

Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus*

Questa è la versione Post print del seguente articolo:

Original

Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus* / Farbo, Maria Grazia; Urgeghe, Pietro Paolo; Fiori, Stefano; Marcello, Angela; Oggiano, Stefania; Balmas, Virgilio; Zahoor Ul Hassan, ; Samir, Jaoua; Migheli, Quirico. - In: INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY. - ISSN 0168-1605. - 284:(2018), pp. 1-10. [10.1016/j.ijfoodmicro.2018.06.023]

Availability:

This version is available at: 11388/215908 since: 2022-05-14T22:10:06Z

Publisher:

Published

DOI:10.1016/j.ijfoodmicro.2018.06.023

Terms of use:

Chiunque può accedere liberamente al full text dei lavori resi disponibili come "Open Access".

Publisher copyright

note finali coverpage

(Article begins on next page)

Manuscript Number: FOOD-D-17-00886R1

Title: Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus*

Article Type: Full Length Article

Keywords: mycotoxin; food safety; gene expression; 2-phenylethanol; post-harvest; biological control; antifungal compounds.

Corresponding Author: Professor Quirico Migheli,

Corresponding Author's Institution: Università degli Studi di Sassari

First Author: Maria Grazia Farbo

Order of Authors: Maria Grazia Farbo; Pietro Paolo Urgeghe; Stefano Fiori; Angela Marcello; Stefania Oggiano; Virgilio Balmas; Zahoor Ul Hassan; Samir Jaoua; Quirico Migheli

Abstract: Many foods and beverages in temperate and tropical regions are prone to contamination by ochratoxin A (OTA), one of the most harmful mycotoxins for human and animal health. *Aspergillus ochraceus* and *Aspergillus carbonarius* are considered among the main responsible for OTA contamination. We have previously demonstrated that four low or non-fermenting yeasts are able to control the growth and sporulation of OTA-producing *Aspergilli* both in vitro and on detached grape berries: the biocontrol effect was partly due to the release of volatile organic compounds (VOCs). Aiming to further characterise the effect of VOCs produced by biocontrol yeast strains, we observed that, beside vegetative growth and sporulation, the volatile compounds significantly reduced the production of OTA by two *A. carbonarius* and *A. ochraceus* isolates. Exposure to yeast VOCs also affected gene expression in both species, as confirmed by downregulation of polyketide synthase, non-ribosomal peptide synthase, monooxygenase, and the regulatory genes *laeA* and *veA*. The main compound of yeast VOCs was 2-phenylethanol, as detected by Headspace-Solid Phase Microextraction-Gas Chromatography-Tandem Mass Spectrometry (HS-SPME-GC-MS) analysis. Yeast VOCs represent a promising tool for the containment of growth and development of mycotoxigenic fungi, and a valuable aid to guarantee food safety and quality.



UNIVERSITÀ DEGLI STUDI DI SASSARI
Dipartimento di Agraria – Sezione di *Patologia vegetale ed Entomologia*
Via Enrico De Nicola, 9 - 07100 Sassari
ITALIA

Quirico Migheli - Tel: ++39 079 229295; Fax: ++39 079 229316; e-mail: qmigheli@uniss.it

Professor Luca Cocolin

Editor-in-Chief, International Journal of Food Microbiology

Dear Professor Cocolin,

we are pleased to submit a revised version of manuscript entitled: "Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus carbonarius* and *A. ochraceus*", by Maria Grazia Farbo, Pietro Paolo Urgeghe, Stefano Fiori, Angela Marcello, Stefania Oggiano, Virgilio Balmas, Zahoor Ul Hassan, Samir Jaoua, and myself.

In this revised version, we have met all the reviewers' requirements, as indicated in the detailed response to reviewers. Changes in text are highlighted in red.

We hope that this new version shall be suitable for publication in *IJFM*.

On behalf of the authors,

Yours faithfully,

Professor Quirico Migheli

A handwritten signature in black ink, appearing to read 'Quirico Migheli', written in a cursive style.

Author's response to Reviewers' comments:

Reviewer #1: The authors analyzed volatile organic compounds (VOCs) produced by four yeast species that they previously isolated. The major VOC was identified to be 2-phenylethanol, a compound known to inhibit fungal growth and development, and production of secondary metabolites. The VOCs of the yeasts reduced both vegetative growth and conidiation as well as ochratoxin A (OTA)-production of *Aspergillus carbonarius* and *Aspergillus ochraceus*. The significant reduction in OTA production was correlated with decreased expression of OTA biosynthesis genes and other related regulatory genes. The work is straightforward. Results may aid current biocontrol of toxigenic fungi in food and feed.

The following are some suggestions for authors' consideration in revising the manuscript-

1. Results presented in Table 2 are somewhat redundant since Figure 1 shows time-course changes of radial growth of colonies. Moreover, Table 4 also shows colony diameters after seven days at 25 °C on PDA. If necessary, briefly state the "reduction" in the text.

R: Table 2 was deleted and the results summarised in the text. Figure 1 was retained as it shows the growth inhibition over 10 days of incubation. Table 4 (now renamed as Table 3) was retained since it allows comparing growth inhibition and OTA inhibition within the same experiments.

2. Lines 281-286 can be presented in the introduction section. No need to describe the general information about VOCs in the discussion section.

R: Done.

3. Lines 316-319 basically are results and can be merged into section 3.3. Give it a strong start by directly discussing the major compound, 2-phenylethanol.

R: Done.

4. OTA production is a major focus of this study. Consider move the part describing OTA pathway and gene structure/organization (lines 333-346) to the introduction section. Discuss only how LaeA and VeA may regulate OTA production.

R: The part related to the description of OTA pathway has been moved to the Introduction section. In the Discussion section, the main differences between *A. carbonarius* and the less studied *A. ochraceus* are highlighted, with focus on the genes investigated in each species.

Reviewer #2: The manuscript Number FOOD-D-17-00886

Title: Effect of yeast volatile organic compounds on ochratoxin A producing *Aspergilli*

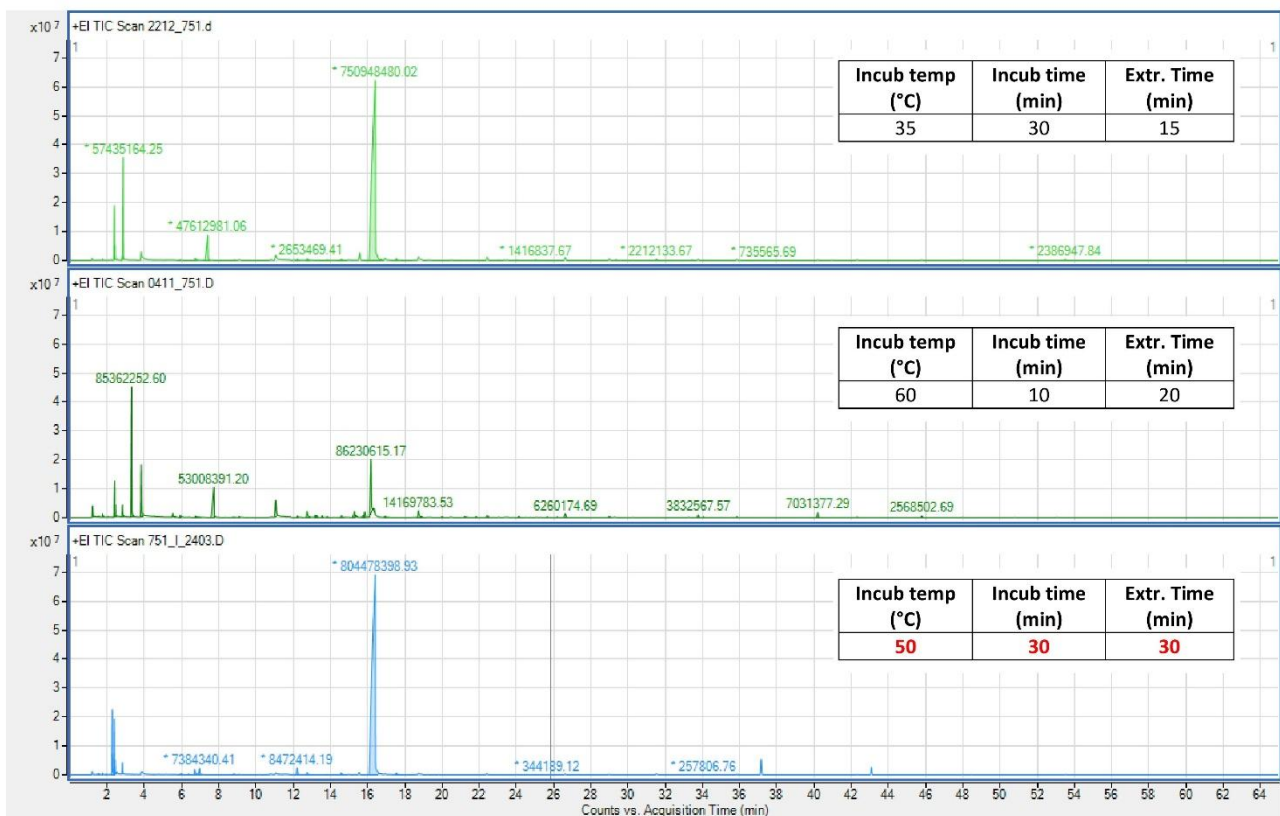
General comments:

The manuscript FOOD-D-17-00886 describes the control effect that VOCs produced by yeasts have on vegetative growth and sporulation by both *A. carbonarius* and *A. ochraceus*. The volatile compounds significantly reduced the production of OTA by both species and affected gene expression in *A. carbonarius*. The manuscript is well presented and the results well presented. There are though some questions and comments that would need to be addressed by the authors before publication is granted.

Specific comments:

With regard to the analysis of the VOCs: The extraction and trapping of the VOCs has been done at a much higher temperature than the experiments were run, 50°C. In this case, could it be possible that we are overestimating the importance of 2-phenylethanol? Would the proportion of volatiles be the same at lower temperatures as the ones used to grow the fungi?

R: We agree that the temperature of extraction and the time of extraction can influence the volatile pattern one can obtain during the subsequent GC-MS analysis, therefore an optimization of these parameters is advisable. To this aim, during the optimization step we tested some different time-temperature combinations, as reported in this Figure:



As it can be seen, performing the incubation either at 35°C or 50°C did not result in any significant difference, being the 2-phenylethanol the main peak of the chromatogram, while increasing the incubation temperature to 60 °C caused a depletion of its peak area (please note that some peaks in the region of 2 minutes of retention time are referable to stationary phase bleeding). Our objective was to obtain a fairly balanced profile, trying to enhance the response of main peaks, and taking also into account that this is a semi-quantitative analysis, being the instrumental response in GC-MS strongly dependent on the structure of single analytes.

The volatile analysis has been performed at a specific time. Does the pattern of VOC from the yeasts change during the time the experiment was running? Have the authors been able to check this?

R: We have not checked whether the VOCs pattern may change over time. Based on the biocontrol data, we decided to measure VOCs after 7 days of yeast growth.

Some of the volatiles the authors have mentioned are not related at all with living organisms. For example, styrene is normally found in laboratory samples since most plastics will contain styrene. Thus, when you heat at 50oC it will be quite common to find styrene in the samples.

R: We thank the reviewer for this suggestion. We have carefully revised Table 5 by removing the analytes that are unlikely to be related to yeast metabolism or YPD. We have also corrected some mistakes in the mean calculations.

The paper looks unbalanced from the point of view that only the gene expression from *A. carbonarius* has been considered. Are VOC modifying the expression of the OTA synthetic pathway in general or is just an effect on this species. This is a very important lack of content in the paper. Nothing has been mentioned in the discussion about why this has happened. This data will need to be included in order to balance the content.

R: The manuscript has been integrated with new expression studies on *A. ochraceus*. Based on available literature, the expression of different genes involved in OTA biosynthesis was evaluated in *A. carbonarius* MPVA566 (*acOTApks* and *acOTAnrps*) and in *A. ochraceus* MPVA703 (*ao-pks* and *nrps-west*) by qRT-PCR. An *acpks* gene as well as two regulatory genes (*laeA* and *veA*) were considered in *A. carbonarius*, whereas two monooxygenase genes (*p450-H11* and *p450-B03*) and a halogenase gene (*hal-west*) were evaluated in *A. ochraceus*. The expression levels of the target genes were normalised by the expression of the reference genes *18S* and *g3pdh* for *A. carbonarius* and *A. ochraceus*, respectively, these being the most stable among four potential reference genes (namely, β - tubulin, actin, *18S* and *g3pdh*) used in preliminary studies.

The explanation of the statistical methods used looks very short and succinct. Have the authors checked the normality and homoscedasticity of the datasets? Where all datasets normally distributed allowing for the use of ANOVA?

R: Normality of residuals and homoscedasticity of variances were indeed verified prior to performing ANOVA. The section has been completed and rephrased as follows: "Data from each experiment were subject to one-way analysis of variance (ANOVA) followed by multiple comparisons by Tukey's test, using the Statgraphics Centurion XVI software. Prior to performing ANOVA, normality of residuals was verified by the Shapiro-Wilk test, whereas assumption of homoscedasticity was checked by the Levene test."

The title is quite broad taking into account that only 2 species of *Aspergillus* has been used and only one has been fully studied.

R: The title has been changed and now refers solely to the two species of *Aspergillus* considered in this study.

- Food and feed can be contaminated by ochratoxin A (OTA) produced by *Aspergillus* spp.
- Non- or low-fermenting yeast selected strains are able to control OTA-producing *Aspergillus ochraceus* and *A. carbonarius*
- Yeast volatile organic compounds (VOCs) are responsible for reduction of vegetative growth and sporulation
- Yeast VOCs also reduce OTA production and OTA-biosynthetic gene expression
- The main component of yeast VOCs is 2-phenylethanol

1 **Effect of yeast volatile organic compounds on ochratoxin A-producing *Aspergillus***
2 ***carbonarius* and *A. ochraceus***

3

4 Maria Grazia Farbo^{1§}, Pietro Paolo Urgeghe^{1§}, **Stefano Fiori^{1§}**, Angela Marcello¹, Stefania
5 Oggiano¹, Virgilio Balmas¹, Zahoor Ul Hassan², Samir Jaoua², Quirico Migheli^{1,3}

6

7 ¹ *Dipartimento di Agraria, Università degli Studi di Sassari, Viale Italia 39, I-07100*
8 *Sassari, Italy.*

9 ² *Department of Biological & Environmental Sciences, College of Arts and Sciences,*
10 *Qatar University, P.O. Box: 2713, Doha, Qatar.*

11 ³ *Unità di Ricerca Istituto Nazionale di Biostrutture e Biosistemi, Università degli Studi*
12 *di Sassari, Viale Italia 39, I-07100 Sassari, Italy.*

13

14 § The first **three** authors have equally contributed to the present work.

15

16 **Corresponding author:**

17 Quirico Migheli, Dipartimento di Agraria, Università degli Studi di Sassari

18 Viale Italia 39, I - 07100 Sassari, Italy

19 Phone: +39 079 229295; Fax: +39 079 229316; E-mail: qmigheli@uniss.it

20

21 **Key words:** mycotoxin, food safety, gene expression, 2-phenylethanol, post-harvest,
22 **biological control, antifungal compounds**

23

24 **Abstract**

25 Many foods and beverages in temperate and tropical regions are prone to contamination
26 by ochratoxin A (OTA), one of the most harmful mycotoxins for human and animal health.

27 *Aspergillus ochraceus* and *Aspergillus carbonarius* are considered among the main
28 responsible for OTA contamination. We have previously demonstrated that four low or
29 non-fermenting yeasts are able to control the growth and sporulation of OTA-producing
30 *Aspergilli* both *in vitro* and on detached grape berries: the biocontrol effect was partly due
31 to the release of volatile organic compounds (VOCs). Aiming to further characterise the
32 effect of VOCs produced by biocontrol yeast strains, we observed that, beside vegetative
33 growth and sporulation, the volatile compounds significantly reduced the production of
34 OTA by **two** *A. carbonarius* and *A. ochraceus* isolates. Exposure to yeast VOCs also
35 affected gene expression in **both species**, as confirmed by downregulation of polyketide
36 synthase, non-ribosomal peptide synthase, **monooxygenase**, and the regulatory genes
37 *laeA* and *veA*. The main compound of yeast VOCs was 2-phenylethanol, as detected by
38 Headspace-Solid Phase Microextraction-Gas Chromatography-Tandem Mass
39 Spectrometry (HS-SPME-GC-MS) analysis. Yeast VOCs represent a promising tool for the
40 containment of growth and development of mycotoxigenic fungi, and a valuable aid to
41 guarantee food safety and quality.

42

43

44 **1. Introduction**

45 After aflatoxins, ochratoxin A (OTA) is the second most frequent mycotoxin found in food
46 and feed products (European Commission, 2012). The OTA structure consists in the
47 amino acid phenylalanine linked by an amide bond to a pentaketide dihydroisocoumarin
48 (Huffman et al., 2010). Some species of *Aspergillus* and *Penicillium* are the main source of
49 OTA in warm and tropical regions, and in particular *Aspergillus carbonarius* (Bainier)
50 Thom. is considered one of the most relevant OTA producers in food and feed (Abarca et
51 al., 2003; Duarte et al., 2010; Kogkaki et al., 2015). OTA is classified as a group 2B
52 carcinogen by the World Health Organization (Cabañes et al., 2013; IARC, 1993; JECFA,

53 2008; Van der Merwe et al., 1965). Studies are still under way to confirm whether OTA is
54 responsible for the so-called Balkan Endemic Nephropathy (Castegnaro et al., 1998;
55 Krogh, 1978). In most countries, strict regulatory limits are set for the presence of OTA in
56 food commodities. The European Union has set the maximum OTA level at 2 mg/kg in
57 wine, grape juice, and other grape products, and at 3 mg/kg for all products derived from
58 cereal, including processed cereal products and cereal grains for human consumption
59 (Duarte et al., 2010; European Commission, 2012).

60 Inhibiting the growth of OTA-producing fungi on sensitive commodities is by far the most
61 reliable method to prevent OTA contamination of food and feed. Fungicides can control the
62 growth of OTA-producing fungi and OTA contamination, but the European Union has
63 established a strict legislation concerning the maximum residue levels of pesticides in
64 agricultural commodities. Moreover, continuous application of specific active substances
65 favours the selection of resistant OTA-producing *Aspergillus* spp. (Malandrakis et al. 2013;
66 Zhang et al., 2016), and often growth inhibition achieved by improper use of fungicides can
67 be accompanied by an unwanted induction of toxin biosynthesis (Schmidt-Heydt et al.
68 2013).

69 Many studies were focused on alternative biological control methods, which may be most
70 appropriate to reduce infection and mycotoxin production by different fungal pathogens
71 both in the field and during the postharvest phases.

72 Among the biological antagonists, yeasts are particularly promising in different
73 commodities, as they have several properties that can be manipulated to improve their use
74 and efficiency. Many yeast species have simple nutritional requirements, they are adapted
75 to colonise wounds as well as dry surfaces and can grow quickly on a broad range of
76 substrates in bioreactors. Furthermore, yeasts do not produce allergenic compounds or
77 secondary metabolites like many filamentous fungi or bacterial antagonists do (Droby et
78 al., 2009; Janisiewicz et al., 2010; Liu et al., 2013).

79 A number of yeast strains were selected and evaluated for use as a pre- or postharvest
80 biological treatment of grape against OTA-producing *Aspergilli* (Bleve et al., 2006; Cubaiu
81 et al., 2012; De Curtis et al., 2012; Ponsone et al., 2011; Zhu et al., 2015).

82 Besides other mechanisms of action, the biocontrol ability of some antagonistic yeast
83 strains has been at least partly attributed to the production of volatile organic compounds
84 (VOCs). VOCs are typically lipophilic substances with low molecular weight (<300 Da),
85 high vapour pressure and low polarity (Werner et al., 2016), and they are able to inhibit
86 mycelial growth and sporulation in many fungi (Buzzini et al., 2005; Chang et al., 2015; Di
87 Francesco et al., 2015; Fiori et al., 2014; Huang et al., 2012; Parafati et al., 2017). VOCs
88 may derive from different biosynthetic pathways, hence the term “volatilome” has been
89 proposed to describe their broad chemical complexity (Maffei et al., 2011). So far, the
90 production of volatile compounds by industrially relevant yeasts has been mainly explored
91 for technological purposes (Passoth et al., 2006; Romano et al., 2015; Wriessenegger and
92 Pichler 2013).

93 Yeast VOCs may also modulate the expression of genes involved in the OTA biosynthesis
94 (Chang et al., 2015). Hence, VOCs released by selected yeasts deserve attention for their
95 ability to reduce spore germination, mycelial growth, and mycotoxin production in
96 preventive food safety strategies.

97 The biosynthetic pathway of OTA, as described by Huff and Hamilton (1979) has not yet
98 been fully explained and only few genes were discovered so far (Abbas et al., 2009; Bacha
99 et al., 2009; Gallo et al., 2014; Geisen et al., 2006; Karolewicz et al., 2005; Niessen et al.,
100 2005; O’ Callaghan et al., 2003; O’ Callaghan et al., 2013; Wang et al., 2015). According
101 to the OTA structure, the biosynthesis pathway includes a polyketide synthase (*pks*) and a
102 non-ribosomal peptide synthase family (*nrps*), but also other genes such as regulators
103 (Bayram et al., 2008) and monooxygenases (O’ Callaghan et al., 2006) are likely to play a
104 key role in the mycotoxin production.

105 In *A. carbonarius*, the *acpks* gene encodes a conserved ketosynthase and acyl transferase
106 domains (Gallo et al., 2009). The *acOTApks* gene encodes a component of the PKS
107 family, and contains a methyltransferase domain responsible for the addition of a methyl
108 group to the OTA polyketide structure (Gallo et al., 2014). Another gene implicated in OTA
109 biosynthesis in *A. carbonarius*, *AcOTAnrps*, is located about 900 nt upstream of *pks* and is
110 transcribed in the same direction, differently from *Penicillium nordicum*, where OTA *pks*
111 and *nrps* genes are transcribed in the opposite direction (Gallo et al., 2009, 2012;
112 Karolewicz et al., 2005). Furthermore, in *A. carbonarius* two other genes are implicated in
113 the regulation of OTA biosynthesis, *laeA* and *veA*. *LaeA* encodes a methyltransferase, and
114 was described for the first time in *Aspergillus nidulans*, in *Aspergillus terreus* and in
115 *Aspergillus fumigatus* (Bok et al., 2004; Linde et al., 2016). *VeA* codes for a regulatory
116 protein, which is transported from the cytoplasm to the nucleus in response to illumination.
117 These two highly conserved proteins are considered as global regulators in fungi,
118 modulating the sporulation capacity and mycotoxin production in *Aspergillus* spp. (Bayram
119 et al., 2008). Deletion of these genes induces a drastic decrease of OTA production and a
120 downregulation in the *nrps* gene expression (Crespo-Sempere et al., 2013).

121 We have previously demonstrated that four low or non-fermenting yeasts are able to
122 control the growth and sporulation of OTA-producing *A. carbonarius* both *in vitro* and on
123 detached grape berries (Fiori et al., 2014). This biological effect was at least partly due to
124 the release of VOCs. The objectives of the present study were: 1) to further determine the
125 effect of VOCs produced by selected yeast strains on vegetative growth and sporulation of
126 OTA-producing *A. carbonarius*, and *A. ochraceus*; 2) to identify the main component(s) of
127 VOCs released by yeasts; 3) to evaluate the capability of yeast VOCs to inhibit OTA
128 production; 4) to evaluate the effect of yeast VOCs on the expression level of key genes in
129 the OTA biosynthetic pathway.

130

131

132 **2. Materials and Methods**

133 *2.1. Fungal and yeast strains and culture conditions*

134 *A. carbonarius* Bainier Thom. MPVA566 and *A. ochraceus* G. Wilh. MPVA703 (courtesy of
135 Professor Paola Battilani, Università Cattolica del Sacro Cuore, Piacenza, Italy) strains are
136 maintained in the mycological collection of the Dipartimento di Agraria, Università di
137 Sassari (Italy). The two strains were tested for their potential to produce OTA on PDA
138 (potato dextrose agar; Sigma-Aldrich, St. Louis, MO, USA), after incubation at 25 °C for 7
139 days, as described by Bragulat et al. (2001).

140 Four yeast strains, namely two non-fermenting (*Cyberlindnera jadinii* 273 and *Candida*
141 *friedrichii* 778) and two low-fermenting (*Candida intermedia* 235 and *Lachancea*
142 *thermotolerans* 751) were selected based on their ability to control the growth and
143 sporulation of *A. carbonarius* (Fiori et al., 2014) and for their OTA-adsorption properties
144 (Farbo et al., 2016).

145

146 *2.2. Inhibition of fungal growth and OTA production by yeast strains*

147 A spore suspension (10^5 spores/mL) of each strain of *A. carbonarius* MPVA566 and *A.*
148 *ochraceus* MPVA703, grown on PDA for seven days at 25 °C, was prepared in Ringer's
149 solution containing 0.1% Tween 20 (Sigma) to prevent spore clumping.

150 The four yeasts were routinely grown on YPD agar (1% yeast extract, 2% peptone, 2%
151 dextrose, 2% agar; Sigma-Aldrich, St. Louis, MO, USA) and stored at 4 °C until use. Two
152 days before each trial, yeast were grown on PDA agar at 25 °C and a loopful of fresh cells
153 was further grown overnight at 25°C in 100 mL YPD broth (1% yeast extract, 2%
154 bacteriological peptone, 2% dextrose; Sigma-Aldrich, St. Louis, MO, USA). Cells were
155 recovered by centrifugation, washed, resuspended in Ringer's solution (0.9% NaCl) and
156 counted in a Thoma haemocytometer to obtain a final concentration of 1×10^8 CFU/mL.

157 To evaluate the efficacy of yeast VOCs against *Aspergillus* spp., 100 μ L of yeast cell
158 suspension (10^8 CFU/mL) was evenly spread on YPD agar plates by using a disposable
159 sterile inoculation loop and incubated at 25 °C for 24 h. After 24 h, the lid of the plate was
160 replaced by a base plate containing PDA. Twenty μ L of a spore suspension (10^7 CFU/mL)
161 of each pathogen were spotted onto the centre of the PDA plate. The two base plates
162 were sealed immediately with a double layer of Parafilm[®] and one layer of Scotch[®] tape
163 and incubated at 25 °C for 7-10 days. The plates were examined daily to measure radial
164 growth and any change in morphology of *Aspergillus* colonies was recorded. A control
165 treatment was represented by plates inoculated only with *Aspergillus*. There were three
166 replicates for each treatment and the experiment was repeated three times.

167

168 2.3. Analysis of yeast VOCs

169 A loopful of freshly grown yeast cells was transferred into a 20 mL amber headspace vial
170 containing 5 mL of YPD agar slant and incubated at 26 °C for seven days. Yeast VOCs
171 composition was qualitatively evaluated by head-space solid phase microextraction (HS-
172 SPME) followed by gas chromatography coupled with mass spectrometry (GC-MS)
173 analysis. Prior to extraction the vials were equilibrated under agitation for 30 min at 50 °C.
174 The extraction of VOCs was performed on a 1-cm PDMS 50/30 Stableflex SPME fiber
175 (Supelco, Milano, Italy). The extraction time was fixed at 30 min, after which it was
176 desorbed for 2 min into a Gerstel CIS6 PTV injector operating at 250 °C in a splitless
177 mode. Before and after each extraction, the fiber underwent a bake-out step for 5 min at
178 250 °C on a Gerstel bake-out station.

179 The GC-MS analysis was carried out using an Agilent 7890 GC equipped with a Gerstel
180 MPS autosampler, coupled with an Agilent 7000C MSD detector. The chromatographic
181 separation was performed on a HP-5MS capillary column (30 m \times 0.25 mm, film thickness
182 0.17 μ m) with the following temperature program: 60 °C hold for 3 min, then increased to

183 210 °C at a rate of 4 °C/min, then held at 210 °C for 15 min, then increased to 280 °C at a
184 rate of 10 °C/min. Helium was used as carrier gas, at a constant flow of 1 mL/min. The
185 data were analysed using a MassHunter Workstation B.07.00 SP1. The identification of
186 the components was performed by comparing their retention indices with the built-in
187 libraries or literature data and by matching their spectra on mass spectral libraries
188 (NIST/EPA/NIH 2008).

189

190 *2.4. OTA extraction and HPLC analysis*

191 OTA production was assessed after seven days of incubation according to the method
192 described by Bragulat et al. (2001). Briefly, three PDA agar plugs (6 mm diameter each)
193 were removed with a sterile cork borer, across the diameter of the mycelium. Samples
194 were weighed, placed into a 2.0 mL Eppendorf tube, 1 mL methanol was added and the
195 tubes were briefly hand-shaken. Then, they were left still for one h, extracts were filtered
196 through a 0.2 µm filter (Millex, Millipore Co., Bedford, Mass.) and stored at 4°C until HPLC
197 analysis. OTA was analysed using an Agilent 1200 HPLC system (Agilent Technologies,
198 Palo Alto, CA, USA) equipped with a quaternary pump with integrated vacuum degasser
199 (G1311C), autosampler (G1329B), column oven (G1316A) and fluorescence detector
200 (G1321A). Separation of OTA was performed on a Zorbax column SB-C18, 4.6 x 150 mm,
201 5-Micron (Agilent, Santa Clara, CA, USA). The isocratic mobile phase composition was a
202 mixture of acetonitrile, water and acetic acid (49.5:49.5:1 v/v/v, respectively). The constant
203 flow and column temperature were set to 1 mL/min and 25°C, respectively. The excitation
204 and the emission wavelengths were set to 333 and 460 nm, respectively. OTA
205 identification was achieved by comparing the retention time of the pure standard (5.9 min)
206 with that of the target peak on samples. The injection volume was 100 µL. A linear external
207 calibration over a range from 0.6 to 60 µg/L, with a mean correlation coefficient of 0.99995,
208 was used for the quantification step. Results were then expressed as ng per g of sample.

209 In a preliminary experiment, a time course of mycelium growth and OTA production by
210 *Aspergilli* cultured on PDA at 25°C, with no contact with yeast cells, was assessed by
211 evaluating the radial growth and by analysing 3 plugs cut at 1, 2, 3 and 7 days from
212 duplicate colonies as described previously.

213

214 2.5. Gene expression analysis in *A. carbonarius* MPVA566 and *A. ochraceus* MPVA703

215 The expression of different genes involved in OTA biosynthesis was evaluated in *A.*
216 *carbonarius* MPVA566 (*acOTApks* and *acOTAnrps*) and in *A. ochraceus* MPVA703 (*ao-*
217 *pks* and *nrps-west*) by qRT-PCR. An *acpks* gene as well as two regulatory genes (*laeA*
218 and *veA*) were considered in *A. carbonarius*, whereas two monooxygenase genes (*p450-*
219 *H11* and *p450-B03*) and a halogenase gene (*hal-west*) were evaluated in *A. ochraceus*.

220 The expression levels of the target genes were normalised by the expression of the
221 reference genes *18S* and *g3pdh* for *A. carbonarius* and *A. ochraceus*, respectively, and
222 hence expressed relatively to the control. The two genes *18S* and *g3pdh* were chosen as
223 reference being the most stable among four potential reference genes (namely, β - tubulin,
224 actin, *18S* and *g3pdh*) used in preliminary studies (not shown). For gene expression
225 analysis, 20 μ L of spore suspension (10^7 CFU/mL) of *A. carbonarius* MPVA566 or *A.*
226 *ochraceus* MPVA703 were inoculated in PDB and incubated at 25 °C for five days as
227 previously described. After 120 h, the mycelium was carefully harvested, ground in liquid
228 nitrogen and stored at - 80 °C. Total RNA was extracted from frozen mycelium using the
229 GeneMATRIX® Universal RNA Purification Kit (URx Ltd, Gdansk, Poland) following the
230 manufacturer's protocol and then reverse-transcribed into cDNA using the iScript® cDNA
231 Synthesis Kit (Bio-Rad, Hercules, CA, USA).

232 Gene-specific primers are listed in Table 1. The PCR efficiencies for each primer pair were
233 determined by a 5-fold serial cDNA dilution of cDNA, and calculated from the slopes of the
234 curve realised by the CFX96 Touch Real time PCR detection system software (version

235 3.1, 2010; Bio-Rad). The reactions were held using CFX96 Touch Real Time PCR
236 detection system Bio-Rad (version 3.1, 2010) using SsoAdvanced Universal SYBR[®] Green
237 Supermix (Bio-Rad), by starting with 10 ng/μL of synthesised cDNA. The PCR programme
238 was as follows: 95 °C for 3 min, 40 cycles of 95 °C for 10 s and 58 °C for 1 min. The
239 melting curve was defined by 0.5°C increments of the slow ramp rate between 55 °C and
240 95 °C after the real-time PCR cycles. Specificity of the PCR amplification was confirmed
241 by dissociation curve analysis. Real time PCR derived data as normalised expression
242 ($\Delta\Delta C_q$) were quantified by using the measured expression level of one reference gene
243 (target) as a normalisation factor. Reaction were prepared in triplicate 96-well reaction
244 plates, and three replicates of control sample without cDNA were also included in the runs.
245

246 2.6. Statistical analysis

247 Data from each experiment were subject to one-way analysis of variance (ANOVA)
248 followed by multiple comparisons by Tukey's test, using the Statgraphics Centurion XVI
249 software. Prior to performing ANOVA, normality of residuals was verified by the Shapiro-
250 Wilk test, whereas assumption of homoscedasticity was checked by the Levene test.

251

252 3. Results

253 3.1. Yeast VOCs reduce vegetative growth and sporulation of *A. carbonarius* and *A.*
254 *ochraceus*

255 All the four yeast strains were able to inhibit mycelium development of both *A. carbonarius*
256 MPVA566 and *A. ochraceus* MPVA703. Colony diameter was reduced to 30-50%
257 compared to the untreated control after 10 days of incubation with no contact between
258 bionts (Figure 1).

259 *Aspergillus* spp. colonies exposed to yeast VOCs did not sporulate, and were
260 characterised by a white mycelium. The colony border was undefined, with elongated

261 hyphae irregularly branched compared to unexposed control (Figure 2). Both *A.*
262 *carbonarius* MPVA566 and *A. ochraceus* MPVA703, upon exposure to VOCs emitted by *L.*
263 *thermotolerans* 751, produced hyphae containing coagulated cytoplasm, with swollen
264 hyphal tips exhibiting frequent lysis (Figures 2-3).

265

266 3.2. Yeast VOCs inhibit OTA production in vitro

267 A preliminary experiment was set to monitor the time course of OTA production by the two
268 *Aspergilli*. After seven days of growth on PDA medium at 25 °C, *A. carbonarius* MPVA566
269 produced 8,316.6 ng/g of OTA, whereas *A. ochraceus* MPVA703 produced 29,679.9 ng/g
270 (Table 2).

271 Three separate experiments were set up to evaluate the ability of yeast strains to inhibit
272 both growth and OTA production when co-cultured with *A. carbonarius* MPVA566 or *A.*
273 *ochraceus* MPVA703. The reduction of radial growth after 7 days incubation on PDA at 25
274 °C ranged from 56 to 74%, with *C. intermedia* 253 and *L. thermotolerans* 751 being the
275 most effective inhibitors of colony growth (Table 3).

276 The vegetative growth inhibition was accompanied by a striking reduction of OTA
277 production as an effect of VOCs release by the yeast strains: in *A. carbonarius* MPVA566
278 the OTA content released in the medium dropped from 7,613-13,883 ng/g in the
279 unexposed control to 0.1-135 ng/g upon exposure to yeast volatiles, whereas in *A.*
280 *ochraceus* MPVA703 OTA release was reduced from 19,609-42,960 to 2.7-940 ng/g.
281 VOCs emitted by *L. thermotolerans* 751 provided the most consistent inhibition of OTA
282 production in both *Aspergillus* species (Table 3).

283

284 3.3. 2-phenylethanol is the main component in yeast VOCs

285 The HS-SPME/GC-MS analysis allowed to identify about 20 compounds, belonging to
286 different chemical classes, such as alcohols, aldehydes, hydrocarbons and terpenes.

287 Table 4 summarizes, for each of the four tested yeast strains, the absolute area ($\times 10^6$) of
288 each analyte. Most of these volatiles showed a negligible area, and were also detected in
289 the control headspace, possibly due to the presence of yeast extract in the YPD medium.
290 The main component of all the yeast volatile fraction was 2-phenylethanol, which was not
291 detectable in the YPD medium. Other volatile compounds showed a significantly lower or
292 negligible area, compared with 2-phenylethanol; most of these compounds were also
293 found in the control YPD agar medium headspace (Figure 4).

294

295 3.4. *Aspergillus carbonarius* and *Aspergillus ochraceus* genes involved in OTA 296 biosynthesis are downregulated by yeast VOCs

297 In *A. carbonarius* MPVA566, yeast VOCs dramatically affected the expression level of all
298 the tested OTA biosynthetic genes *AcOTApks*, *AcOTAnrps*, *acpks*, as well as the two
299 regulatory genes *veA* and *laeA* (Figure 5). The expression of *acpks* was almost totally
300 abolished (99%) when the fungus was co-cultured with *C. intermedia* 253, *C. jadinii* 273, *L.*
301 *thermotolerans* 751 and *C. friedrichii* 778. Similarly, *acOTApks* and *AcOTAnrps* expression
302 levels were both equally reduced by 99.9% by the four yeasts. Expression of the
303 regulatory genes *veA* and *laeA* was affected by 97% by *C. intermedia* 253 and by 99.9%
304 by the other three yeast strains (Figure 5).

305 In *A. ochraceus* MPVA703 the expression level of tested OTA biosynthetic genes was far
306 less influenced by yeast VOCs, with the strongest reduction of *pks* expression observed
307 when the fungus was co-cultured with *C. intermedia* 253 (37%) and *L. thermotolerans* 751
308 (25%). *L. thermotolerans* 751 was also the major inhibitor of *nrps* (35%) and one of the
309 strongest repressors of H11 and B03 (both 30%) while the *hal* gene resulted the most
310 affected by all four yeasts, with a reduction of its expression level ranging from 27% (by *L.*
311 *thermotolerans* 751) to 38% (by *C. intermedia* 253; Figure 6).

312

313

314

315 **4. Discussion**

316 The presence of OTA in food and feed chain is a major concern for food safety and health
317 authorities (Ponsone et al., 2011). In a previous investigation (Fiori et al., 2014), we proved
318 the high antagonistic efficacy of two non-fermenting (*Cyberlindnera jadinii* 273 and
319 *Candida friedrichii* 778) and two low-fermenting yeasts (*Candida intermedia* 235 and
320 *Lachancea thermotolerans* 751) in controlling *A. carbonarius* *in vitro* and on grape berries.
321 The biological effect was at least partly ascribed to the release of VOCs. The present work
322 focuses on the chemical composition and role of VOCs produced by these four yeast
323 strains and on their inhibitory effect on *A. carbonarius* and *A. ochraceus* growth and
324 sporulation, OTA production, and OTA biosynthetic gene expression.

325 Since the first exploitation in biocontrol of the VOCs producer *Muscodor albus* (Worapong
326 et al., 2001; Strobel et al., 2001), a panoply of VOCs produced by isolated soil microbes
327 showed antifungal activity against plant pathogens (Kanchiswamy et al., 2015; Werner et
328 al., 2016; Zeilinger et al., 2016). Many biocontrol yeast strains have the ability to produce
329 VOCs and in some instances the volatiles were shown to be implicated as main drivers of
330 the antagonistic capacity. For instance, VOCs emitted by *Candida intermedia*,
331 *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* inhibited *Botrytis cinerea* growth
332 *in vitro* and reduced disease on strawberries inoculated with this pathogen (Suwannarach
333 et al., 2010; Huang et al., 2011; Parafati et al., 2015). A mixture of VOCs produced by
334 *Candida sake* was able to reduce the incidence of apple rot caused by *Penicillium*
335 *expansum* and *B. cinerea* (Arrarte et al., 2017). VOCs released by different strains of
336 *Pichia anomala*, *Pichia kluyveri*, and *Hanseniaspora uvarum* inhibited *A. ochraceus* growth
337 and OTA production during processing of *Coffea arabica* (Masoud et al., 2005; Masoud &
338 Kaltoft, 2006). On the contrary, the role of VOCs released by biocontrol strains of *Candida*

339 *zemplanina*, *Saccharomyces cerevisiae*, *P. kluyveri*, and *Metschnikowia aff. fructicola* in the
340 *in vitro* and *in vivo* inhibition of *A. carbonarius* was not proven (Zhu et al., 2015).

341 The present study demonstrates that the production of VOCs, and particularly 2-
342 phenylethanol, plays an essential role in the antagonistic activity of four yeasts strains
343 against *A. carbonarius* MPVA566 and *A. ochraceus* MPVA703. All the experiments were
344 performed under airtight conditions, and any contact between *Aspergilli* and yeast strains
345 was prevented. Therefore, the antifungal activity of yeasts can be definitely attributed to
346 their VOCs release. While all the four yeast tested were biologically active, two strains (*C.*
347 *intermedia* 253 and *L. thermotolerans* 751), were most effective in reducing mycelial
348 growth, sporulation and *in vitro* OTA production by *A. carbonarius* and *A. ochraceus*.

349 Remarkably, in both *Aspergilli*, hyphae containing coagulated cytoplasm, with swollen
350 hyphal tips and frequent lysis were observed upon exposure to *L. thermotolerans* 751
351 volatilome. To the best of our knowledge, this study reveals for the first time the ability of
352 the yeast species *C. jadinii*, *C. friedrichii*, *C. intermedia* and *L. thermotolerans* to reduce
353 the production of OTA by two *Aspergilli* through the release of VOCs.

354 The GC-MS analysis allowed us to identify several compounds, including alcohols,
355 aldehydes, hydrocarbons and terpenes, with a marked variability on their absolute area.

356 The foremost compound detected in our work, 2-phenylethanol, has been previously
357 observed as the main volatile produced by other yeasts, such as *S. cerevisiae* with a
358 potential to control the pathogen *Sclerotinia sclerotiorum* *in vitro* and in bean seeds (Fialho
359 et al., 2010), *Kloeckera apiculata* controlling *P. italicum* mold in citrus fruit (Liu et al.,
360 2014), and *P. anomala* able to inhibit spore germination and aflatoxin production by
361 *Aspergillus flavus* (Chang et al. 2015; Hua et al., 2014). This compound is present in
362 nature, particularly in extracts of perfumed flowers and essential oils, and represents one
363 of the nine most dominant volatile compounds of the rose scent (Yan et al. 2011). Chang
364 et al. (2015) reported that 2-phenylethanol has a lethal effect against *A. flavus* and inhibits

365 the production of aflatoxin at sublethal dosage. High concentrations of 2-phenylethanol
366 may cause alterations in amino acid and protein biosynthesis, in mitochondria and the
367 nuclei of both fungi and bacteria (Rosenkranz, et al., 1965; Liu et al., 2014).

368 Our results **indicate** that yeast volatiles are able to downregulate key genes implicated in
369 OTA biosynthesis. **In *A. carbonarius*, so far the best described OTA-producing *Aspergillus***
370 **species, expression of all the tested OTA biosynthetic (*AcOTApks*, *AcOTAnrps*, *acpks*) as**
371 **well as the two regulatory genes *veA* and *laeA* was almost completely suppressed by**
372 **yeast VOCs. In *A. ochraceus*, instead, knowledge of the OTA biosynthetic gene**
373 **organisation is less understood. Therefore, alongside the polyketide synthase gene *pks*, a**
374 **non-ribosomal peptide synthase *nrps* and a halogenase protein that could be responsible**
375 **for chlorination in OTA biosynthesis (Gil-Serna et al., 2018) were evaluated in the present**
376 **study using primers previously tested on the phylogenetically similar species *A.***
377 ***westerdijkiae*. In addition, we tested the expression of two putative p450 type**
378 **monooxygenase genes, namely *p450-H11* and *p450-B03*, known for having a role in other**
379 **mycotoxin biosynthetic pathways (O'Callaghan et al., 2006). Compared to *A. carbonarius*,**
380 **the expression level of tested OTA biosynthetic genes in *A. ochraceus* was less affected,**
381 **with inhibition levels comprised between 25 and 38%. Whether this discrepancy indicates**
382 **a differential sensitivity to VOCs by the two species/tested isolates or is dependent on the**
383 **primers used is still questionable.**

384 **In a previous report (Chang et al., 2015) based on *A. flavus* exposed to low concentrations**
385 **of 2-phenylethanol, this compound decreased the aflatoxin production by reducing the**
386 **expression levels of all aflatoxin gene pathway. This specific effect on secondary**
387 **metabolism may explain the fact that while vegetative growth was reduced by 50-70%,**
388 **OTA release in the medium was almost completely repressed upon exposure to yeast**
389 **VOCs.**

390 Overall, the present report provides additional evidence to the suitability of selected yeast
391 strains able to produce VOCs as important tools for postharvest management of
392 mycotoxin-producing fungi. Acting as biodegradable fumigants, VOCs leave no residues
393 and do not need any direct contact between yeast cells and the surface of the target
394 commodity (Parafati et al. 2017). To further develop yeast VOCs application in postharvest
395 disease control, it will be crucial to increase their stable production over time by identifying
396 optimal growing and storage conditions. Moreover, there is a need to develop new carriers
397 for efficient and inexpensive application and compatibility with standard postharvest
398 handling pipeline (Parafati et al. 2017). Finally, those compounds that are exclusively
399 responsible for fungal inhibition should be further characterised to allow the design of
400 improved artificial VOCs mixtures with high efficiency and low toxicity towards non-target
401 organisms.

402

403 **Acknowledgments**

404 This publication was made possible by NPRP grant # 8-392-4-003 from the Qatar National
405 Research Fund (a member of Qatar Foundation). The findings achieved herein are solely
406 the responsibility of the authors. **We acknowledge constructive comments provided by**
407 **anonymous reviewers.**

408

409

410 **References**

411 Abarca, M.L., Accensi, F., Bragulat, M.R., Castella, G., Cabañes, F.J., 2003. *Aspergillus*
412 *carbonarius* as the main source of ochratoxin A contamination in dried vine fruits from the
413 Spanish market. J. Food Prot. 66, 504-506.

414 Abbas, A., Valez, H., Dobson, A.D.W., 2009. Analysis of the effect of nutritional factors on
415 OTA and OTB biosynthesis and polyketide synthase gene expression in *Aspergillus*
416 *ochraceus*. Int. J. Food Microbiol. 135, 22-27.

417 Arrarte, E., Garmendia, G., Rossini, C., Wisniewski, M., Vero, S., 2017. Volatile organic
418 compounds produced by Antarctic strains of *Candida sake* play a role in the control of
419 postharvest pathogens of apples. Biol. Control 109, 14-20.

420 Bacha, N., Atoui, A., Mathieu, F., Liboz, T., Lebrihi, A., 2009. *Aspergillus westerdijkiae*
421 polyketide synthase gene “aoks1” is involved in the biosynthesis of ochratoxin A. Fungal
422 Genet. Biol. 46, 77-84.

423 Bayram, Ö., Krappmann, S., Ni, M., Bok, J.W., Helmstaedt, K., Valerius, O., Braus-
424 Stromeyer, S., Kwon, N.J., Keller, N.P., Yu, J.H., Braus, G.H., 2008. VeIB/VeA/LaeA
425 complex coordinates light signal with fungal development and secondary metabolism.
426 Science 320, 1504-1506.

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

430 Bok, J.W., Keller, N.P., 2004. *LaeA*, a regulator of secondary metabolism in *Aspergillus*
431 *spp.* Eukaryot. Cell 3, 527-535.

432 Bragulat, M.R., Abarca, M.L., Cabañes, F.J., 2001. An easy screening method for fungi
433 producing ochratoxin A in pure culture. Int. J. Food Microbiol. 71, 139-144.

434 Buzzini, P., Romani, S., Turchetti, B., Vaughan, A., Pagnoni, U.M., Davoli, P. 2005.
435 Production of volatile organic sulfur compounds (VOCs) by basidiomycetous yeasts.
436 FEMS Yeast Res. 5, 379-385.

437 Cabañes, F.J., Bragulat, M.R., Castellá, G., 2013. Characterization of nonochratoxigenic
438 strains of *Aspergillus carbonarius* from grapes. Food Microbiol. 36, 135-141.

439 Castegnaro, M., Mohr, U., Pfohl-Leszkowicz, A., Estève, J., Steinmann, J., Tillmann, T.,
440 Michelon, J., Bartsch, H., 1998. Sex- and strain-specific induction of renal tumors by
441 ochratoxin A in rats correlated with DNA adduction. *Int. J. Cancer* 77, 70-75.

442 Chang, P.K., Hua, S.S.T., Sarreal, S.B.L., Li, R.W., 2015. Suppression of aflatoxin
443 biosynthesis in *Aspergillus flavus* by 2-phenylethanol is associated with stimulated growth
444 and decreased degradation of branched-chain amino acids. *Toxins* 7, 3887-3902.

445 Crespo-Sempere, A., Marin, S., Sanchis, V., Ramos, A.J., 2013. VeA and LaeA
446 transcriptional factors regulate ochratoxin A biosynthesis in *Aspergillus carbonarius*. *Int. J.*
447 *Food Microbiol.* 166, 479-486.

448 Cubaiu, L., Abbas, H., Dobson, A.D.W., Budroni, M., Migheli, Q., 2012. A *Saccharomyces*
449 *cerevisiae* wine strain inhibits growth and decreases ochratoxin A biosynthesis by
450 *Aspergillus carbonarius* and *Aspergillus ochraceus*. *Toxins* 4, 1468-1481.

451 De Curtis, F., de Felice, D.V., Ianiri, G., De Cicco, V., Castoria, R., 2012. Environmental
452 factors affect the activity of biocontrol agents against ochratoxigenic *Aspergillus*
453 *carbonarius* on wine grape. *Int. J. Food Microbiol.* 159, 17-24.

454 Di Francesco, A., Ugolini, L., Lazzeri, L., Mari, M., 2015. Production of volatile organic
455 compounds by *Aureobasidium pullulans* as a potential mechanism of action against
456 postharvest fruit pathogens. *Biol. Control* 81, 8-14.

457 Droby, S., Wisniewski, M., Macarisin, D., Wilson, C., 2009. Twenty years of postharvest
458 biocontrol research: Is it time for a new paradigm? *Postharvest Biol. Technol.* 52, 137-145.

459 Duarte, S.C., Pena, A., Lino, C.M., 2010. A review on ochratoxin A occurrence and effects
460 of processing of cereal and cereal derived food products. *Food Microbiol.* 27, 187-198.

461 European Commission (EC), 2012. Rapid Alert System for Food and Feed (RASFF).
462 Annual Report. Directorate-General for Health and Consumers. Available:
463 http://ec.europa.eu/food/food/rapidalert/docs/rasff_annual_report_2012_en.pdf.

464 Farbo M.G., Urgeghe P.P., Fiori S., Marceddu S., Jaoua S., Migheli Q., 2016. Adsorption
465 of ochratoxin A from grape juice by yeast cells immobilised in calcium alginate beads. *Int.*
466 *J. Food Microbiol.* 217, 29-34.

467 Fialho, M.B., Toffano, L., Pedroso, M.P., Augusto, F., Pascholati, S.F., 2010. Volatile
468 organic compounds produced by *Saccharomyces cerevisiae* inhibit the *in vitro*
469 development of *Guignardia citricarpa*, the causal agent of citrus black spot. *World J.*
470 *Microbiol. Biotechnol.* 26, 925-932.

471 Fiori, S., Urgeghe, P.P., Hammami, W., Razzu, S., Jaoua, S., Migheli, Q., 2014. Biocontrol
472 activity of four non- and low-fermenting yeast strains against *Aspergillus carbonarius*
473 and their ability to remove ochratoxin A from grape juice. *Int. J. Food Microbiol.* 189, 45-
474 50.

475 Gallo, A., Perrone, G., Solfrizzo, M., Epifani, F., Abbas, A., Dobson, A.D., Mulè, G., 2009.
476 Characterisation of a *pks* gene which is expressed during ochratoxin A production by
477 *Aspergillus carbonarius*. *Int. J. Food Microbiol.* 129, 8-15.

478 Gallo, A., Bruno, K.S., Solfrizzo, M., Perrone, G., Mulè, G., Visconti, A., Baker, S.E., 2012.
479 New insight into the ochratoxin A biosynthetic pathway through deletion of a nonribosomal
480 peptide synthetase gene in *Aspergillus carbonarius*. *Appl. Environ. Microbiol.* 78, 8208-
481 8218.

482 Gallo, A., Knox, B.P., Bruno, K.S., Solfrizzo, M., 2014. Identification and characterization
483 of the polyketide synthase involved in ochratoxin A biosynthesis in *Aspergillus*
484 *carbonarius*. *Int. J. Food Microbiol.* 179, 10-17.

485 Geisen, R., Schmidt-Heydt, M., Karolewicz, A., 2006. A gene cluster of the ochratoxin A
486 biosynthetic genes in *Penicillium*. *Mycotoxin Res.* 22, 134-141.

487 Gil-Serna, J., García-Díaz, M., González-Jaén, M.T., Vázquez, C., Patiño, B., 2018.
488 Description of an orthologous cluster of ochratoxin A biosynthetic genes in *Aspergillus* and
489 *Penicillium* species. A comparative analysis. *Int. J. Food Microbiol.* 268, 35-43.

490 Hua, S.S.T., Beck, J.J., Sarreal, S.B. L., Gee, W., 2014. The major volatile compound 2-
491 phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of
492 aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycotoxin Res.*, 30, 71-78.

493 Huang, R., Li, G.Q., Zhang, J., Yang, L., Che, H.J., Jiang, D.H., Huang, H.C., 2011.
494 Control of postharvest *Botrytis* fruit rot of strawberry by volatile organic compounds of
495 *Candida intermedia*. *Phytopathology* 101, 859-869.

496 Huang, R., Che, H.J., Zhang, J., Yang, L., Jiang, D.H., Li, G.Q., 2012. Evaluation of
497 *Sporidiobolus pararoseus* strain YCXT3 as biocontrol agent of *Botrytis cinerea* on post-
498 harvest strawberry fruits. *Biol. Control* 62, 53-63.

499 Huff, W.E., Hamilton, P.B., 1979. Mycotoxins - Their biosynthesis in fungi: ochratoxins-
500 metabolites of combined pathways. *J. Food Prot.* 42, 815-820.

501 Huffman, J., Gerber, R., Du, L., 2010. Recent advancements in the biosynthetic
502 mechanisms for polyketide-derived mycotoxins. *Biopolymers* 93, 764-776.

503 IARC, 1993. Ochratoxin A. IARC Monographs on the Evaluation of Carcinogenic Risks to
504 Humans. 56. IARC, Lyon, France.

505 Janisiewicz, W.J., Kurtzman, C.P., Buyer, J.S., 2010. Yeasts associated with nectarines
506 and their potential for biological control of brown rot. *Yeast* 27, 389-398.

507 JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2008. Safety Evaluation of
508 Certain Mycotoxins in Food. WHO Food Additives Series 59. Available
509 http://whqlibdoc.who.int/publications/2008/9789241660594_eng.pdf.

510 Kanchiswamy, C.N., Malnoy, M., Maffei, M.E., 2015. Chemical diversity of microbial
511 volatiles and their potential for plant growth and productivity. *Front. Plant. Sci.* 6, 151.

512 Karolewicz, A., Geisen, R., 2005. Cloning a part of the ochratoxin A biosynthetic gene
513 cluster of *Penicillium nordicum* and characterization of the ochratoxin polyketide synthase
514 gene. *Syst. Appl. Microbiol.* 28, 588-595.

515 Kogkaki, E.A., Natskoulis, P.I., Magan, N., Panagou, E.Z., 2015. Effect of interaction
516 between *Aspergillus carbonarius* and non-ochratoxigenic grape-associated fungal isolates
517 on growth and ochratoxin A production at different water activities and temperatures. Food
518 Microbiol. 46, 521-527.

519 Krogh, P., 1978. Casual associations of mycotoxic nephropathy. Acta Path. Micro. Im. A
520 269, 1-28.

521 Linde, T., Zoglowek, M., Lübeck, M., Frisvad, J.C., Lübeck, P.S., 2016. The global
522 regulator LaeA controls production of citric acid and endoglucanases in *Aspergillus*
523 *carbonarius*. J. Ind. Microbiol. Biotechnol. 43, 1139-1147.

524 Liu, J., Sui, Y., Wisniewski, M., Droby, S., Liu, Y., 2013. Review: Utilization of antagonistic
525 yeasts to manage postharvest fungal diseases of fruit. Int. J. Food Microbiol. 167, 153-
526 160.

527 Liu, P., Cheng, Y., Yang, M., Liu, Y., Chen, K., Long, C.A., Deng, X., 2014. Mechanisms of
528 action for 2-phenylethanol isolated from *Kloeckera apiculata* in control of *Penicillium* molds
529 of citrus fruits. BMC Microbiol. 14, 242.

530 Maffei, M.E., Gertsch, J., Appendino, G., 2011. Plant volatiles: production, function and
531 pharmacology. Nat. Prod. Rep. 28, 1359-1380.

532 Malandrakis, A.A., Vattis, K.N., Doukas, E.G., Markoglou, A.N., 2013. Effect of
533 phenylpyrrole-resistance on fitness parameters and ochratoxin production in *Aspergillus*
534 *carbonarius*. Int. J. Food Microbiol. 165, 287-294.

535 Masoud, W., Poll, L., Jakobsen, M., 2005. Influence of volatile compounds produced by
536 yeasts predominant during processing of *Coffea arabica* in East Africa on growth and
537 ochratoxin A (OTA) production by *Aspergillus ochraceus*. Yeast 22, 1133-1142.

538 Masoud, W., Kaltoft, C.H., 2006. The effects of yeasts involved in the fermentation of
539 *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus*
540 *ochraceus*. Int. J. Food Microbiol. 106, 229-234.

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

543 Niessen, L., Schmidt, H., Mühlencoert, E., Färber, P., Karolewicz, A., Geisen, R., 2005.
544 Advances in the molecular diagnosis of ochratoxin A-producing fungi. *Food Addit.*
545 *Contam.* 22, 324-334.

546 O'Callaghan, J., Caddick, M.X., Dobson, A.D.W., 2003. A polyketide synthase gene
547 required for ochratoxin A biosynthesis in *Aspergillus ochraceus*. *Microbiology* 149, 3485-
548 3491.

549 O'Callaghan, J., Stapleton, P.C., Dobson, A.D.W., 2006. Ochratoxin A biosynthetic genes
550 in *Aspergillus ochraceus* are differentially regulated by pH and nutritional stimuli. *Fungal*
551 *Genet. Biol.* 43, 213-221.

552 O'Callaghan, J., Coghlan, A., Abbas, A., García-Estrada, C., Martín, J.F., Dobson, A.D.W.,
553 2013. Functional characterization of the polyketide synthase gene required for ochratoxin
554 A biosynthesis in *Penicillium verrucosum*. *Int. J. Food Microbiol.* 161, 172-181.

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

558 Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2017. Performance evaluation of volatile
559 organic compounds by antagonistic yeasts immobilized on hydrogel spheres against gray,
560 green and blue postharvest decays. *Food Microbiol.* 63, 191-198.

561 Passoth, V., Fredlund, E., Druvefors, U.Ä., Schnürer, J., 2006. Biotechnology, physiology
562 and genetics of the yeast *Pichia anomala*. *FEMS Yeast Res.* 6, 3-13.

563 Ponsone, M.L., Chiotta, M.L., Combina, M., Dalcerro, A., Chulze, S., 2011. Biocontrol as a
564 strategy to reduce the impact of ochratoxin A and *Aspergillus* section *Nigri* in grapes. *Int. J.*
565 *Food Microbiol.* 151, 70-77.

566 Romano, A., Capozzi, V., Spano, G., Biasioli, F., 2015. Proton transfer reaction-mass
567 spectrometry: online and rapid determination of volatile organic compounds of microbial
568 origin. *Appl. Microbiol. Biotechnol.* 99, 3787-3795.

569 Rosenkranz, H.S., Carr, H.S., Rose, H.M., 1965. Phenethyl alcohol. I. Effect on
570 macromolecular synthesis of *Escherichia coli*. *J. Bacteriol.* 89, 1354-1369.

571 Schmidt-Heydt, M., Stoll, D., Geisen, R., 2013. Fungicides effectively used for growth
572 inhibition of several fungi could induce mycotoxin biosynthesis in toxigenic species. *Int. J.*
573 *Food Microbiol.* 166, 407-412.

574 Strobel, G.A., Dirkse, E., Sears, J., Markworth, C., 2001. Volatile antimicrobials from
575 *Muscodor albus*, a novel endophytic fungus. *Microbiology* 147, 2943-2950.

576 Suwannarach, N., Bussaban, B., Hyde, K.D., Lumyong, S., 2010. *Muscodor cinnamon*, a
577 new endophytic species from *Cinnamomum bejolghota*. *Mycotaxon* 114, 15-23.

578 The National Center for Biotechnology Information advances science and health by
579 providing access to biomedical and genomic information. Available online: [http://](http://www.ncbi.nlm.nih.gov/)
580 www.ncbi.nlm.nih.gov/

581 Van den Doo, H., Kratz, P.D., 1963. A generalization of the retention index system
582 including linear temperature programmed gas-liquid partition chromatography. *J.*
583 *Chromatography A* 11, 463-471.

584 Van der Merwe, K.J., Steyn, P.S., Fourie, L., Scott, D.B., Theron, J.J., 1965. Ochratoxin A,
585 a toxic metabolite produced by *Aspergillus ochraceus* Wilh. *Nature* 205, 1112-1113.

586 Wang, L., Wang, Y., Wang, Q., Liu, F., Selvaraj, J.N., Liu, L., Xing, F., Zhao, Y., Zhou, L.,
587 Liu, Y., 2015. Functional characterization of new polyketide synthase genes involved in
588 ochratoxin A biosynthesis in *Aspergillus ochraceus* fc-1. *Toxins* 7, 2723-2738.

589 Werner, S., Polle, A., Brinkmann, N., 2016. Belowground communication: impacts of
590 volatile organic compounds (VOCs) from soil fungi on other soil-inhabiting organisms.
591 *Appl. Microbiol. Biotechnol.* 100, 8651-8665.

592 Worapong, J., Strobel, G.A., Ford, E.J., Li, J.Y., Baird, G., Hess, W.M., 2001. *Muscodor*
593 *albus* anam. gen. et sp. nov., an endophyte from *Cinnamomum zeylanicum*. *Mycotaxon*
594 79, 67-79.

595 Wriessnegger, T., Pichler, H., 2013. Yeast metabolic engineering-targeting sterol
596 metabolism and terpenoid formation. *Prog. Lipid Res.* 52, 277-293.

597 Yan, H., Zhang, H., Wang, Q., Jian, H., Qiu, X., Wang, J., Tang, K., 2011. Isolation and
598 identification of a putative scent-related gene RhMYB1 from rose. *Mol. Biol. Rep.* 38,
599 4475-4482.

600 Zeilinger, S., Gruber, S., Bansal, R., Mukherjee, P.K., 2016. Secondary metabolism in
601 *Trichoderma* - Chemistry meets genomics. *Fungal Biol. Rev.* 30, 74-90.

602 Zhang, H., Apaliya, M.T., Mahunu, G.K., Chen, L., Li, W., 2016. Control of ochratoxin A-
603 producing fungi in grape berry by microbial antagonists: a review. *Trends Food Sci. Tech.*
604 51, 88-97.

605 Zhu, C., Shi, J., Jiang, C., Liu, Y., 2015. Inhibition of the growth and ochratoxin A
606 production by *Aspergillus carbonarius* and *Aspergillus ochraceus* *in vitro* and *in vivo*
607 through antagonistic yeasts. *Food Control* 50, 125-132.

608

609 **Figure captions**

610 Figure 1. Biocontrol activity of four yeast strains (253, *Candida intermedia*; 273,
611 *Cyberlindnera jadinii*; 751, *Lachancea thermotolerans*; 778, *Candida friedrichii*) against
612 *Aspergillus ochraeus* (A) or *Aspergillus carbonarius* (B). Results are expressed as colony
613 diameter (cm) over 10 days of incubation at 25 °C. Vertical bars indicate standard error (n
614 = 5).

615
616 Figure 2. Colony growth and morphology of *Aspergillus carbonarius* MPVA566 (A) and
617 *Aspergillus ochraceus* MPVA703 (F) after seven days of incubation on PDA alone, or in
618 the presence of VOCs produced by yeast strains *Candida intermedia* 253 (B, G),
619 *Cyberlindnera jadinii* 273 (C, H), *Lachancea thermotolerans* 751 (D, I), or *Candida*
620 *friedrichii* 778 (E, J). Microscopic detail (10X) of the colony margin is displayed below each
621 *Aspergillus*-yeast combination.

622 Figure 3. Microphotography (40X) of *A. ochraceus* MPVA703 marginal hyphae exposed to
623 VOCs emitted by *Lachancea thermotolerans* 751: the presence of coagulated cytoplasm
624 (white arrows) and swollen hyphal tips exhibiting lysis (black arrows) is shown.

625 Figure 4. HS-SPME/GC-MS chromatograms of yeast strains, highlighting the peak of 2-
626 phenylethanol, the main compound released by the *Candida friedrichii* 778 (A), *Lachancea*
627 *thermotolerans* 751 (B), *Candida intermedia* 235 (C), *Cyberlindnera jadinii* 273 (D) and not
628 detectable in YPD medium (E).

629
630 Figure 5. Relative expression analysis by qRT-PCR of *AcOTAnrps*, *AcOTApks*, *acpks*,
631 *laeA* and *veA* genes in *A. carbonarius* MPVA566 after 5 days growth on PDB at 25 °C.

632 Figure 6. Relative expression analysis by qRT-PCR of *pks*, *nrps*, *p450-H11*, *p450-B03* and
633 *hal* genes in *A. ochraceus* MPVA703 after 5 days growth on PDB at 25 °C.

634

635

636

637

638

639

640

641

642

643

644

645

646

647

648

649

650

651

652

653

654

655

656

657

658 Table 1. List of the primers used in this study.

Primer name	Sequence (5'→3')	References
<i>acpks-F</i>	GAGTCTGACCATCGACACGG	Gallo <i>et al.</i> , 2009
<i>acpks-R</i>	GGCGACTGTGACACATCCAT	
<i>acOTApks-F</i>	CGTGTCCGATACTGTCTGTGA	Gallo <i>et al.</i> , 2014
<i>acOTApks-R</i>	GCATGGAGTCCTCAAGAACC	
<i>acOTAnrps-F</i>	ATCCCCGGAATATTGGCACC	Gallo <i>et al.</i> , 2012
<i>acOTAnrps-R</i>	CCTTCGATCAAGAGCTCCCC	
<i>laeA-F</i>	CACCTATAACAACCTCCGAACC	Crespo-Sempere <i>et al.</i> , 2013
<i>laeA-R</i>	GGTTCGGCCAACCGACGACGC	
<i>veA-F</i>	TCCCGGTTCTCACAGGCGTA	Crespo-Sempere <i>et al.</i> , 2013
<i>veA-R</i>	GCTGTCCTTGGTCTCCTCGTA	
<i>18S-F</i>	GCAAATTACCCAATCCCGAC	NCBI
<i>18S-R</i>	GAATTGCCGCGGCTGCTG	
<i>ao-pks2-F</i>	TTCTCTACTGCGCTTCTCACATC	O'Callaghan <i>et al.</i> , 2006
<i>ao-pks2-R</i>	AACATCATAGCCATAAGAGGTCAACA	
<i>nrps-west-F</i>	GCTTGCTGACAAGCCGATGAC	Gil-Serna <i>et al.</i> , 2018
<i>nrps-west-R</i>	GGTCGTCAGTCGCTCATCCA	
<i>ao-p450-B03-F</i>	CTCGGTGACATCAGGGGTATC	O'Callaghan <i>et al.</i> , 2006
<i>ao-p450-B03-R</i>	AGCGTATTCAGTCACTCATTGAGA	
<i>ao-p450-H11-F</i>	AGAACGGGATGCCAAAACAGTGAG	O'Callaghan <i>et al.</i> , 2006
<i>ao-p450-H11-R</i>	AAGAATGCGAGGGATGGGATAACC	
<i>hal-west-F</i>	AGGAGGGAGAGGATGGGTTC	Gil-Serna <i>et al.</i> , 2018
<i>hal-west-R</i>	GCTCTTGCTGAAGGCGACAG	
<i>g3pdh-F</i>	TCGTCAACGGCAAGAAGATT	O'Callaghan <i>et al.</i> , 2006
<i>g3pdh-R</i>	TAGCAAGGGGAGCAAGGCAGT	

659

660

661

662 Table 2. Time course of OTA production by *Aspergillus carbonarius* MPVA566 and
 663 *Aspergillus ochraceus* MPVA703 incubated on PDA medium for 1-7 days. Results are
 664 expressed as colony diameter (cm) and OTA concentration (ng/g) \pm standard error. Values
 665 in the same column followed by different letters indicate significant differences as per
 666 Tukey's test ($P < 0.05$).

Day	Colony diameter (cm)		OTA (ng/g)	
	<i>A.carbonarius</i>	<i>A.ochraceus</i>	<i>A.carbonarius</i>	<i>A.ochraceus</i>
1	1.2 \pm 0	0.9 \pm 0	10.2 \pm 3.0 ^c	13.7 \pm 3.1 ^b
2	1.8 \pm 0	1.4 \pm 0	111.7 \pm 36.1 ^c	29.6 \pm 1.0 ^b
3	2.3 \pm 0	2.0 \pm 0	3,059.9 \pm 25.5 ^b	4,624.8 \pm 226.6 ^b
7	4.6 \pm 0	5.0 \pm 0	8,316.6 \pm 90.4 ^a	29,679.9 \pm 2,421.8 ^a

667

668 Table 3. Growth of *Aspergillus carbonarius* MPVA566 and *Aspergillus ochraceus*
669 MPVA703 and their ochratoxin A (OTA) production in PDA medium when incubated alone
670 or in co-culture with antagonistic yeast strains after seven days at 25 °C. Data from three
671 independent experiments are expressed as the colony diameter (cm), whereas OTA
672 production is expressed in ng/g \pm standard error. Values in the same column followed by
673 different letters indicate significant differences as per Tukey's test ($P < 0.05$).

Tested combinations	Experiment 1		Experiment 2		Experiment 3	
	Colony diameter (cm)	OTA (ng/g)	Colony diameter (cm)	OTA (ng/g)	Colony diameter (cm)	OTA (ng/g)
<i>A. carbonarius</i>	5 \pm 0 ^a	13883 \pm 2197	5 \pm 0 ^a	7866 \pm 1214 ^a	5 \pm 0 ^a	7613.9 \pm 643 ^a
<i>C. intermedia</i> 253	1.3 \pm 0.2 ^c	< 0.9	2.8 \pm 0.2 ^{bc}	135 \pm 16.3 ^b	2.5 \pm 0.2 ^b	0.1 \pm 0.1 ^b
<i>C. jadinii</i> 273	2.2 \pm 0.3 ^b	8.9 \pm 1.5	3.2 \pm 0.2 ^{bc}	93.4 \pm 28.1 ^b	2.3 \pm 0.2 ^b	13.7 \pm 6.0 ^b
<i>L. thermotolerans</i> 751	1.9 \pm 0.1 ^{bc}	< 0.4	3.4 \pm 0.1 ^b	13.6 \pm 15.5 ^b	2.2 \pm 0.2 ^b	< 0.8
<i>C. friedrichii</i> 778	2.1 \pm 0.3 ^b	< 0.2	2.6 \pm 0.1 ^c	51 \pm 14.3 ^b	2.0 \pm 0.3 ^b	2.4 \pm 1.4 ^b
<i>A. ochraceus</i>	4.5 \pm 0 ^a	42960 \pm 4112 ^a	4.5 \pm 0 ^a	28269 \pm 3811 ^a	4.5 \pm 0 ^a	19609 \pm 10492 ^a
<i>C. intermedia</i> 253	1.3 \pm 0.1 ^c	3.2 \pm 0.1 ^b	1.7 \pm 0.1 ^b	14.1 \pm 6.8 ^b	1.7 \pm 0.2 ^b	4.5 \pm 0.8 ^a
<i>C. jadinii</i> 273	1.6 \pm 0.1 ^b	2.7 \pm 0.1 ^b	2.2 \pm 0.2 ^b	939.7 \pm 145.9 ^b	1.8 \pm 0.2 ^b	75.2 \pm 31.7 ^a
<i>L. thermotolerans</i> 751	1.4 \pm 0.1 ^{bc}	3.0 \pm 0.3 ^b	1.9 \pm 0.1 ^b	7.5 \pm 2.3 ^b	1.6 \pm 0.3 ^b	5.0 \pm 0.5 ^a
<i>C. friedrichii</i> 778	1.5 \pm 0 ^{bc}	3.4 \pm 0.8 ^b	2.1 \pm 0.1 ^b	50.3 \pm 16.0 ^b	1.3 \pm 0.3 ^b	5.3 \pm 0.8 ^a

674

675 Table 4. VOCs produced by yeast strains.

Cpd ID	Name	LRI	Area (x10 ⁶ , n=3)						
			273		253		751		778
1	Methanethiol	641	0.44 ± 0.09	1.50 ± 1.08	0.87 ± 0.46	0.72 ± 0.59	0.13 ± 0.11		
2	Ethanol	643	0.32 ± 0.13 ^{ab}	0.26 ± 0.05 ^b	ND	0.21 ± 0.02 ^b	0.61 ± 0.17 ^a		
3	2-Butanone	663	ND	0.23 ± 0.07	0.17 ± 0.10	0.21 ± 0.06	0.27 ± 0.10		
4	3-Methylbutanal	681	ND	0.13 ± 0.03	0.38 ± 0.21	0.22 ± 0.05	0.51 ± 0.26		
5	3-Methyl-butanol	726	11.80 ± 4.30	10.30 ± 11.19	20.06 ± 10.75	9.99 ± 3.41	7.57 ± 0.54		
6	2-Methylbutanol	729	3.50 ± 2.21	Tr	4.93 ± 3.79	3.09 ± 0.93	4.16 ± 0.48		
7	Dimethyl disulfide	737	0.50 ± 0.22 ^b	3.00 ± 1.37 ^a	tr	0.76 ± 0.13 ^b	0.93 ± 0.97 ^{ab}		
8	2-Heptanone	883	ND	1.67 ± 0.95	0.71 ± 0.47	0.71 ± 0.13	0.90 ± 0.65		
9	2-Heptanol	895	0.92 ± 0.73	Tr	0.46 ± 0.34	0.44 ± 0.03	ND		
10	2,5-Dimethylpyrazine	905	8.27 ± 2.80 ^{ab}	7.20 ± 1.07 ^b	7.36 ± 1.09 ^{ab}	8.05 ± 0.75 ^{ab}	11.52 ± 1.23 ^a		
11	Benzaldehyde	950	0.52 ± 0.14 ^b	0.49 ± 0.06 ^b	1.07 ± 0.63 ^{ab}	1.83 ± 0.51 ^a	1.23 ± 0.54 ^{ab}		
12	D-Limonene	1019	0.45 ± 0.10	0.49 ± 0.13	0.41 ± 0.02	0.52 ± 0.05	1.02 ± 1.23		
13	2-Ethylhexanol	1024	7.76 ± 2.38 ^{ab}	4.17 ± 0.74 ^b	6.49 ± 2.24 ^{ab}	9.95 ± 1.37 ^a	10.82 ± 0.11 ^a		
14	Benzeneacetaldehyde	1035	0.39 ± 0.02 ^b	7.09 ± 5.37 ^a	2.28 ± 0.98 ^{ab}	1.66 ± 0.57 ^{ab}	0.68 ± 0.39 ^b		
15	Acetophenone	1057	0.25 ± 0.13 ^c	1.08 ± 0.43 ^{ab}	0.30 ± 0.11 ^c	0.50 ± 0.09 ^{bc}	1.42 ± 0.28 ^a		
16	3-ethyl-2,5-dimethylpyrazine	1072	3.02 ± 0.14	3.31 ± 0.32	2.66 ± 1.23	3.48 ± 0.22	3.35 ± 0.16		
17	2-Phenylethanol	1110	508.67 ± 57.46	499.46 ± 136.90	581.97 ± 283.88	306.55 ± 32.89	ND		
18	Camphor	1134	2.52 ± 0.05	2.16 ± 0.16	1.56 ± 1.07	2.46 ± 0.22	2.94 ± 0.71		
19	2,5-dimethyl-3-(3-methylbutyl)pyrazine	1310	0.31 ± 0.06	0.39 ± 0.07	0.26 ± 0.08	0.33 ± 0.03	0.26 ± 0.02		
20	Nerylacetone	1439	ND	0.94 ± 0.08	1.13 ± 0.93	1.29 ± 0.31	ND		
21	Stilbene	1495	0.43 ± 0.12 ^a	0.38 ± 0.03 ^a	0.21 ± 0.14 ^b	0.30 ± 0.01 ^{ab}	0.31 ± 0.06 ^{ab}		

676

677 Presented data show mean \pm standard deviation of the mean for 3 replicate experiments, for each yeast. Values within a row not sharing a common superscript differ
678 significantly ($P < 0.05$).

Figure 1

[Click here to download high resolution image](#)

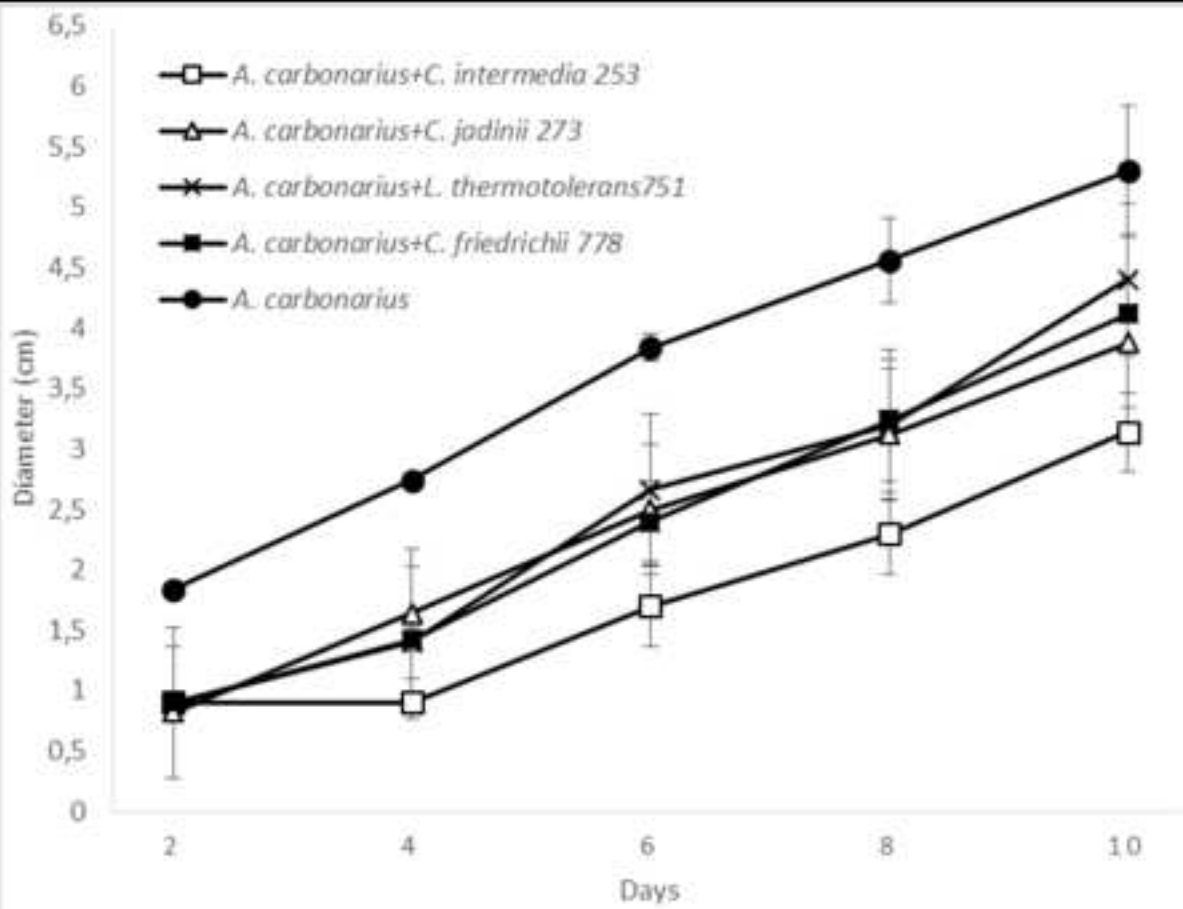
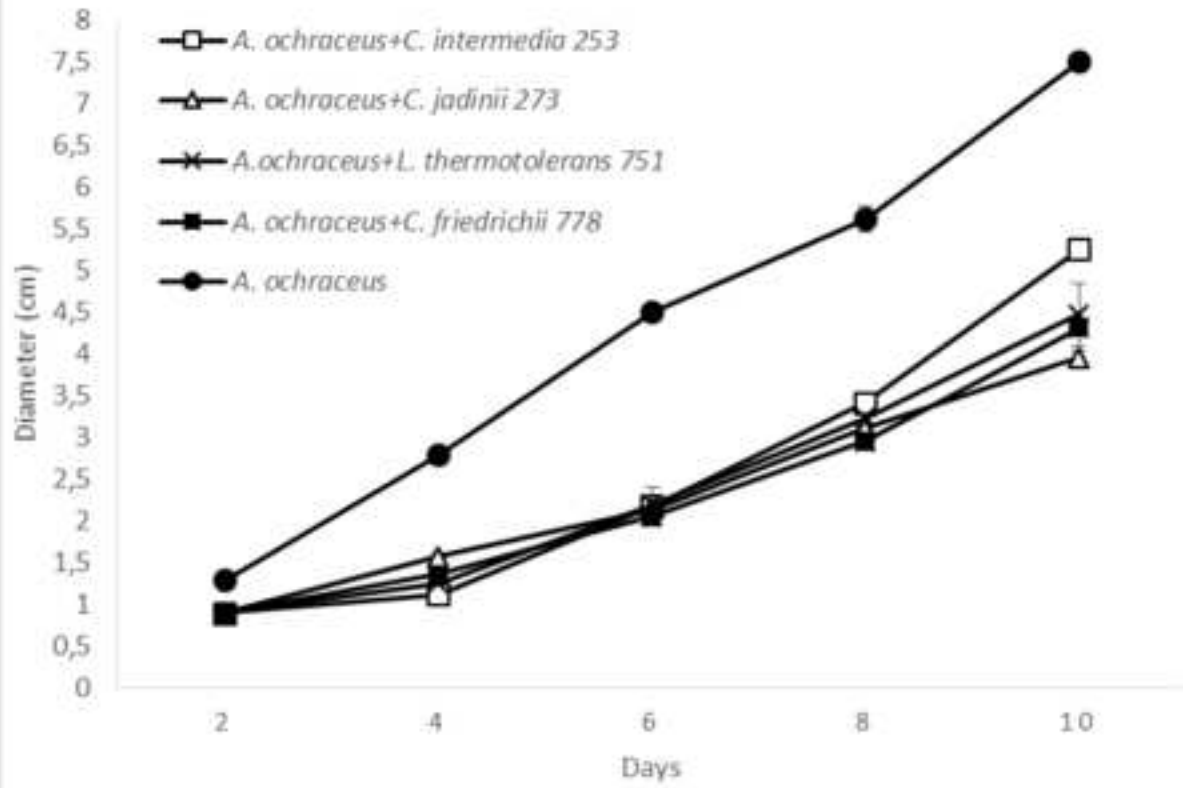


Figure 2
[Click here to download high resolution image](#)

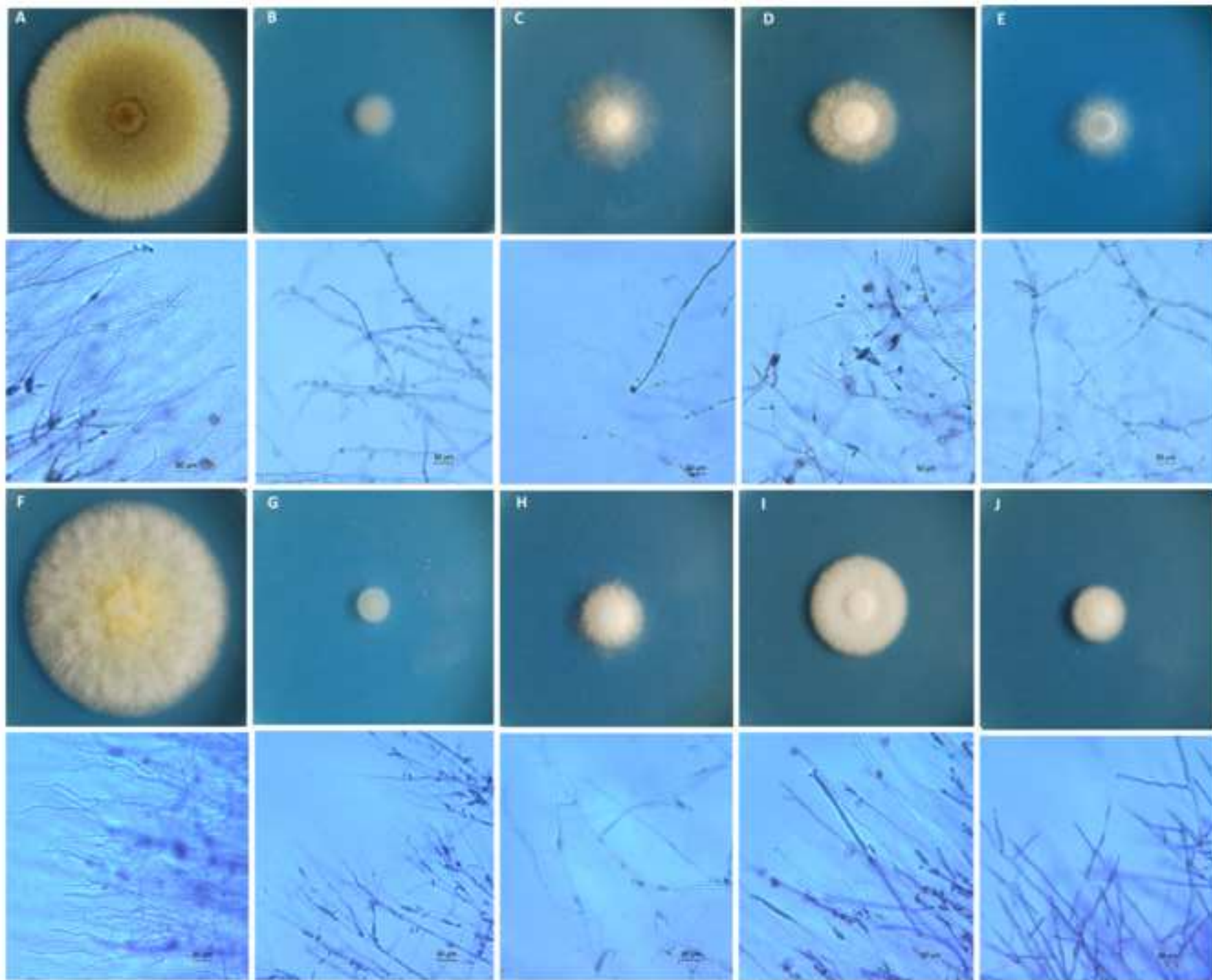


Figure 3
[Click here to download high resolution image](#)

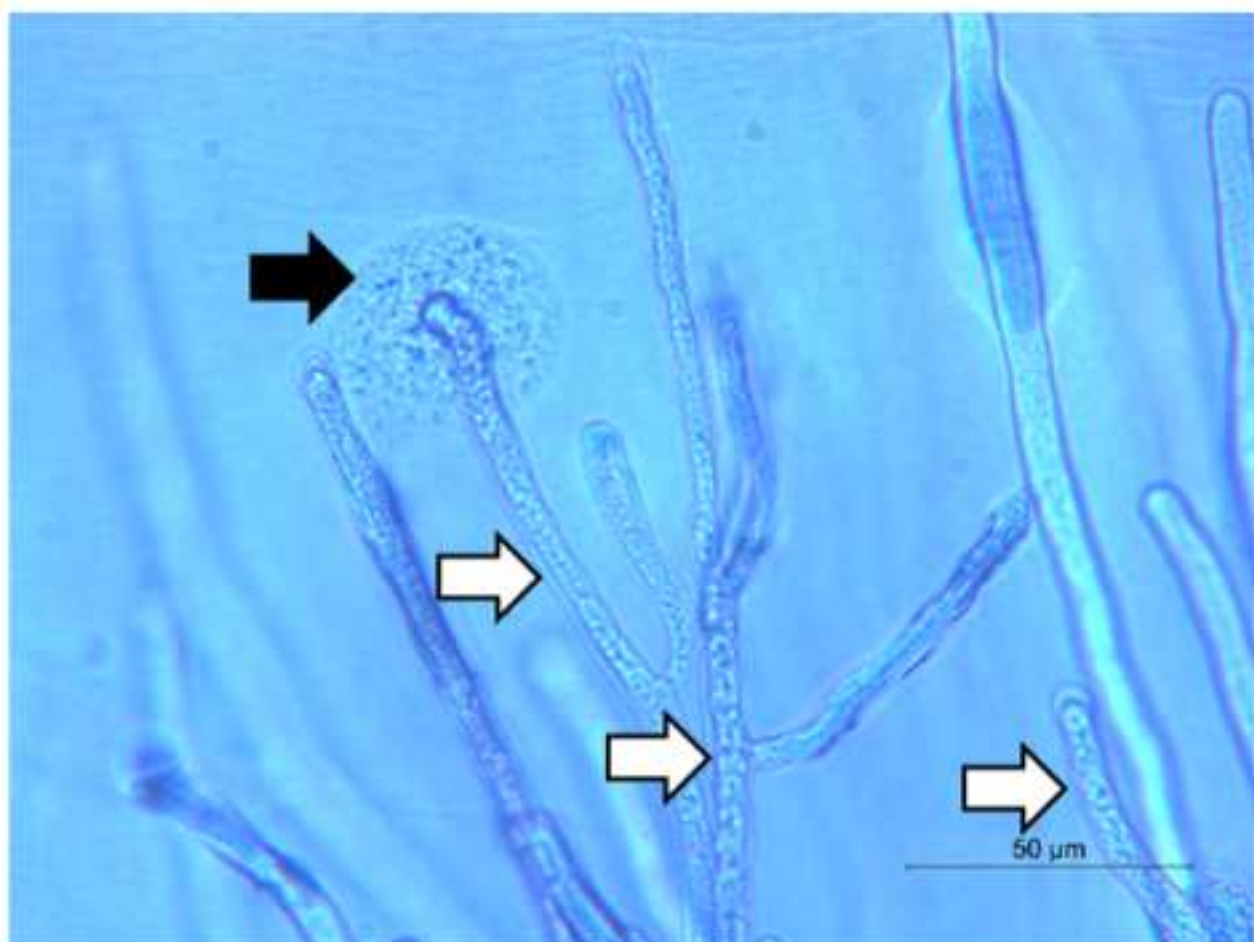
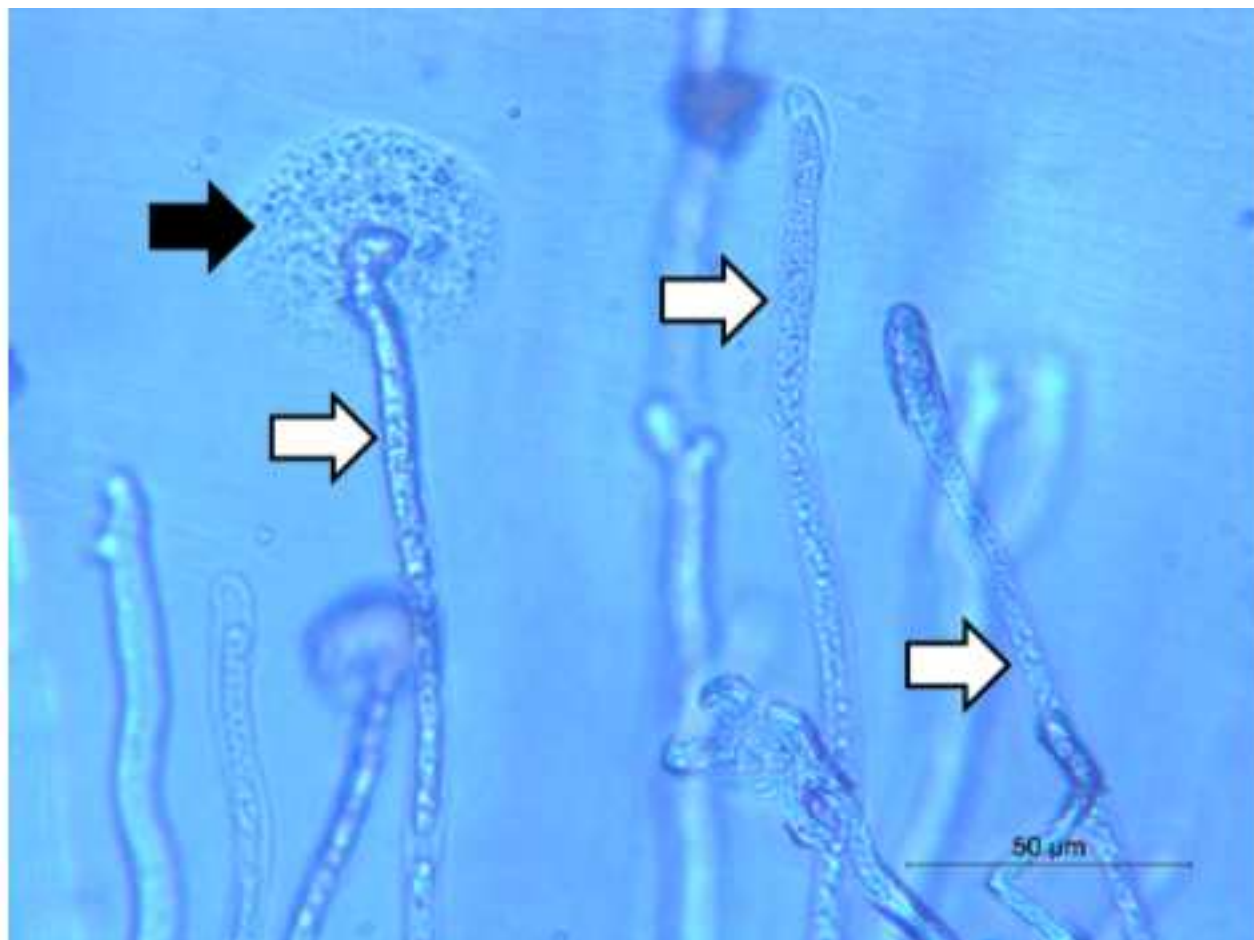


Figure 4
[Click here to download high resolution image](#)

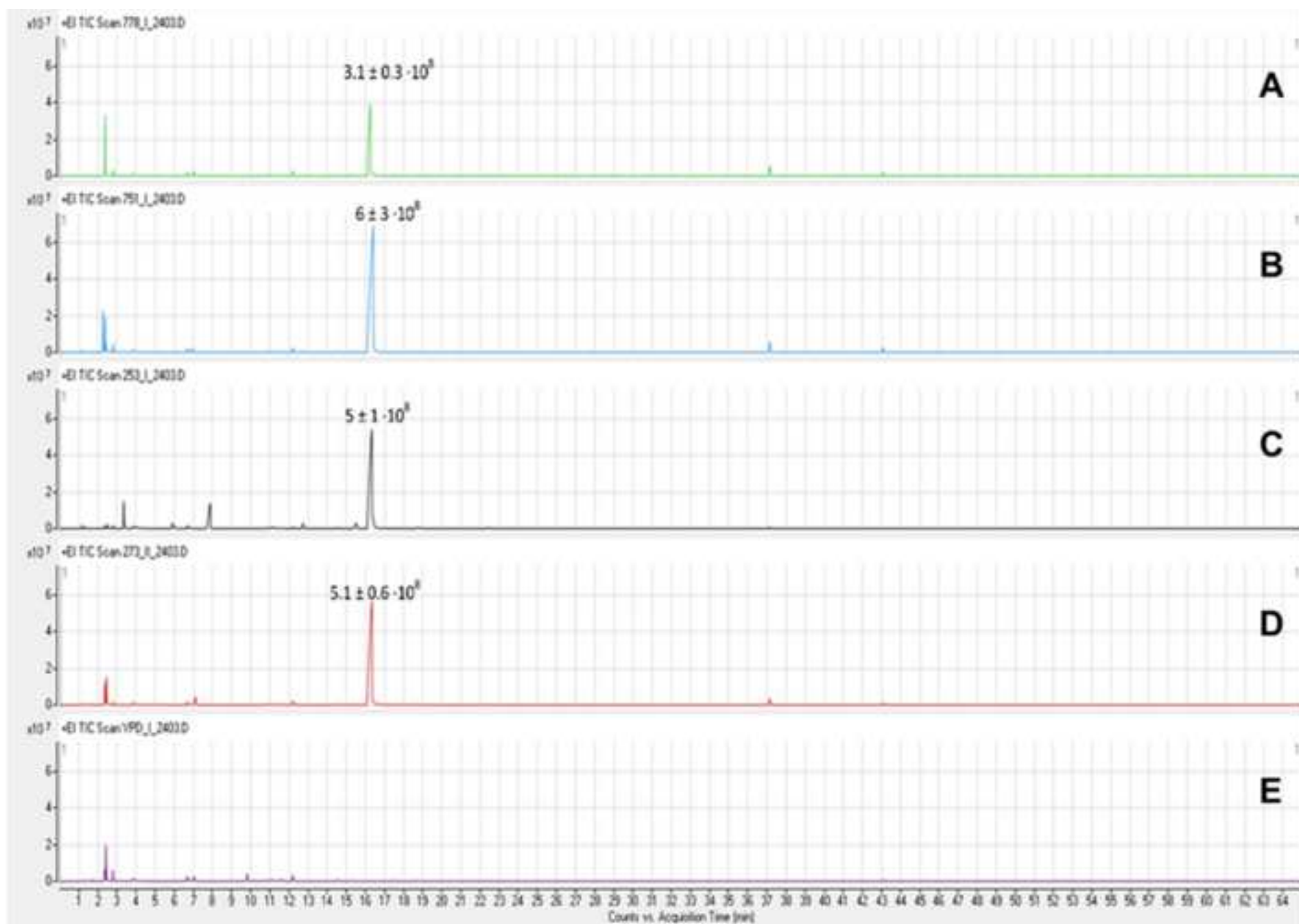


Figure 5
[Click here to download high resolution image](#)

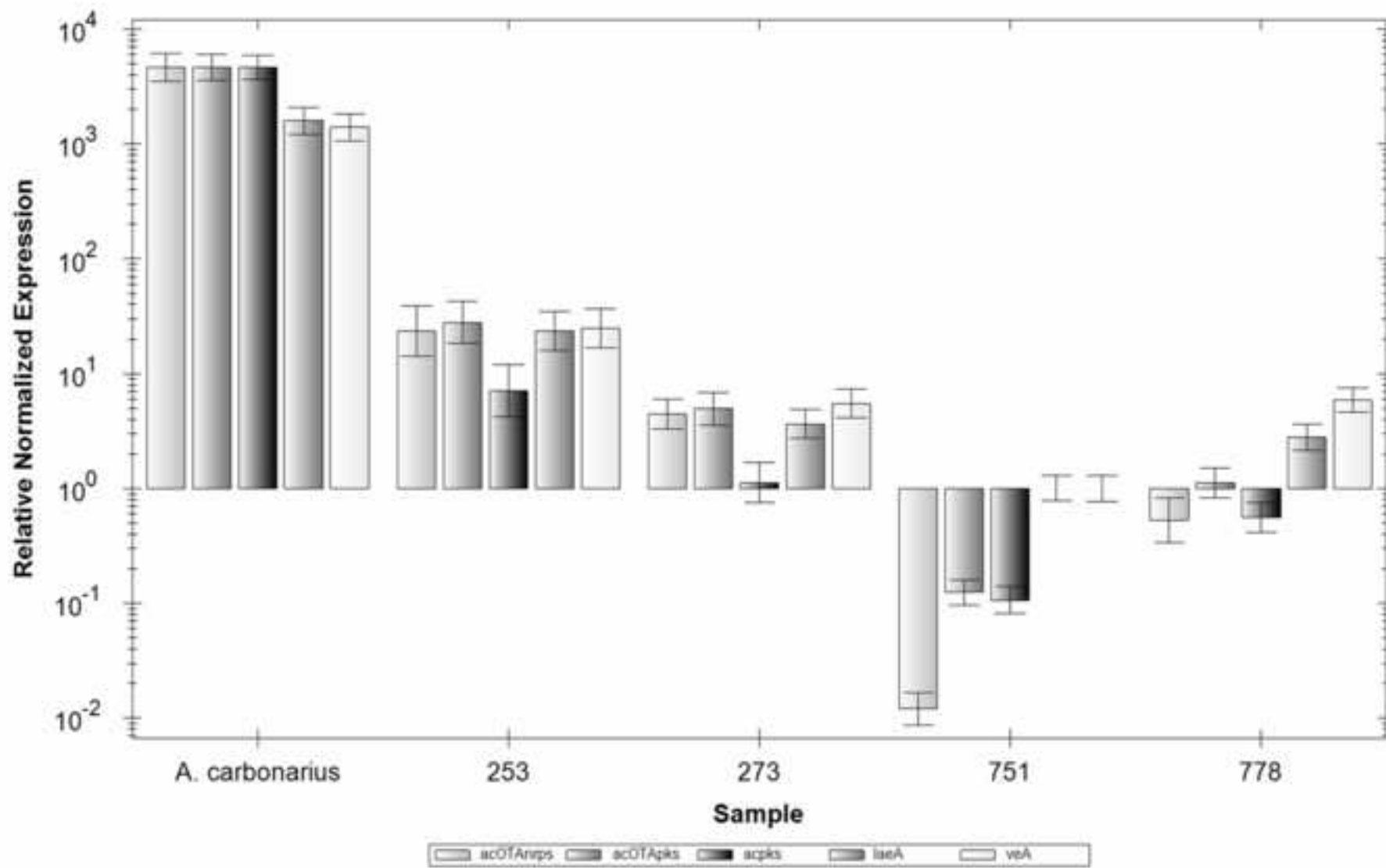


Figure 6
[Click here to download high resolution image](#)

