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Survival of Spray-Dried *Rhodotorula mucilaginosa* Isolated from Natural Microbiota of Murta Berries and Antagonistic Effect on *Botrytis cinerea*

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SUMMARY

The aim of this study is to evaluate the survival rate and effective antagonistic activity against *Botrytis cinerea*, responsible for grey mould on harvested fruits and vegetables, of yeast *Rhodotorula mucilaginosa*, isolated and identified from the natural microbiota of murta (Chilean guava) berries, after spray drying at different inlet air temperatures, mass per volume ratio of encapsulating agent (maltodextrin) and feed flow rates. The 100 % survival of the yeast was obtained after spray drying with 18 % maltodextrin at 130 °C inlet temperature and a feed flow rate of 9.25 mL/min. The dried yeast obtained under such conditions had the highest antagonistic activity *in vitro* and *in vivo* on apples, which showed that spray drying is a valid method to produce active dried cells of *R. mucilaginosa* that can be used for biocontrol of grey mould spoilage. It was also found that the encapsulating agent maltodextrin improved the *in vitro* antagonistic activity of *R. mucilaginosa*.

Key words: dehydration, yeast, grey mould, phytopathogenic fungus, antagonistic activity, biocontrol

INTRODUCTION

Botrytis cinerea is a phytopathogenic fungus responsible for grey mould spoilage of many fruit and vegetable products around the world (*1*). It is a latent postharvest hazard that can provoke spoilage of fruit and vegetable products during storage and transport, when unfavourable conditions, such as physical damage, cellular senescence or high relative humidity of air, may occur and increase the vulnerability of vegetable products (*1–3*). The main strategy to control postharvest diseases is still through the use of chemical fungicides (*4*). However, their application is associated with many drawbacks and can induce fungicide resistance in pathogens (*5,6*), affect the biota *via* toxic mechanisms, accumulate in living organisms or in underground water, have harmful effect on human health or cause environmental pollution (*6*). Due to a high genetic variability, a short life cycle and a prolific reproduction, *B. cinerea* is considered a highly hazardous pathogen that develops resistance to chemical fungicides (*7*), especially if applied too frequently (*1*). This is one of the main reasons for the emergence of biocontrol agents that are being applied as biological and natural alternatives to chemical fungicides in reducing disease incidence provoked by phytopathogens in vegetable products (*8–11*).

The biocontrol agents are mostly living microorganisms (*12*) used to counteract the effect of pathogens on the plants by reducing their growth and activity, thus preventing development of diseases that cause huge financial loss in the agricultural sector (*9,10*). Yeasts stand out as biocontrol agents against pathogens in many agricultural products, due to their versatile characteristics, such as genetic stability, safe application, wide antagonistic range (*11*), tolerance to extreme pre- and postharvest conditions, high adaptability to vegetable microenvironment, and rapid growth on complex and cheap substrates in fermentors, which all leads to easy production in large quantity (*13,14*). Among the yeast species that have proved to be effective against various pathogens, including *B. cinerea*, the following can be mentioned among others: *Metschnikowia fructicola* against *Penicillium expansum*

(*15*), *Candida oleophila* against *P. expansum* and *B. cinerea* (*16*), *Pichia guilliermondii* against *B. cinerea* (*17*) or *Pichia membranifaciens* against *Colletotrichum gloeosporioides* (*18*). This has led to the development of many commercial products for application within a biological control strategy against plant pathogens, which have been registered in countries of the European Union (Germany, Spain) and Israel (*19*). Many other yeast species have also been studied and have shown biocontrol activity against postharvest diseases; however, their development to a commercial product has not been achieved, due to low efficiency and stability and failure to meet the requirement for application on vegetable crops (*3*). Moreover, from a commercial standpoint high yield and high antagonistic efficiency depend on a strict monitoring of production, formulation and stabilization of the microorganisms during packing, storage and application (*19*). Frequently, the microbial biocontrol agents are found active in liquid formulations, which have the main disadvantage of large volume and require refrigeration during storage and transport (*20*). Therefore, to enable the development of a stable commercial product, the liquid formulations must be submitted to a drying process that does not affect the biocontrol activity of the fresh cells (*21*). Various drying processes have been applied, among others, freeze drying of *Candida sake* CPA-1 (*22*), *Pichia anomala* J121 (*23,24*), *Cryptococcus laurentii* and *Rhodotorula glutinis* (*25,26*), fluidized bed drying on *Cryptococcus flavescens* OH 182.9 (*27*), *P. anomala* J121 (*24*) and *Aureobasidium pullulans* (*28*) and spray drying on *C. sake* CPA-1 (*20,21*). Freeze drying is considered the most appropriate and least harmful method to dry microorganisms (*24*); nonetheless, it is a high-cost process with long drying time and high energy consumption (*29*). On the other hand, spray drying is the main drying process used on an industrial scale (*29–32*), due to its high production rate and low operation cost (*33,34*). However, the survival rate of the microorganisms during spray drying is generally very low and measures must be taken to protect them (*35*). Therefore, we conducted assays to assess the effect of spray drying on survival rate and effective antagonistic activity of *Rhodotorula mucilaginosa* yeast *in vitro* and *in vivo* against *B. cinerea,* identified in the natural microbiota of murta (Chilean guava) berries. The spray drying was evaluated at different inlet air temperatures, mass per volume ratios of maltodextrin as encapsulating agent and feed flow rates.

MATERIALS AND METHODS

Microorganisms and their preparation for antagonistic assays

Rhodotorula mucilaginosa was isolated from the surface of fresh murta (*Ugni molinae* Turcz) berries, obtained in the neighborhood of Curanilahue, in the Province of Arauco, Region of Biobío, Chile (37°28'S, 73°21'W). It was initially unidentified among around 168 yeast strains obtained from the surfaces of different plants in a preliminary study on biofungicidal control of grey mould. The yeast was selected due to its vigorous growth and its origin from the native Chilean guava (murta) berries. It was kept in glycerol (Calbiochem, San Diego, CA, USA) mixed with diluted yeast extract-peptone-dextrose (YPD) broth (yeast extract 2 g/L, peptone 4 g/L and dextrose 4 g/L; Becton Dickinson and Company, Franklin Lakes, NJ, USA), adjusted to pH=6.0 and maintained at –20 °C until use in the antagonistic assays. To prepare the yeast culture for the assays, it was first reactivated in 20 mL of diluted YPD broth in a 100-mL conical flask and incubated at 30 °C on a shaker (IS-971; Lab Companion, Seoul, Republic of Korea) at 200 rpm for 22 h. The preculture of the yeast was then inoculated in diluted YPD broth to a total volume of 200 mL and further incubated under the same conditions.

Grey mould *Botrytis cinerea* was isolated from the surface of infected murta berries. The culture was maintained at 4 °C on potato dextrose agar (PDA; Becton Dickinson and Company) until use in the antagonistic assays, when they were first incubated for 7 days at 25 °C on PDA. The suspensions of *B. cinerea* spores required for the antagonistic assays were then prepared by adding sterile water to the sporulating edges of the culture and separating the mycelium from the spores by filtration through four layers of sterile cotton gauze. Spore count was determined using the Neubauer chamber.

Identification of antagonistic yeast found on murta berries

To identify the yeast *R. mucilaginosa*, 5.8S internal transcribed spacer, ITS1-5.8s-ITS2, was first amplified using polymerase chain reaction (PCR). Total DNA was extracted using the extraction kit E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-tek, Inc., Norcross, GA, USA). The primers used were: 5'-TCCGTAGGTGAACCTGCGG-3' for ITS1 and 5'-TCCTCCGCTTATTGATATGC-3' for ITS4. The PCR operating conditions were: 95 °C for 2 min denaturation; 30 thermal cycles comprising 30 s at 95 °C, 30 s at 56.8 °C annealing temperature and 1 min at 72 °C; 2 min at 72 °C and finally 5 min at 4 °C. The PCR product was excised from the agarose gel and purified with Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The DNA sequencing was done by Macrogen, Inc. (Seoul, Republic of Korea). The obtained nucleotide sequence was identified by comparison with available nucleotide sequences of the GenBank (*36*), using the Basis Local Alignment Search Tool (BLAST) algorithm (*37*).

Spray drying, survival rate, moisture content and yeast powder yield

The spray drying was performed using the laboratory scale spray dryer (SD-Basic; Lab Plant, Hunmanby, UK). Assays were conducted at two inlet temperatures (130 and 140 °C), using two feed flow rates (6.30 and 9.25 mL/min) and 18 and 26 % maltodextrin (PRINAL, Santiago, Chile) as encapsulating agent. *R. mucilaginosa* yeast cells were first centrifuged (Himac CR 21G II; Hitachi, Tokio, Japan) at 6000×*g* and 4 °C for 6 min and then resuspended in sterile distilled water with the corresponding mass of maltodextrin to obtain the suspension that was then dried in the spray dryer. In each assay survival rate, moisture content and yeast powder recovery yield were determined.

Survival rate was determined as a percentage of living yeast cells in the dried powder with respect to the living yeast cells in the initial feed suspension before spray drying:

$$\text{Survival rate}=(N_p/N_s)\cdot 100 \qquad /1/$$

where N_p and N_s are yeast cell counts in the dried powder and in the feed suspension respectively, expressed as living cells per g of dry matter. Yeast cell count was determined using plate count method on PDA. Survival rate can also be expressed in terms of decimal reduction (*20*,*21*) as follows:

$$\text{Survival rate}=105.07\cdot e^{2.3035 \log (N_p/N_s)} \qquad /2/$$

Moisture and dry matter contents were determined in 1 g of the spray-dried powder or in 1 mL of the feed suspension using a convective oven (ZRD A5055; Zhicheng, Shangai, PR China) at 105 °C for 24 h. Yeast powder yield was calculated as a percentage of the dry matter in the spray-dried yeast powder to dry matter in the feed suspension.

Determination of in vitro antagonistic activity

The antagonistic activity of the yeast was determined according to the method described by Zhang *et al.* (*38*) with some modifications. The PDA plate with a centred well of 12 mm in diameter was prepared by first pouring 10 mL of sterile PDA on a 90 mm×15 mm Petri dish. After solidification, a sterile 12-mm plastic ring was placed in the centre of the dish before pouring another 25 mL of PDA. The ring was removed before inoculation with 100 µL of yeast suspension (10^7 cells/mL) obtained from fresh or dried cells. The samples of yeast suspension were taken at the stationary phase from 200 mL of diluted YPD culture broth of the fresh or the reactivated dehydrated yeast cells of *R. mucilaginosa*. In the control well, 100 µL of sterile water were added. After 2 h, 100 µL of the *B. cinerea* suspension, containing 10^5 spores/mL, were added and incubated at 28 °C for 5 days, after which the colony diameter of *B. cinerea* was measured.

Determination of in vivo antagonistic activity

The *in vivo* antagonistic activity of spray-dried *R. mucilaginosa* against *B. cinerea* on apples was assayed using the samples with the best survival rate. For the assays, three sets of 40 apples were used; one set, treated with a commercial biocontrol agent (Trichonativa®, Bio Insumos Nativa SPA, Talca, Chile) based on *Trichoderma* spp. and *B. cinerea* served as a control, the second set was inoculated only with *B. cinerea* and the third set was inoculated with both *R. mucilaginosa* and *B. cinerea*. Before the assays, the apples were first disinfected by spraying with 70 % aqueous ethanol (Winkler Ltda., Santiago, Chile) and then dipped in 1 % sodium hypochlorite solution (Winkler Ltda.) for 1 min, followed by thorough rinsing with sterile water for 5 min. For the assays, the disinfected apples were punctured at three equidistant spots on the equatorial line to cause three wounds of 2 mm, then inoculated with 10 µL of a suspension of *R. mucilaginosa* (10^7 cells/mL) or *Trichoderma* spp. (10^9 CFU/mL) and left to dry in a laminar flow cabinet (AHC-4D; ESCO, Singapore) for 2 h. Afterwards, the apples were inoculated with 10 µL of a *B. cinerea* suspension (10^6 spores/mL) and incubated in humidity chamber (*38*) consisting of a closed plastic box to maintain a high relative humidity (about 95 %) and incubated at (18±2) °C for 10 days. Antagonistic activity was determined by measuring the diameter of the lesion.

Statistical analysis

The data acquired in triplicate were analyzed by a one-way analysis of variance (ANOVA) using the statistical software Statgraphics Centurion XVI.II (Statistical Graphics Corp., Herndon, VA, USA) for three factors (inlet temperature, maltodextrin mass per volume ratio and feed flow rate) at two levels, with a total of 24 assays (2^3×3). Normality and homogeneity of variance were analyzed using respectively the Shapiro-Wilk and Levene tests. The Kruskal-Wallis test was applied for non-compliance analysis of mean values.

RESULTS AND DISCUSSION

Identification of the antagonistic yeast

Most antagonistic microorganisms used in the biocontrol of postharvest diseases come from natural sources, usually from the microbiota on fruit and vegetable surfaces, which are then isolated, reproduced and prepared for application on the products infected by the pathogen (*8,13,39*). In this study, the antagonistic yeast for biocontrol of grey mould *Botrytis cinerea*, isolated from the surface of murta berries, was identified as *Rhodotorula mucilaginosa* (NCBI accession number KM822749.1). This yeast species had previously been isolated in different parts of the world from surfaces of peaches and apples and its antagonistic activity has also been evaluated satisfactorily against several phytopathogenic moulds, including *P. expansum*, *Rhizopus stolonifer* and *B. cinerea*, reducing the incidence of the corresponding diseases (*40–45*). However, the *R. mucilaginosa* isolated from the Chilean native murta berries has not been investigated so far and our results have shown its promising potential for biocontrol of grey mould.

Results of spray drying

The spray drying considered three independent variables (inlet air temperature, mass per volume ratio of maltodextrin as encapsulating agent and feed flow rate), and the effects of drying on the yeast *R. mucilaginosa* were evaluated in terms of three response variables, namely survival rate, moisture content and yeast powder yield. As expected, the temperature profile during drying depended on the inlet air temperature and feed flow rate that defined the residence time within the dryer. Therefore, the lowest survival rate of the yeast was at

the highest inlet air temperature and the lowest feed flow rate (Table 1), with the lowest moisture content of the powdered product (Fig. 1). It was also observed that all three variables had a combined effect on the outlet temperature (Table 1). Higher content of maltodextrin and higher feed flow rate implied a higher content of the total mass of solid and liquid, leading to higher thermal capacity. Consumption of the initial thermal load, determined by inlet air temperature, by more solids reduced the availability of energy used up as latent heat of vapourization, leading to lower removal of water. Moreover, as feed flow rate increased, residence time tended to decrease, resulting in a higher outlet temperature and lower removal of moisture. Higher moisture content of the powdered product leads to a lower yeast survival rate due to its higher susceptibility to temperature (*21,30*).

Yeast survival rate

As can be seen in Table 1, the highest survival rate (113.6 %) of dried powdered yeast was observed at an inlet temperature of 130 °C, 18 % encapsulating agent and a feed flow of 9.25 mL/min. The Kruskal-Wallis test applied to the survival data in an analysis of median showed that significant differences ($p<0.05$) exist among the determined survival rates. All three process parameters have significant effects. Survival rate was the highest at high feed flow, low temperature and low mass per volume ratio of maltodextrin. When the inlet temperature was increased to 140 °C, the most favourable survival rate was achieved with the lower mass per volume ratio of maltodextrin (18 %) and higher feed rate (9.25 mL/min) (Table 1). Different processing and biological factors have been reported to affect cell viability. The main process parameters include outlet air temperature, residence time and feed flow rate (*31,46*), while the biological factors may include genus, species or strain of the dehydrated microorganisms (*31*). The outlet temperature during spray drying depends on residence time, which in turn depends on the inlet air temperature and feed flow rate (*31*). In this respect, various research works have considered the use of spray drying on yeasts (Table 2 (*20,21,35,47,48*)). In two studies (*20,21*), *C. sake* CPA-1, a known postharvest biocontrol agent

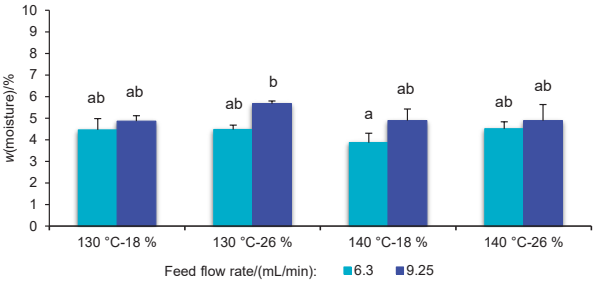

Fig. 1. Moisture content of a dried yeast powder of *Rhodotorula mucilaginosa* obtained under different spray drying conditions. Different letters indicate highly significant differences at $p<0.01$

with antagonistic activity against *B. cinerea*, *P. expansum* and *Rhizopus nigricans*, was spray dried, but its survival rate was below 20 % in both cases (Table 2), therefore it was concluded that this yeast strain was inappropriate for the production of economically viable dehydrated product. On the other hand, Romano *et al.* (*35*) applied 3 % skimmed milk as a protective agent during spray drying of wine industry yeast *Saccharomyces cerevisiae* and achieved a survival rate of 62.4 % at inlet and outlet temperatures of 120 and 60 °C, respectively. On the contrary, Chandralekha *et al.* (*48*) achieved survival rates over 80.5 % during spray drying of *S. cerevisiae,* but with 10 % maltodextrin as encapsulating agent at inlet and outlet temperatures of 100 and 65 °C respectively. These studies indicate the positive protective effect of an encapsulating agent on the survival rate during spray drying. Aponte *et al.* (*47*) investigated spray drying of *S. cerevisiae* with 3.5 % starch and 1.75 % maltose as protective agents at inlet and outlet air temperatures of 110 and 55 °C, respectively. A higher survival rate (Table 2) was achieved with maltodextrin than with starch and maltose, which would indicate its better suitability for use as a protective encapsulating agent. However, the efficacy of maltodextrin as protective agent is not proportional to its quantity. In this study a higher survival rate was achieved with 18 % maltodextrin and it decreased at the same feed flow rate and inlet temperature when maltodextrin mass per volume ratio was increased to 26 %.

Table 1. Effect of independent variables (maltodextrin mass per volume ratio, feed flow rate and inlet air temperature) on the outlet air temperature and survival rate of *Rhodotorula mucilaginosa*

(*m*(maltodextrin)/*V*(suspension))/%	Feed flow rate/(mL/min)	Temperature/°C		Yeast survival rate**/%	log(N_p/N_s)**
		Inlet air	Outlet air*		
18	6.3	130	$(73.3\pm2.3)^{de}$	$(3.4\pm0.3)^{e}$	$(-1.47\pm0.04)^{f}$
26	6.3	130	$(73.3\pm0.6)^{de}$	$(15.2\pm1.4)^{d}$	$(-0.82\pm0.04)^{d}$
18	6.3	140	$(76.3\pm1.5)^{de}$	$(0.7\pm0.2)^{f}$	$(-2.2\pm0.1)^{g}$
26	6.3	140	$(77.0\pm1.0)^{e}$	$(0.5\pm0.0)^{f}$	$(-2.33\pm0.01)^{h}$
18	9.25	130	$(62.0\pm1.0)^{a}$	$(113.6\pm6.7)^{a}$	$(0.05\pm0.03)^{a}$
26	9.25	130	$(64.3\pm2.1)^{ab}$	$(62.9\pm13.0)^{b}$	$(-0.21\pm0.09)^{b}$
18	9.25	140	$(67.7\pm1.2)^{bc}$	$(31.8\pm4.5)^{c}$	$(-0.50\pm0.06)^{c}$
26	9.25	140	$(71.7\pm0.6)^{cd}$	$(13.0\pm1.2)^{d}$	$(-1.0\pm0.2)^{e}$

*Different letters indicate highly significant differences at $p<0.01$
**Different letters indicate significant differences at $p<0.05$

Table 2. Survival rate, moisture content and powder yield for assays with different yeasts and different protective agents

Yeast	Temperature/°C (inlet/outlet air)	*w*(protective agent)/%	Yeast survival rate/%	$\log(N_p/N_s)$	*w*(moisture)/%	*Y*(yeast powder)/%	Ref.
Candida sake CPA-1	150/n.d.	skimmed milk 10	17.0	(–0.79)	n.d.	n.d.	(*20*)
Candida sake CPA-1	150/77.8	skimmed milk 10	(8.5)	–1.09	7.9	43.8	(*21*)
Saccharomyces cerevisiae	120/60	skimmed milk 3	62.4	(–0.23)	n.d.	n.d.	(*35*)
Saccharomyces cerevisiae	110/55	starch 3.5 with maltose 1.75	33.2/52.6	–0.3/–0.5	5.3/5.6	n.d.	(*47*)
Saccharomyces cerevisiae	100/65	maltodextrin 10	80.5	(–0.12)	3.2	25.8	(*48*)

n.d.=not determined. Values of survival rate in brackets were calculated according to Eq. 2

In this work, total survival of *R. mucilaginosa* during spray drying under the above-mentioned conditions was shown to be possible. This may partly be a result of the applied drying conditions as well as of the innate physiological characteristics of the yeast species. It has been observed that the resistance of yeast cells would increase under various types of stress situations as they tend to produce trehalose during the stationary phase (*20,25,26*). In our study, we determined a high content of trehalose of 169.5 mg/g in the freeze-dried cells of our cultivated *R. mucilaginosa*. This was much higher than <20 mg/g reported for spray-dried *C. sake* CPA-1, which was also used as a biocontrol agent, but with a survival rate of only 17 % (*20*).

Moisture content

Moisture content achieved after spray drying was evaluated by an analysis of variance that showed significant differences between some treatments at a confidence level of 99 % ($p<0.01$). Contrary to survival rate, low moisture content (below 10 %) of the biocontrol yeast powder is necessary to obtain its microbiological stability and prolonged shelf-life (*48*). The lowest moisture content of 3.9 % was observable at the highest inlet temperature of 140 °C and lowest feed flow of 6.30 mL/min (**Fig. 1**). However, under these conditions, the survival rate was only 0.7 % (**Table 1**). Using conditions for the best survival rate, as shown in **Table 1**, a moisture content of 4.9 % was obtained (**Fig. 1**), which is also favourable for microbiological stability. Abadias *et al*. (*21*) considered the moisture content below 8 % for practical applications, so that the survival rate may well serve as criterion to set drying conditions (*21*).

Yeast powder yield

In the spray drying assays, yeast powder yield did not significantly ($p>0.05$) depend on the process parameters. The highest yeast powder yield of 50.3 % (**Fig. 2**) was observed at an inlet temperature of 140 °C with 18 % maltodextrin and feed flow rate of 6.30 mL/min. This may be due to the lowest moisture content of 3.9 % also observed under these conditions, which imparted best flowability of the powder over a glass surface.

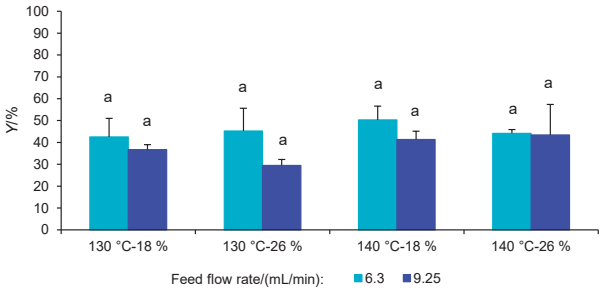

Fig. 2. Yeast powder yield of *Rhodotorula mucilaginosa* obtained under different spray drying conditions. Different letters indicate significant differences ($p>0.05$) among treatments

On the other hand, under the conditions of best survival rate, the yeast powder yield was only 36.7 %. The higher moisture content of 4.9 % could be unfavourable for flow characteristics together with the hygroscopic nature of maltodextrin (*48*), which may provoke stickiness and higher adhesiveness to the glass surface of the dryer, reducing the yield efficiency. However, in an industrial spray dryer special devices, like hammering devices or electric or mechanic vibrators, are available and they could improve the separation process and increase the yield of yeast powder.

Antagonistic activity of R. mucilaginosa against B. cinerea

To further evaluate the antagonistic activity of *R. mucilaginosa* against *B. cinerea*, the dried products with the highest and lowest survival rate were analyzed. The best *in vitro* antagonistic activity compared with the control sample was observed with the yeast powder obtained at an inlet temperature of 130 °C with 18 % maltodextrin and a feed flow rate of 9.25 mL/min (**Fig. 3**). Zhang *et al.* (*38*) reported similar results after performing an *in vitro* assay on PDA with *R. mucilaginosa* against *B. cinerea*. However, a cell count of *R. mucilaginosa* below 10^8 cells/mL was used against a cell count >10^5 spores/mL of *B. cinerea*. Moreover, Zhang *et al.* (*38,44*) also showed that the reduction of incidence of grey mould disease on strawberries by *R. mucilaginosa* could be further improved using phytic acid or chitosan. In the present study,

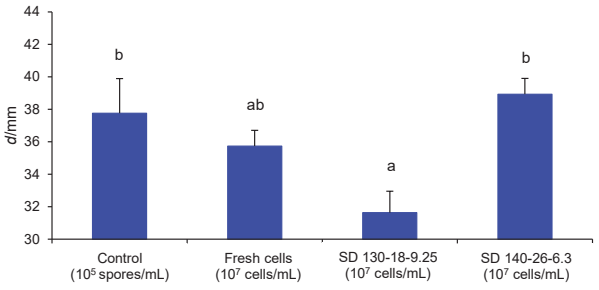

Fig. 3. Antagonistic activity *in vitro* of the spray-dried (SD) *Rhodotorula mucilaginosa* compared to that of the fresh yeast cells. Numbers 130–18–9.25 and 140–26–6.3 refer to inlet drying temperature (°C), maltodextrin mass per volume ratio (%) and feed flow rate (mL/min). Different letters indicate highly significant differences ($p<0.01$) among treatments

it was observed that maltodextrin was not only efficient as a shield to increase the cell viability, which was reflected in the high yeast survival rate, but it also improved the antagonistic activity of *R. mucilaginosa* against *B. cinerea*. Higher antagonistic activity of spray-dried cells encapsulated with 18 % maltodextrin was observed than of fresh ones (**Fig. 3**), which may be due to an unreported interaction between *R. mucilaginosa* and maltodextrin. A possible mechanism of action may be related to the effect of maltodextrin as a protective encapsulating agent. It has been reported that the components for biological control like potassium bicarbonate, sodium carbonate and bicarbonate could reduce the turgidity of the fungal cells, impeding sporulation of the phytopathogen fungus (*49,50*). An increased adherence of maltodextrin to the hyphae of the fungus may also be responsible for curtailing the growth of the phytopathogen fungus (*18*). Further study of the influence of maltodextrin on the antagonistic activity of *R. mucilaginosa* should be considered.

The *in vivo* antagonistic activity was tested (**Fig. 4**) on wounded apples using the dried cells of *R. mucilaginosa* (10^7 cells/mL) that achieved a survival rate of 100 % during spray drying at 130 °C inlet temperature, 18 % maltodextrin and a flow rate of 9.25 mL/min. As shown in **Fig. 4**, the biocontrol efficacy of *R. mucilaginosa* (10^7 cells/mL) was significantly

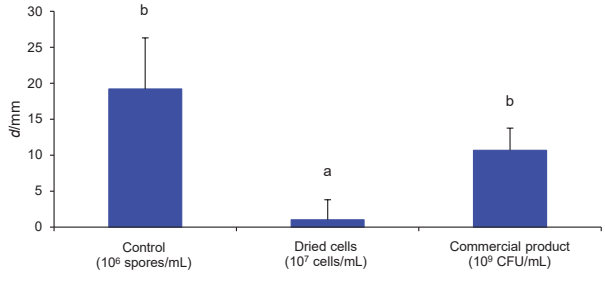

Fig. 4. Antagonistic activity *in vivo* of the spray dried *Rhodotorula mucilaginosa* on wounded apples compared to that of the commercial product (Trichonativa). Different letters indicate significant differences ($p<0.05$) among treatments

higher ($p<0.05$) than that of the commercial product containing *Trichoderma* spp. (10^9 CFU/mL) as biocontrol agent; the control sample with 10^6 spore/mL also showed a significantly ($p<0.05$) lower biocontrol efficacy. The spray-dried *R. mucilaginosa* inhibited grey mould growth by 94.8 % compared to the commercial biocontrol agent that inhibited the growth by only 44.4 %. The growth inhibition was the ratio of the difference in lesion diameter of the treated and control samples to the lesion diameter of control sample, expressed as a percentage. Similar results have been reported by Liu *et al.* (*16*) for *in vivo* antagonistic activity of fresh cells of *C. oleophila* (10^7 cells/mL) on apples, with a significant reduction of the wound diameter compared to the control with 10^4 spores/mL.

CONCLUSIONS

An antagonistic yeast identified as *Rhodotorula mucilaginosa* belongs to the natural surface microbiota of murta (*Ugni molinae* Turcz) berries and acts as a protection against the phytopathogen fungus *Botrytis cinerea*. The performed spray drying assays have shown that it is possible to obtain a dried yeast powder of *R. mucilaginosa* that retains a survival rate of 100 %, using an inlet temperature of 130 °C with 18 % maltodextrin as encapsulating agent at a feed flow rate of 9.25 mL/min. The spray drying process can produce a yeast powder with a moisture content below 8 %, necessary for practical application, with improved *in vitro* antagonistic activity compared to the fresh yeast cells, as well as an *in vivo* antagonistic activity with an inhibition rate of 94.8 % on grey mould spoilage in apples. The use of maltodextrin may also be effective in improving the *in vitro* antagonistic activity of *R. mucilaginosa*.

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