrieco, A., Visconti, A., 2006. Isolation of epiphytic yeast with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. Int. J. Food Microbiol. 108, 204–209.

Bouzerda, L., Boubaker, H., Boudyach, E.H., Akhayat, O., Aoumar, A.A., 2003. Selection of antagonistic yeasts to green mold disease of Citrus in Morocco. Food Agric. Environ. 1, 215–218.

Cañamás, T.P., Viñas, I., Usall, J., Magan, M., Solsona, C., Teixidó, N., 2008. Impact of mild heat treatments on induction of thermotolerance in the biocontrol yeast *Candida sake* CPA-1 and viability after spray-drying. J. Appl. Microbiol. 104, 767–775.

Cassone, M., Serra, P., Mondello, F., Giolamo, A., Scafetti, S., Pistella, E., Venditti, M., 2003. Outbreak of *Saccharomyces cerevisiae* subtype *boulardii* fungemia in patients neighbouring those treated with a probiotic preparation of the organism. J. Clin. Microbiol. 41, 5340–5343.

Chanchaichaovivat, A., Ruenwongsa, P., Panijpan, B., 2007. Screening and identification of yeast strains from fruits and vegetables: Potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*). Biol. Control 42, 326–335.

Chen, W.B., Han, Y.F., Jong, S.C., Chang, S.C., 2000. Isolation purification and characterization of a killer protein from *Schwanniomyces occidentalis*. Appl. Environ. Microbiol. 66, 5348–5352.

Commenil, P., Belingheri, L., Bauw, G., Dehorter, B., 1999. Molecular characterization of a lipase induced in *Botrytis cinerea* by components of grape berry cuticle. Physiol. Mol. Plant Pathol. 55, 37–43.

de Llanos, R., Fernandez-Espinar, M.T., Querol, A., 2006. A comparison of clinical and food Saccharomyces cerevisiae isolates on the basis of potential virulence factors. Antonie van Leeuwenhoek 90, 221–231.

de Llanos Frutos, R., 2007. Estudio comparativo entre aislados clínicos y no clínicos de S. cerevisiae y su papel como patógeno emergente. Tesis doctoral. Instituto de Agroquímica y tecnología de los alimentos (CSIC), Facultad de Ciencias Biológicas, Departamento de Microbiología y Ecología, Universidad de Valencia, España, pp. 7.

Díaz, R., Krauz, C., 2002. Aislamiento, caracterización e identificación de los agentes causales de pudrición ácida de los racimos en vides de mesa cv. Red globe, 37. In: XI Congreso Nacional de Fitopatología, Argentina, pp. 67–108.

Diekema, D.J., Petroelje, B., Messer, S.A., Hollis, R.J., Pfaller, M.A., 2005. Activities of available and investigational antifungal agents against *Rhodotorula* species. J. Clin. Microbiol. 43, 476–478.

Diguta, C.F., Vincent, B., Guilloux-Benatier, M., Alexandre, H., Rousseaux, S., 2011. PCR ITS-RFLP: a useful method for identifying filamentous fungi isolates on grapes. Food Microbiol. 28, 1145–1154.

Donoso, A., Latorre, B.A., 2006. Caracterización del moho azul causado por *Penicillium* spp, en uva de mesa almacenada en frío. Cien. Inv. Agr. 33, 143–155.

Droby, S., Hofstein, R., Wilson, C.L., Wisniewski, M., Fridlender, B., Cohen, L., Weiss, B., Daus, A., Timar, D., Cahlutz, E., 1993. Pilot testing of *Pichia guilliermondii*: A biocontrol agent of postharvest diseases of citrus fruit. Biol. Control 3, 47–59.

El-Ghaouth, A., Wilson, C.L., Wisniewski, M., 1998. Ultrastructural and cytochemical aspects of the biological control of *Botrytis cinerea* by *Candida saitoana* in apple fruit. Phytopathology 88, 282–291.

El-Ghaouth, A., Smilanick, J.L., Wilson, C.L., 2000. Enhancement of the performance of *Candida saitoana* by the addition of glycolchitosan for the control of postharvest decay of apple and citrus fruit. Post. Biol. Technnol. 19, 103–110.

Enache-Angoulvant, A., Hennequin, C., 2005. Invasive *Saccharomyces* infection: a comprehensive review. Clin. Infect. Dis. 41, 1559–1568.

Fravel, D.R., Deahl, K.L., Stommel, J.R., 2005. Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides. Biol. Control 34, 165–169.

García Bayona, L., Grajales, A., Cardenas, M.E., Sierra, R., Cepero de Garcia, M.C., Bernal, A., Jimenez, P., Restrepo, S., 2011. First report of *Fusarium oxysporum* causing potato dry rot in *Solanum tuberosum* in Colombia. New Dis. Rep. 24, 14.

García-Martos, P., Hernandez-Molina, J.M., Galan, F., Ruiz-Henestrosa, J.R., Garcia-Agudo, R., Palomo, M.J., Mira, J., 1999. Isolation of *Hanseniaspora uvarum* (*Kloeckera apiculata*) in humans. Mycopathologia 144, 73–75.

Garmendia, G., Garat, F., Silvera, E., de Aurrecoechea, I., Vero, S., 2005. Levaduras nativas como controladores de enfermedades postcosecha de frutas en Uruguay. Agrocienca 1/2, 327–335.

Guerzoni, M.E., Marchetti, R., 1982. Microflora associata al marciume ácido della vite e modificazioni indotte dalla malattia sulla composizione di uve e mosti. Difesa Piante 4, 231–246.

Guerzoni, E., Marchetti, R., 1987. Analysis of yeast flora associated with grape Sour rot and of the chemical disease markers. Appl. Environ. Microbiol. 53, 571–576.

Gust, A.A., Brunner, F., Nurnberger, T., 2010. Biotechnological concepts for improving plant innate immunity. Curr. Opin. Biotechnol. 21, 204–210.

Guzzon, R., Widmann, G., Settanni, L., Malacarne, M., Francesca, N., Larcher, R., 2011. Evolution of yeast populations during different biodynamic winemaking processes. S. Afr. J. Enol. Viticult. 32, 2.

Hashim-Buckey, J., Mlikota Gabler, F., Mansour, M.F., Schrader, P., Pryor, M., Margosan, D.A., Smilanick, J.L., 2008. Effectiveness of preharvest applications of fungicides on preharvest Bunch rot and postharvest Sour Rot of 'Redglobe'. In: Grapes Proceedings of the 2nd Annual National Viticulture Research Conference, University of California, Davis, pp. 33–36.

Hewstone, N., Valenzuela, J., Muñoz, C., 2007. Nueva variedad de uva de mesa. Agricultura Técnica 67, 201–204.

INTA, 2012. Instituto Nacional de Tecnología Agropecuaria. Argentina. www.inta.gob.ar

Kantarcioglu, A.S., Yucel, A., 2002. Phospholipase and protease activities in clinical *Candida* isolates with reference to the sources of strains. Mycoses 45, 160–165.

Karabulut, O.A., Baykal, N., 2003. Biological control of postharvest diseases of peaches and nectarines by yeast. J. Phytopathol. 151, 130–134.

Krcmery, V., Barnes, A.J., 2002. Non- albicans *Candida* spp causing fungaemia: pathogeneity and antifungal-resistance. J. Hospital Infect. 50, 243–260.

Latorre, B., Franck, J., Zoffoli, J.P., Viertel, S., 2002. Pudrición Ácida de la vid. Revista Frutícola (Chile) 23, 53–58.

Lherm, T., Monet, C., Nougiere, B., Soulier, M., Larbi, D., Le Gall, C., Caen, D., Malbrunot, C., 2002. Seven cases of fungemia with *Saccharomyces bouldardii* in critically ill patients. Intens. Care Med. 28, 797–801.

Lima, G., Arru, S., De Curtis, F., Arras, G., 1999. Influence of antagonist, host fruit and pathogen on the biological control of postharvest fungal diseases by yeast. J. Int. Microbiol. Biotechnol. 23, 223–229.

Lisperguer, S., Piontelli, E., Campos, V., 2003. Aislamiento y determinación de microorganismos presentes en la pudrición acida de uvas de mesa, variedad Red Globe. In: XII Congreso Nacional de fitopatología Puerto Varas, X Región, Chile.

Lutwick, L.I., Phaff, H.J., Stevens, D.A., 1980. Kluyveromyces fragilis as an opportunistic fungal pathogen in man. Sabouraudia 18, 69–73.

Martínez- Romero, D., Guillén, F., Valverde, J.M., Bailén, G., Zapata, P., Serrano, M., Castillo, S., Valero, D., 2007. Influence of carvacrol on survival of *Botrytis cinerea* inoculated in table grapes. Int. J. Food Microbiol. 115, 144–148.

Masih, E.I., Slezack-Deschaumes, S., Marmaras, I., Ait Barka, E., Vernet, G., Charpentier, C., Adholeya, A., Paul, B., 2001. Characterization of the yeast *Pichia membranifaciens* and its possible use in the biological control of *Botrytis cinerea*, causing the grey mould disease of grapevine. FEMS Microbiol. Lett. 202, 227–232.

Mateluna Estay, R.A., 2006. Estudio de actividad antibacteriana de potenciales biocontroles sobre bacterias acéticas involucradas en la pudrición ácida de la uva. Tesis para optar al título de Ingeniero en Alimentos. Universidad de Chile, Facultad de Ciencias Químicas y Farmacéuticas, Chile, pp. 15.

Mercier, J., Wilson, C.L., 1994. Colonization of apple wound by occurring microflora and introduced *Candida oleophila* and their effect on infection by *Botrytis cinerea* during storage. Biol. Control 4, 138–144.

Mlikota Gabler, F., Smilanick, J.L., 2001. Postharvest control of table grape gray mold on detached berries with carbonate and bicarbonate salts and disinfectants. Am. J. Enol. Viticult. 52, 12–20.

Nally, M.C., Pesce, V.M., Maturano, Y.P., Muñoz, C.J., Combina, M., Toro, M.E., Castellanos de Figueroa, L.I., Vazquez, F., 2012. Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina. Post. Biol. Technol. 64, 40–48.

National Committee for Clinical Laboratory Standards (NCCLS), 1997. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts—Approved Standard M27-A. NCCLS, Villanova, PA.

Nielsen, H., Stenderup, J., Bruun, B., 1990. Fungemia with *Saccharomycetaceae*. Report of four cases and review of the literature. Scand. J. Infect. Dis 22, 581–584.

Oriolani, E., Rodriguez, M., Combina, M., 2007. Etiología de la podredumbre ácida de los racimos de la vid en Mendoza y San Juan, Argentina. In: 30° Congreso Argentino de Horticultura, La Plata, Argentina.

Oriolani, E., Romera Rodríguez, M., Combina, M., 2009. La Podredumbre ácida de los racimos de la vid, en los viñedos de San Juan y Mendoza, Argentina. In: II Simposio Internacional de Uva de Mesa y Pasa. San Juan, Argentina.

Palou, L., Smilanick, J.L., Droby, S., 2008. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Rev. 1, 1.

Payne, C., Bruce, A., Staines, A., 2000. Yeast and bacteria as biological biontrol agents against fungal discolouration of *Pinus sylvestris* blocks in laboratory-based tests and the role of antifungal volatiles. Hozoforschung 54, 563–569.

Petersson, S., Schnurer, J., 1995. Biocontrol of mold growth in high- moisture wheat stored under airtight conditions by *Pichia anomala*, *Pichia guilliermondii* and *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 61, 1027–1032.

Piarroux, R., Millon, L., Bardonnet, K., Vagner, O., Koenig, H., 1999. Are live *Saccharomyces* yeasts harmful to patients. The Lancet 353, 1851–1852.

Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage, 2nd ed. Blackie Academic and Professional, London.

Ponsone, M.L., Chiotta, M.L., Combina, M., Dalcero, A., Chulze, S., 2011. Biocontrol as a strategy to reduce the impact of ochratoxin A and *Aspergillus* section Nigri in grapes. Int. J. Food Microbiol. 15, 70–77.

Rabosto, X., Carrau, M., Paz, A., Boido, E., Dellacassa, E., Carrau, F.M., 2006. Grapes and vineyard soils as sources of microorganisms for biological control of *Botrytis cinerea*. Am. J. Enol. Viticult. 57, 332–338.

Salmon, J.M., 2009. Evaluación de la pulverización de levaduras como herramienta para reducir las enfermedades fúngicas en las uvas para vino. Revista internet de Viticultura y Enología N° 11/2 www.infowine.com

SENASA, 2012. Servicio Nacional de Sanidad y Calidad Agroalimentaria (National Health Service and Food Quality). www.senasa.gov.ar

Shanmugam, V., Kanoujia, N., Singh, M., Singh, S., Prasad, R., 2011. Biocontrol of vascular wilt and corm rot of gladiolus caused by *Fusarium oxysporum* f. sp. gladioli using plant growth promoting rhizobacterial mixture. Crop Protect. 7, 807–813.

Suzzi, G., Romano, P., Ponti, I., Montuschi, C., 1995. Natural wine yeast as biocontrol agents. J. Appl. Bacteriol. 78, 304–308.

Taqarort, N., Echairi, A., Chaussod, R., Nouaim, R., Boubaker, H., Benaoumar, A.A., Boudyach, E., 2008. Screening and identification of epiphytic yeasts with potential for biological control of green mold of citrus fruits. World J. Microbiol. Biotechnol. 24, 3031–3038.

Utkhede, R., Bogdanoff, C., McNevin, J., 2001. Effects of biological and chemical treatments on *Botrytis* stem canker and fruit yield of tomato under greenhouse conditions. Can. J. Plant Pathol. 23, 253–259.

Validov, S., Kamilova, F., Qi, S., Stephan, D., Wang, J.J., Makarova, N., Lugtenberg, B., 2007. Selection of bacteria able to control *Fusarium oxysporum* f sp. radicis-lycopersici in stonewool substrate. J. Appl. Microbiol. 102, 461–471.

World Health Organization (WHO), 2004. Laboratory Biosafety Manual, 3rd ed, Available at: http://www.who.int/en/ (accessed online 2006).

Zahavi, T., Cohen, L., Weiss, B., Schena, L., Daus, A., Kaplunov, T., Zutkhi, J., Ben- Arie, R., Droby, S., 2000. Biological control of *Botrytis Aspergillus* and *Rhizopus* rots on table and wine grapes in Israel. Post. Biol. Technol. 20, 115–124.

Zhou, T., Schneider, K.E., Li, X.Z., 2008. Development of biocontrol agents from food microbial isolates for controlling post-harvest peach brown rot caused by *Monilinia fructicola* I. J. Food Microbiol. 126, 180–185.