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Volatile organic compounds from *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* inhibit growth of decay causing fungi and control postharvest diseases of strawberries

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1           **Volatile organic compounds from *Wickerhamomyces anomalus*,**  
2           ***Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* inhibit growth of**  
3           **decay causing fungi and control postharvest diseases of strawberries**

4

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14

15 **Abstract**

16

17 The effectiveness of *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and  
18 *Saccharomyces cerevisiae* as biocontrol agents on postharvest decay of strawberry (*Fragaria* ×  
19 *ananassa*, cv. ‘Alba’) fruit, and their inhibitory activities on some decay-causing fungi were  
20 evaluated. Volatile organic compounds from these yeasts decreased mycelial growth of *Botrytis*  
21 *cinerea* by 69%, and by less for *Monilinia fructicola*, *Alternaria alternata*, *Aspergillus*  
22 *carbonarius*, *Penicillium digitatum*, *Cladosporium* spp., and *Colletotrichum* spp. Strawberry  
23 fruit exposed to 6-day-old liquid cultures of *W. anomalus*, *M. pulcherrima* and *S. cerevisiae* for  
24 48 h showed 89%, 40%, and 32% reductions, respectively, in ~~gray mold~~ McKinney Index.  
25 Vapours of ethyl acetate, the main volatile organic compound of these yeasts, completely  
26 inhibited *B. cinerea* growth at 8.97 mg/mL, and suppressed ~~gray mold grey mould~~ on  
27 strawberry fruit at 0.718 mg/mL. The biocontrol activities of these yeasts can be ascribed to  
28 ethyl acetate, which can be used for control of postharvest ~~gray mold~~ of strawberry fruit.

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30

31 **Keywords:** *Botrytis cinerea*, ethyl acetate, *Fragaria* × *ananassa*, ~~gray mold grey mould~~, yeast  
32 volatile organic compounds

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35 **Highlights**

36 Yeast volatile organic compounds can reduce *in vitro* growth of decay-causing fungi

37 Strawberry exposure to yeast producing volatile organic compounds reduces gray mold ~~grey~~

38 ~~mould~~

39 The main volatile organic compound of the three selected yeasts is ethyl acetate

40 Ethyl acetate at 8.97 mg/mL completely inhibits *B. cinerea* growth in the *in vitro* trials

41 Ethyl acetate at 0.717 mg/mL can control gray mold ~~grey mould~~ on strawberry fruit

42 ~~Ethyl acetate at 8.97 mg/mL completely inhibits *B. cinerea* growth in the *in vitro* trials~~

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## 1. Introduction

The use of yeasts as biocontrol agents for prevention of pre-harvest and postharvest diseases of fruit is an alternative strategy to the use of synthetic fungicides (Spadaro and Droby, 2016). Various yeasts genera have been widely used for control of decay caused by pathogens such as *Botrytis* spp., *Aspergillus* spp., *Penicillium* spp., *Monilinia* spp. and *Colletotrichum* spp. (Janisiewicz and Korsten, 2002). In a previous trials, the three yeasts *Wickerhamomyces anomalus* (formerly *Hansenula anomala* and *Pichia anomala*), *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* were applied as pre-harvest treatments to tree canopies, and they were effective for reduction of sweet cherry postharvest decay that was mainly caused by the fungus *Monilinia laxa* (Oro et al., 2014).

Generally, the biocontrol ability of these yeasts can be related to different mechanisms through which they interact with pathogens and fruit tissues including: competition with pathogens for space and nutrients (Bencheqroun et al., 2007); parasitism and production of extracellular proteins (Lima et al., 2013); killer activity; induction of resistance in fruit tissues through elicitors that are either secreted by the biocontrol agent (Spadaro and Droby, 2016). Moreover, several studies have demonstrated that the production of volatile organic compounds (VOCs) by yeasts, has a significant role in their antagonistic activities (Huang et al., 2012; Di Francesco et al., 2015; Huang et al., 2012). In particular, ethyl acetate, commonly found in food and beverages, has proven antimicrobial properties (Vimala et al., 2007) (<https://www.nlm.nih.gov/>). These antimicrobial properties change across species, and are affected by yeast growth stage, culture mode, temperature, medium composition, and other factors (Martins et al., 2010). The aim of the present study was to evaluate the effectiveness of

87 the VOCs from these three yeasts *in vitro* and ~~postharvest~~controlling of the postharvest decay  
88 of strawberry fruit.

89

## 90 **2. Materials and methods**

91

### 92 *2.1. Yeast and strawberry*

93 The antagonist yeasts *W. anomalus* Disva 2, *M. pulcherrima* Disva 267, and *S. cerevisiae*  
94 Disva599 from the collection of the Department of Life and Environmental Sciences  
95 (Polytechnic University of Marche, Ancona, Italy) were used for the *in vitro* and *in vivo* assays.  
96 Yeast strains were grown in yeast potato dextrose (YPD) medium (prepared with 10 g/L yeast  
97 extract, 10 g/L peptone, 20 g/L glucose). All of the strains were sub-cultured at 3-month  
98 intervals on YPD agar medium and maintained at 4 °C.

99 The *in vivo* trials were carried out on strawberry (*Fragaria × ananassa*, cv. ‘Alba’) fruit  
100 harvested from an organic farm in Ancona, central-eastern Italy, with the strawberry fruit  
101 selected for uniformity in size and colour, and absence of defects.

102

103

### 104 *2.2. Profiles of yeast volatile organic compounds and higher alcohols, and their production* 105 *over time*

106 The yeast VOC compositions were quantitatively evaluated using head-space solid-phase  
107 microextraction with a gas chromatograph (GC-2014; Shimadzu, Kyoto, Japan). Analyses were  
108 performed after 1 day and 6 days of growth of *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*.  
109 Five millilitres of each yeast culture were heated to 35 °C, during which time the headspace  
110 volatiles were collected with divinylbenzene/ carboxen/ polydimethylsiloxane fibre (Supelco,

111 Bellefonte, PA, USA) following the procedures of Ando et al. (2012). The chemical analyses  
112 were performed in triplicate, and are expressed as means  $\pm$  standard deviation.

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114

### 115 2.3. *In vitro* antimicrobial activity of yeast volatile organic compounds against decay-causing 116 fungi

117 The experimental assays were based on a dual culture method to test antagonistic activities  
118 without contact between cultures, of the yeast VOCs against some postharvest pathogens of  
119 fruit. The yeast antimicrobial activities were targeted for seven decay-causing fungi from the  
120 culture collection of the Department of Life and Environmental Sciences (Polytechnic  
121 University of Marche, Ancona, Italy): *B. cinerea*, *Monilinia fructicola*, *Alternaria alternata*,  
122 *Aspergillus carbonarius*, *Penicillium digitatum*, *Cladosporium* spp. and *Colletotrichum* spp..

123 Yeast potato dextrose agar medium plates were seeded with each yeast, and after a 24-  
124 h incubation at 25 °C, the lids of the Petri dishes were replaced with plates of potato dextrose  
125 agar (PDA), with the plates inoculated with the fungal pathogen alone. These plates with the  
126 two colonies were sealed (Parafilm) and incubated at 25 °C for 7 days, after which the fungal  
127 growth was recorded. Per each fungus, the control treatment was represented by the Petri dishes  
128 that did not have the yeast cultures. The percentage of growth inhibition of the fungal mycelia  
129 by the yeasts was calculated as compared to the control. Three replicates were used for each  
130 yeast/ fungus combination.

131

### 132 2.4. *In vitro* antimicrobial activity of ethyl acetate

133 To determine the antimicrobial activity of ethyl acetate, a plug of the fungus *B. cinerea*  
134 was placed on the base of a Petri dish containing PDA, and a sterile absorbent paper disk that  
135 was soaked with different concentrations of ethyl acetate (from 0.897 to 17.94 mg/mL) was

136 placed in the lid of this Petri dish. The control Petri dishes were prepared with the same  
137 procedure, but the sterile absorbent paper disk was soaked with 1 mL water. For each ethyl  
138 acetate concentration, three Petri dish replicates were used. The Petri dishes were kept for 7  
139 days at  $25 \pm 1$  °C, after which the fungal growth was recorded. The percentage of growth  
140 inhibition of the fungal mycelia was calculated as compared to the control.

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142

### 143 2.5. *Effects of yeast volatile organic compounds on postharvest decay of strawberry fruit*

144 *Wickerhamomyces anomalus*, *M. pulcherrima* and *S. cerevisiae* strains were pre-grown  
145 separately on 30 mL YPD medium for 24 h at 25 °C, and then used to inoculate 1500 mL YPD  
146 medium in each trial, with incubations for 1 or 6 days at 25 °C. Petri dishes containing the liquid  
147 culture of each yeast were placed in plastic boxes (70.5 × 40 × 16.5 cm) together with the  
148 strawberry fruit. Each plastic box contained eight trays with six strawberries each (48 fruit per  
149 box), with 500 mL growing yeast culture placed in open 18-mm-diameter glass Petri dishes  
150 above the fruit. The plastic boxes were closed with a plastic lid and sealed immediately, then  
151 left under shaking at 100 rpm and 25 °C for 48 h. Plastic boxes with the same number of  
152 strawberries and with containers including water were used as the controls. After 48 h, the Petri  
153 dishes containing the growing yeast cultures were removed from the boxes, and the strawberries  
154 were kept at  $25 \pm 1$  °C for 2 days. The percentages of decayed strawberry fruit were recorded  
155 daily, and disease severity was recorded according to an empirical scale with six ~~levels~~degrees:  
156 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3,  
157 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5,  $\geq 81\%$  fruit surface  
158 infected ~~and showing sporulation~~. The infection index, or McKinney's Index, incorporates both  
159 the incidence and severity of the disease, and it ~~is~~ expressed ~~as~~ the weighted means of the

160 disease, as a percentage of the maximum possible level (McKinney, 1923). Specifically, it is  
161 calculated according to Equation (1):

162

$$163 \quad I = [(d \times f)/(N \times D)] \times 100 \quad (1),$$

164

165 where  $d$  is the category of rot intensity scored on the strawberry, and  $f$  is its frequency,  $N$  is the  
166 total number of strawberry fruit examined (healthy and rotted), and  $D$  is the highest category of  
167 disease intensity that occurred on the empirical scale (Romanazzi et al., 2001). ~~Two replicates~~  
168 ~~were carried out.~~

169

## 170 2.6. *In vivo* effects of ethyl acetate for the control of strawberry postharvest decay

171 The *in vivo* antifungal activity of ethyl acetate was studied for strawberry postharvest decay.  
172 Preliminary trials were carried out to evaluate the optimal concentration of ethyl acetate, which  
173 would be effective against postharvest decay and avoid phytotoxic effects on strawberry fruit.  
174 Forty-eight strawberry fruit were placed in the plastic boxes used previously in the *in vivo* VOC  
175 efficacy evaluation, testing different concentrations of ethyl acetate, as 0.0718, 0.718 and 7.18  
176 mg/mL ~~(the higher concentrations tested in *in vitro* test caused damage to the fruit by changing~~  
177 ~~the fruit's consistency)~~. Boxes containing strawberry fruit and water were used as the controls.  
178 Four replicates were used for each treatment. After 48 h, flasks containing ethyl acetate or water  
179 were removed and the boxes were kept for 3 d at  $25 \pm 1$  °C, after which the decay, severity and  
180 McKinney's Index were evaluated as previously reported. Since the highest concentrations  
181 tested in *in vitro* test (7.18 mg/mL) was phytotoxic on strawberries, a second trial was carried  
182 out according to the optimal concentration of ethyl acetate found in the preliminary test. Boxes  
183 containing thirty-four trays with 6 strawberries each were placed with the same arrangement  
184 with 0.718 mg/mL ethyl acetate for 48 h. ~~Same b~~ Boxes containing strawberry fruit and water

185 were used as the controls. After 48 h, flasks containing ethyl acetate or water were removed  
186 and the boxes were kept for 3 d at 25 ±1 °C, ~~after which then~~ the number of infected fruit and  
187 disease severity were recorded. ~~strawberry decay, severity and McKinney's Index were~~  
188 ~~evaluated.~~

189

## 190 2.7. Statistical analysis

191 The data were analysed using the Student *t*-test or one-way ANOVA, followed by Duncan tests  
192 or Fisher least significant difference (LSD) test (Statsoft, USA) at  $p < 0.05$ . The percentage data  
193 were arcsine transformed before analysis, to improve the homogeneity of variance when the  
194 range of percentages was >40. Actual values are shown. All of the trials were repeated at least  
195 twice, with at least three replicates. Data from two or more experiments were pooled, and the  
196 statistical analysis to determine the homogeneity of variances was tested using Levene's test.

197

## 198 3. Results

### 199 3.1. Solid-phase microextraction gas-chromatography analyses of the volatile organic 200 compounds

201 In the 1-day-old and 6-day-old yeast liquid cultures, the most representative VOC was ethyl  
202 acetate. As expected, the 6-day-old yeast liquid cultures produced larger amounts of ethyl  
203 acetate and the other VOCs (Table 1) than seen for the 1-day-old yeast liquid cultures (data not  
204 shown), independent of the yeast species.

205 In these incubations with the 6-day-old cultures, *W. anomalus* and *M. pulcherrima* produced  
206 the highest levels of ethyl acetate, at 792.9 mg/L and 115.0 mg/L, respectively. In *W. anomalus*,  
207 isoamyl acetate was moderately abundant (26.5 mg/L), with low levels for *M. pulcherrima* and  
208 *S. cerevisiae* (1.2 and 5.0 mg/L, respectively). All three of the yeasts produced small amounts  
209 of the other VOCs, where ethyl butyrate, ethyl hexanoate, and phenylethyl acetate ranged from

210 0 mg/L to 5 mg/L. For the higher alcohols, *W. anomalus* produced greater amounts of 2-  
211 phenylethanol (10.0 mg/L) and isobutanol (12.2 mg/L) than the other two yeasts (Table 1).

212

### 213 3.2. *In vitro* antimicrobial activity of yeast volatile organic compounds against the main fungal 214 pathogens of fruit

215 The data from the double plate assays showed that *W. anomalus* had the highest antimicrobial  
216 activity against *B. cinerea*, with 87% growth inhibition. Similar behaviours were observed for  
217 *M. pulcherrima* and *S. cerevisiae*, which reduced the growth of *B. cinerea* by 56% and 63%,  
218 respectively (Table 1). These three yeasts also reduced the development of the fungus *M.*  
219 *fructicola* with 55%, 42% and 57% growth inhibition by *W. anomalus*, *M. pulcherrima*, and *S.*  
220 *cerevisiae*, respectively. *W. anomalus* also inhibited the growth of *A. alternata* and *A.*  
221 *carbonarius* by 47% and 44%, respectively. *S. cerevisiae* also showed antimicrobial activity  
222 against *A. alternata*, with 35% growth inhibition. Little or no antimicrobial activity was  
223 observed for *W. anomalus*, *M. pulcherrima*, and *S. cerevisiae* against *P. digitatum*,  
224 *Cladosporium* spp. and *Colletotrichum* spp. (Table 2).

225

### 226 3.3. *In vitro* antimicrobial activities of ethyl acetate

227 Considering that ethyl acetate was the main representative VOC of the *W. anomalus* and *M.*  
228 *pulcherrima* cultures, synthetic ethyl acetate was also investigated to define a threshold of its  
229 antimicrobial effects.

230 In the *in vitro* trials for the full range of ethyl acetate concentrations, for 8.97 mg/mL ethyl  
231 acetate and above, there was total inhibition of the fungus *B. cinerea*. In the presence of lower  
232 concentrations of ethyl acetate, there was a slight reduction of 15% of mycelial growth, while  
233 the suppressive effect was completely ~~nullified-none~~ with ~~the leastless-~~ concentrations.

234

235 3.4. Effects of exposure to yeast liquid cultures on postharvest decay of strawberry fruit

236 The results of trials with the exposure of strawberry fruit to yeast liquid cultures of strawberry  
237 confirmed the *in vitro* data. The largest effects were seen for the exposure to liquid cultures of  
238 *W. anomalus*, with *M. pulcherrima* and *S. cerevisiae* also showing some inhibitory effects.  
239 When the strawberry fruits were incubated with the 1-day-old liquid yeast cultures, there were  
240 no significant effects seen compared to the control (data not shown). However, following the  
241 incubation with the 6-day-old liquid yeast cultures, the strawberry fruit showed significant  
242 reduction of grey mould, the main cause of postharvest decay (Fig. 1). Here, while only *W.*  
243 *anomalus* significantly reduced the strawberry fruit decay, the McKinney's Index was reduced  
244 by 89%, 40% and 32% by *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*, respectively, as  
245 compared to the control.

246

247 3.5. In vivo effects of ethyl acetate for the control of strawberry postharvest decay

248 The main cause for strawberry postharvest decay was ~~grey~~gray mould. From the  
249 preliminary trials, the 0.718 mg/mL ethyl acetate concentration showed significant reductions  
250 of the strawberry disease parameters while it did not result in any ~~matrix degeneration of the~~  
251 ~~strawberry fruit~~phytotoxic effect, which maintained the integrity of their structure. In contrast,  
252 the 10-fold greater concentration of 7.18 mg/mL ethyl acetate ~~was toxic for these~~damaged  
253 strawberry fruit, ~~as they showed deliquescence with vigorous fluid loss~~. No inhibitory effects  
254 were seen for the lowest concentration ~~of~~(0.0718 mg/mL) of ethyl acetate as compared to the;  
255 ~~indeed, in this case, the development of the postharvest fungi on the strawberry fruit paralleled~~  
256 ~~that of the~~ control (data not shown). Whereas,

257 ~~Differently,~~ the vapours of 0.718 mg/mL ethyl acetate decreased ~~the strawberry disease,~~  
258 ~~as compared to the control~~gray mold (Table 3). In particular, strawberry fruit treated with ethyl

259 acetate had ~~decay~~ disease incidence, ~~disease~~-severity and McKinney's Index, ~~of~~ 36%, 52%, and  
260 52% lower than the control, respectively.

261

262

#### 263 4. Discussion

264 Volatile organic compounds are carbon-based, gas-phase products, and approximately 250  
265 different VOCs have been defined as being produced by fungi. These are produced as mixtures  
266 of simple hydrocarbons, heterocyclics, aldehydes, ketones, alcohols, phenols, thioalcohols and  
267 thioesters, and/or their derivatives (Korpi et al., 2009; Ortiz-Castro et al., 2009). Due to the  
268 small sizes of these molecules and their diffusion through the atmosphere and soil, numerous  
269 studies have been carried out on the potential applications of VOCs in agriculture, industry and  
270 pharmacology, particularly as biocontrol factors (Liu et al., 2008; Arrebola et al., 2010; Morath  
271 et al., 2012; Giorgio et al., 2015; ~~Liu et al., 2008~~; ~~Morath et al., 2012~~).

272 In a recent study, Nally et al. (2015) showed that antifungal VOCs produced by  
273 *Saccharomyces* isolates can inhibit fungal mycelial growth of *B. cinerea*, *Aspergillus*  
274 *versicolor*, *Aspergillus caelatus* and *F. oxysporum*, while the VOCs produced by non-  
275 *Saccharomyces* strains, such as *Candida sake* and *Candida versatilis*, inhibited *Penicillium*  
276 *commune*, *Aspergillus terreus* and *A. carbonarius*. Di Francesco ~~and coworkers~~ et al. (2015)  
277 identified a group of different alcohol VOCs that are produced by *Aureobasidium pullulans* that  
278 can protect apples from ~~grey~~ gray mould. Parafati et al. (2015) ~~also~~ demonstrated inhibitory  
279 effects of the yeast *W. anomalus*, *M. pulcherrima* and *S. cerevisiae* against *B. cinerea* *in vitro*  
280 and *in vivo* on table grapes. They justified these yeast antimicrobial activities through multiple  
281 modes of action, including the production of VOCs.

282 In the present study, the effects of these three yeast, *W. anomalus*, *M. pulcherrima* and  
283 *S. cerevisiae*, previously characterised as biological biocontrol agents ~~for~~ on sweet cherries (Oro

284 et al., 2014), were tested on strawberry fruit, that is highly perishable, ~~that and~~ after harvest  
285 they can easily undergo fungal spoilage (Feliziani et al., 2016).

286 Our preliminary *in vitro* tests, -carried out without contact between yeast/mould cultures,  
287 showed an effectively reduction on the growth of *B. cinerea* and other fungi. -This behaviour  
288 excluded killer activity, competition for nutrients or -other modes of action involving cell to  
289 cell contact, and we focused our attention on VOCs that are toxic to these fungal pathogens,  
290 which would thus represent their probable mode of action. ~~These-This~~ hypothesis ~~were-was~~  
291 confirmed by the *in vivo* tests, where the strawberry fruit exposed to the synthetic VOCs from  
292 these yeasts showed a reduction of ed McKinney's Index for strawberry ~~grey-gray~~ mould,  
293 ~~compared to the control fruit~~. The VOCs naturally produced by *W. anomalus* were more  
294 effective ~~for-on-the-reduction-of~~ postharvest decay of ~~these~~ strawberry fruit as, compared to  
295 those of *M. pulcherrima* and *S. cerevisiae*. Indeed, *W. anomalus* produced the highest quantities  
296 of VOCs that remain confined in the boxes. The yeast with the highest production of  
297 antimicrobial VOCs, *W. anomalus*, -was the one that best better controlled the fungal pathogen.

298 When the strawberry fruit were exposed to the yeast VOCs, reduction of the ~~grey-gray~~  
299 mould was only seen for the 6-day-old yeast cultures since the VOCs produced after 1-day were  
300 not sufficient to control the grey mould effective.

301 In the analysis of the VOC profiles of these yeasts, ethyl acetate was the major  
302 compound produced in terms of the quantity and *W. anomalus* showed the highest production  
303 if compared to *S. cerevisiae* and *M. pulcherrima*. In previous studies, the biocontrol activity of  
304 *W. anomalus* has been attributed to the production of ethyl acetate in terms of grain storage  
305 moulds, such as *Penicillium* spp. (Druvefors et al., 2005), and of 2-phenyl ethanol in terms of  
306 *Aspergillus flavus* (Hua et al., 2014). This would indicate that the active ingredient of *W.*  
307 *anomalus* VOCs against the fungus *B. cinerea* is ethyl acetate even if other volatile molecules  
308 may have an antimicrobial effect. -Indeed, in the present study, ethyl acetate was effective for

309 suppression of *B. cinerea* on strawberry fruit, where the exposure for 48 h to 0.718 mg/mL ethyl  
310 acetate almost halved the McKinney's Index and severity of grey mould. However, further  
311 investigations are needed to assess the potential additive or synergistic effect among the  
312 different VOCs produced by each yeast.

313 Nowadays, biocontrol appears to provide sustainable and reliable alternatives to  
314 chemical fungicides, which have raised serious concerns because of food contamination and  
315 environmental pollution. Here, we have demonstrated that the production of VOCs can have  
316 essential roles in the control of postharvest grey mould of strawberry fruit. However, more  
317 further investigations are necessary to understand how the VOCs produced by *W. anomalus*, or  
318 indeed ethyl acetate, might be integrated into current practice, and if these are also effective for  
319 the control of postharvest diseases of other fruit.

320

#### 321 **Conflict of interest**

322 The authors declare no conflicts of interest.

323 **References**

324

325 Ando, H., Hatanaka, K., Ohata, I., Yamashita-Kitaguchi, Y., Kurata, A., and Kishimoto, N.  
326 2012. Antifungal activities of volatile substances generated by yeast isolated from Iranian  
327 commercial cheese. *Food Control* 26:472–478.

328 Arrebola, E., Sivakumar, D., and Korsten, L. (2010). Effect of volatile compounds produced by  
329 *Bacillus* strains on postharvest decay in citrus. *Biol. Control* 53:122–128.

330 Bencheqroun, S., Bajji, M., Massart, S., Labhilili, M., Jaafari, S., and Jijakli, H. 2007. *In vitro*  
331 and *in-situ* study of postharvest apple blue mold biocontrol by *Aureobasidium pullulans*:  
332 evidence for the involvement of competition for nutrients. *Postharvest Biol. Technol.*  
333 46:128-135.

334 Di Francesco, A., Ugolini, L., Lazzeri, L., and Mari, M. 2015. Production of volatile organic  
335 compounds by *Aureobasidium pullulans* as a potential mechanism of action against  
336 postharvest fruit pathogens. *Biol. Control* 81:8–14.

337 Druvefors, U. A., Passoth, V., and Schnurer, J. 2005. Nutrient effects on biocontrol of  
338 *Penicillium roqueforti* by *Pichia anomala* J121 during airtight storage of wheat. *Appl.*  
339 *Environ. Microbiol.* 71:1865-1869.

340 Feliziani, E., Landi L., and Romanazzi, G. 2015. Preharvest treatments with chitosan and other  
341 alternatives to conventional fungicides to control postharvest decay of strawberry.  
342 *Carbohydr. Polym.* 132:111–117.

343 Giorgio, A., De Stradis, A., Lo Cantore, P., and Iacobellis, N. S. 2015. Biocide effects of  
344 volatile organic compounds produced by potential biocontrol rhizobacteria on *Sclerotinia*  
345 *sclerotiorum*. *Front. Microbiol.* 6:1056.

346 Hua, S. S., Beck, J. J., Sarreal, S. B., and Gee, W., 2014. The major volatile compound 2-  
347 phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression  
348 of aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycotox. Res.* 30:71–78.

349 Huang, R., Che, H. J., Zhang, J., Yang, L., Jiang, D. H., and Li, G. Q. 2012. Evaluation of  
350 *Sporidiobolus pararoseus* strain YCXT3 as biocontrol agent of *Botrytis cinerea* on post-  
351 harvest strawberry fruit. *Biol. Control* 62:53–63.

352 Janisiewicz, W. J., and Korsten, L. 2002. Biological control of postharvest diseases of fruits.  
353 *Annu. Rev. Phytopathol.* 40:411–441.

354 Korpi, A., Jarnberg, J., and Pasanen, A. L. 2009. Microbial volatile organic compounds. *Crit.*  
355 *Rev. Toxicol.* 39:139–193.

356 Lima, J. R., Gondim Oliveira, J. T. A., Oliveira, F. S. A, Gonçalves, L. R. B, and Viana, F. M.  
357 P. 2013. Use of killer yeast in the management of postharvest papaya anthracnose.  
358 [Postharvest Biol. Technol.](#)~~[Post. Biol. Technol.](#)~~ 83:58–64.

359 Liu, W., Wu, W., Zhu, B., Du, Y., and Liu, F. 2008. Antagonistic activities of volatiles from  
360 four strains of *Bacillus* spp. and *Paenibacillus* spp. against soil-borne plant pathogens.  
361 *Agric. Sci. China* 7:1104–1114.

362 Martins, M., Henriques, M., Azeredo, J., Rocha, S. M., Coimbra, M. A., and Oliveira, R. 2010.  
363 *Candida* species extracellular alcohols: production and effect in sessile cells. *J. Basic*  
364 *Microbiol.* 50:S89–S97.

365 Masoud, W., and Kaltonft, C. H. 2006. The effects of yeasts involved in the fermentation of  
366 *Coffea arabica* in east Africa on growth and ochratoxin A (OTA) production by *Aspergillus*  
367 *ochraceus*. *Int. J. Food Microbiol.* 106:229–234.

368 McKinney, H. H. 1923. Influence of soil temperature and moisture on infection of wheat  
369 seedlings by *Helminthosporium sativum*. *J. Agricult. Res.* 26:195–218.

370 Morath, S. U., Hung, R., and Bennett, J. W. 2012. Fungal volatile organic compounds: a review  
371 with emphasis on their biotechnological potential. *Fungal Biol. Rev.* 26:73–83.

372 Nally, M. C., Pesce, V. M, Maturano, Y. P, Rodriguez Assaf, L. A., Toro, M. E., Castellanos  
373 de Figueroa, L. I., and Vazquez, F. 2015. Antifungal modes of action of *Saccharomyces*  
374 and other biocontrol yeasts against fungi isolated from sour and grey rots. *Int. J. Food*  
375 *Microbiol.* 204:91–100.

376 Oro, L., Feliziani, E., Ciani, M., Romanazzi, G., and Comitini, F. 2014. Biocontrol of  
377 postharvest brown rot of sweet cherries by *Saccharomyces cerevisiae* Disva 599,  
378 *Metschnikowia pulcherrima* Disva 267 and *Wickerhamomyces anomalus* Disva 2 strains. ~~Postharvest Biol. Technol.~~ ~~Post. Biol. Technol.~~ 96:64–68.

380 Ortiz-Castro, R., Contreras-Cornejo, H., Macias-Rodriguez, L., and Lopez-Bucio, J. 2009. The  
381 role of microbial signals in plant growth and development. *Plant Sign. Behav.* 4:701-712.

382 Parafati, L., Vitale, A., Restuccia, C., and Cirvilleri, G. 2015. Biocontrol ability and action  
383 mechanism of food-isolated yeast strains against *Botrytis cinerea* causing post-harvest  
384 bunch rot of table grape. *Food Microbiol.* 47:85–92.

385 Romanazzi, G., Nigro, F., Ippolito, A., and Salerno, M. (2001). Effect of short hypobaric  
386 treatments on postharvest rots of sweet cherries, strawberries and table grapes. ~~Postharvest~~  
387 ~~Biol. Technol.~~ ~~Post. Biol. Technol.~~ 22:1–6.

388 Romanazzi, G., Smilanick, J. L., Feliziani, E., and Droby, S. 2016. Integrated management of  
389 postharvest gray mold on fruit crops. ~~Postharvest Biol. Technol.~~ ~~Post. Biol. Technol.~~  
390 113:69–76.

391 Rouissi, W., Ugolini, L., Martini, C., Lazzeri, L., and Mari, M. 2013. Control of postharvest  
392 fungal pathogens by antifungal compounds from *Penicillium expansum*. *J. Food Protect.*  
393 11:1879–1886.

- 394 Spadaro, D., and Droby, S. 2016. Development of biocontrol products for postharvest diseases  
395 of fruit: the importance of elucidating the mechanisms of action of yeast antagonists.  
396 Trends Food Sci. Technol. 47:39–49.
- 397 Vimala, Y., Devi, K. S., and Elizabeth, K. M. 2007. Antimicrobial activity of ethyl acetate  
398 extracts of *D. hamiltonii* on microbial isolates of spoiled vegetables and pathogenic  
399 microorganisms. Asian J. Chem. 19:801–803.

400

401 **Table 1.** VOCs and higher alcohols produced by 6-day-old cultures of *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and  
 402 *Saccharomyces cerevisiae*.

403

Yeast <del>VOC</del> source	VOCs and higher alcohols produced (mg/L)									
	Acetaldehyde	Ethyl acetate	Ethyl butyrate	Isoamyl acetate	Ethyl hexanoate	Phenylethyl acetate	2-Phenyl-ethanol	Isobutanol	Amylic alcohol	Isoamyl alcohol
<i>Wickerhamomyces anomalus</i>	0 ±0 <sup>b</sup>	792.85 ±22.60 <sup>a</sup>	0.730 ±0.028 <sup>a</sup>	26.45 ±0.04 <sup>a</sup>	0.970 ±0.049 <sup>a</sup>	4.80 ±0.19 <sup>a</sup>	10.02 ±0.70 <sup>a</sup>	12.21 ±0.74 <sup>a</sup>	27.02 ±2.16 <sup>a</sup>	18.41 ±0.25 <sup>b</sup>
<i>Metschnikowia pulcherrima</i>	0.82 ±0.03 <sup>a</sup>	115.0 ±5.1 <sup>b</sup>	0.060 ±0.014 <sup>b</sup>	1.170 ±0.014 <sup>c</sup>	0.500 ±0.028 <sup>b</sup>	0 ±0 <sup>b</sup>	4.75 ±0.18 <sup>b</sup>	3.03 ±0.42 <sup>c</sup>	18.22 ±0.31 <sup>b</sup>	24.96 ±2.76 <sup>b</sup>
<i>Saccharomyces cerevisiae</i>	0 ±0 <sup>b</sup>	6.07 ±1.48 <sup>c</sup>	0.045 ±0.007 <sup>b</sup>	5.000 ±0.014 <sup>b</sup>	0 ±0 <sup>c</sup>	0.27 ±0.03 <sup>b</sup>	3.56 ±0.05 <sup>a</sup>	5.43 ±0.82 <sup>a</sup>	24.05 ±5.30 <sup>a</sup>	34.71 ±2.16 <sup>a</sup>

404

405 Different letters show significant differences according to Duncan's tests ( $p < 0.05$ ).

406

407

**Table 2.** *In vitro* antagonistic activity of yeast VOCs against the fungi, expressed as percentages of inhibition of the fungal mycelia, using the dual

408

culture method.

<u>Yeast</u>	<u>Fungal growth inhibition by yeast VOC (%)</u>						
	<u><i>Botrytis</i></u> <u><i>cinerea</i></u>	<u><i>Monilinia</i></u> <u><i>fructicola</i></u>	<u><i>Alternaria</i></u> <u><i>alternata</i></u>	<u><i>Aspergillus</i></u> <u><i>carbonarius</i></u>	<u><i>Penicillium</i></u> <u><i>digitatum</i></u>	<u><i>Cladosporium</i></u> <u>spp.</u>	<u><i>Colletotrichum</i></u> <u>spp.</u>
<u><i>Wickerhamomyces</i></u> <u><i>anomalous</i></u>	<u>87.0 ±3.6<sup>a</sup></u>	<u>55.0 ±11.8<sup>b</sup></u>	<u>47.0 ±8.2<sup>bc</sup></u>	<u>44.0 ±6.9<sup>c</sup></u>	<u>1.5 ±0.5<sup>d</sup></u>	<u>6.0 ±3.5<sup>d</sup></u>	<u>0.5 ±0.5<sup>d</sup></u>
<u><i>Metschnikowia</i></u> <u><i>pulcherrima</i></u>	<u>56.0 ±10.1<sup>a</sup></u>	<u>42.1 ±3.1<sup>b</sup></u>	<u>7.5 ±2.2<sup>c</sup></u>	<u>6.5 ±0.9<sup>cd</sup></u>	<u>0.0 ±0.0<sup>d</sup></u>	<u>4.5 ±1.0<sup>cd</sup></u>	<u>1.0 ±0.0<sup>d</sup></u>
<u><i>Saccharomyces</i></u> <u><i>cerevisiae</i></u>	<u>63.0 ±4.0<sup>a</sup></u>	<u>57.0 ±4.4<sup>a</sup></u>	<u>35.0 ±5.0<sup>b</sup></u>	<u>5.0 ±2.0<sup>c</sup></u>	<u>1.5 ±0.5<sup>c</sup></u>	<u>3.0 ±1.7<sup>c</sup></u>	<u>2.0 ±2.0<sup>c</sup></u>

409

Different letters show significant differences of three yeast VOCs related to different fungal growth inhibitions (Fisher LSD tests; p <0.05).

410

411

412 **Table 2.** *In vitro* antagonistic activity of yeast VOCs against the fungi, expressed as percentages  
 413 of inhibition of the fungal mycelia, using the dual culture method.

414

Yeast VOC source	Fungal growth inhibition by yeast VOC (%)						
	<i>Botrytis cinerea</i>	<i>Monilia fructicola</i>	<i>Alternaria alternata</i>	<i>Aspergillus carbonarius</i>	<i>Penicillium digitatum</i>	<i>Cladosporium</i> spp.	<i>Colletotrichum</i> spp.
<i>Wickerhamomyces anomalous</i>	87.0 ±3.6 <sup>a</sup>	55.0 ±11.8 <sup>b</sup>	47.0 ±8.2 <sup>be</sup>	44.0 ±6.9 <sup>e</sup>	1.5 ±0.5 <sup>d</sup>	6.0 ±3.5 <sup>d</sup>	0.5 ±0.5 <sup>d</sup>
<i>Metschnikowia pulcherrima</i>	56.0 ±10.1 <sup>a</sup>	42.1 ±3.1 <sup>b</sup>	7.5 ±2.2 <sup>e</sup>	6.5 ±0.9 <sup>ed</sup>	0.0 ±0.0 <sup>d</sup>	4.5 ±1.0 <sup>ed</sup>	1.0 ±0.0 <sup>d</sup>
<i>Saccharomyces cerevisiae</i>	63.0 ±4.0 <sup>a</sup>	57.0 ±4.4 <sup>a</sup>	35.0 ±5.0 <sup>b</sup>	5.0 ±2.0 <sup>e</sup>	1.5 ±0.5 <sup>e</sup>	3.0 ±1.7 <sup>e</sup>	2.0 ±2.0 <sup>e</sup>

415

416 Different letters show significant differences of three yeast VOCs related to different fungal  
 417 growth inhibitions (Fisher-LSD tests;  $p < 0.05$ ).

418

419 **Table 3.** Decay, severity and McKinney's Index of postharvest decay, mainly grey mold, of  
420 strawberry fruit exposed for 48 h to 0.8  $\mu\text{L}/\text{mL}$  ethyl acetate, and then kept for 3 d at  $25 \pm 1$  °C.  
421 Different letters indicate significant differences within columns according to the Student's t-  
422 test ( $p < 0.05$ ).

423

<b>Treatment</b>	<b>Decay (%)</b>	<b>Severity (1-5)</b>	<b>McKinney's Index (%)</b>
Control	25.0 <sup>a</sup>	2.5 <sup>a</sup>	15.1 <sup>a</sup>
Ethyl acetate	16.7 <sup>b</sup>	1.2 <sup>b</sup>	7.3 <sup>b</sup>

424

425

426 **Figure caption**

427

428 **Figure 1.** Decay and McKinney's Index of ~~gray~~ mould on strawberry fruit exposed to VOCs  
429 of 6-d old cultures of *W. anomalus*, *M. pulcherrima* and *S. cerevisiae*. Strawberries were kept  
430 at 25 °C for 48 h with the yeast, followed by 2 d of shelf life at 20 ±1 °C, ~~when fruit decay was~~  
431 ~~evaluated~~. Different letters (capital letters to Decay and small letters to McKinney's Index)  
432 show significant differences according to Fisher LSD test ( $p < 0.05$ ).

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