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# Volatile compounds for biotechnological applications produced during competitive interactions between yeasts and fungi

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**Abstract**

Fungi, yeasts and bacteria produce volatile compounds during their metabolism. In this study, the volatile compounds produced by yeast strains (*Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa*) and fungal strains (*Aspergillus carbonarius* and *Aspergillus ochraceus*) during competitive interactions were investigated by solid-phase microextraction coupled with gas chromatography–mass spectrometry. Fifty-six volatile compounds were identified representing alcohols, aldehydes, esters, ketones, aromatic compounds, acids, furans, phenols, and nitrogen compounds, being the largest amount in the class of esters and alcohols. Eight compounds were identified only in interactive culture conditions such as 2-amino-1-propanol, isopropylamine, dimethylamine, pentyl propanoate, ethyl-2-aminopropanoate, acetone, oxalic acid, and β-elemene and five of these were produced in cocultures including *A. carbonarius*. These will be developed for future biotechnological applications such as in the pharmaceutical and biological industry to produce drugs. Antimicrobial and antifungal activities; Solvent and herbicide; flavoring ingredient; solvent, plastic synthesis, nail polish remover and thinner, pesticide and herbicide; important in the complexation of minerals in the soil; and plant-environment interactions, defending predators, pathogens, and competitors.

**KEYWORDS**

*Aspergillus*, microbial interactions, *Rhodotorula mucilaginosa*, *Saccharomyces cerevisiae*, volatile

## 1 | INTRODUCTION

Volatile organic compounds (VOCs) are a large class of carbon-containing compounds characterized by low molecular weight (<300 Da), low polarity, low water solubility, and high volatility [1–3] carrying diverse functional groups including acids, alcohols, aldehydes, aromatics, ketones, terpenes, thiols, and their corresponding derivatives [4].

VOCs are produced by primary and secondary metabolism in yeasts, fungi, and bacteria, through oxidation of glucose, and from various intermediates found in

**Abbreviations:** CCDCA, Culture Collection of Microorganisms from the Food Sciences Department; CCMA, Culture Collection of Agricultural Microbiology; DMA, dimethylamine; F, fungal strains; GC-MS, gas chromatography–mass spectrometry; HS-SPME, headspace solid-phase microextraction; MEA, malt extract agar; RI, retention index; VOCs, volatile organic compounds; Y, yeast strains.

  

biosynthetic aerobic pathways, heterotrophic carbon metabolism, fermentation, amino acid catabolism, terpenoid biosynthesis, fatty acid degradation, and sulfur reduction [5]. Factors including specific strains or species, cell physiological state, available substrates, nutrients, temperature, oxygen availability, humidity, and pH influence production of these compounds [6]. As a result, microbial VOC production is biologically dynamic [7], being influenced by mutualistic, commensal, and competitive microbial interactions. VOCs have different types of functions: ecological (attraction, defense, stress response, and interorganizational communication) [4], and signaling (regulating interactions between cells, microorganisms, and hosts, carbon release mechanisms, and promoters, or inhibitors of microbial growth) [8, 9]. Therefore, it is expected that interactions between different microorganisms will produce different VOC profiles.

Microbial VOCs can play significant roles in antagonistic, commensal, and/or mutualistic interactions between microorganisms occupying the same ecological niche. Interactions can stimulate production of specific compounds. In many cases, negative interactions have been observed such as between yeast species and mycotoxigenic fungi [2, 10]. Such antagonistic interactions can be utilized for biological control [10]. So far it is not clear if VOCs produced during these interactions could be used in food, pharmaceutical, and cosmetic industries. In this sense, this study is to search and analyse volatile compounds that are produced when yeasts and fungi are cocultured and if these compounds are related to growth inhibition of mycotoxigenic fungi and also other applications these compounds may have.

The industrial use of microorganisms to produce VOCs has been shown to be commercially viable, because of ease of production [1, 11]. VOCs can be used to replace synthetic compounds in food and cosmetics. In agriculture, fungal VOCs have been incorporated into biological control strategies to prevent growth of plant pathogens, induce plant growth-promoting effects, and provide biologically important soil components [3, 12]. As a result, VOCs represent a new frontier in bioprospecting, providing new products for human exploitation in various economic sectors.

Interactions between filamentous fungi and yeasts positively impact yield/quality of several fermented products and modulate important industrially bioprocesses of several compounds [13]. However, the production of VOCs in these interactions remains to be explored. Due to the potential use of VOCs produced by microorganisms in different industrial sectors, the objectives of these works were: (1) to identify which VOCs are produced by biological agents under competitive interaction conditions; (2) to identify the influence of strain level on VOC production to provide new microbial sources that can produce key compounds; and (3) point out the possible technological applications of these compounds.

## 2 | MATERIALS AND METHODS

VOCs were obtained from yeast and fungal strains grown in pure culture or two by two (yeast vs. filamentous fungi). Species of these microorganisms were selected based on previous work about biological control at protecting coffee fruits [10, 13].

### 2.1 | Fungi and yeasts

*Aspergillus carbonarius* (CCDCA 10608) and *Aspergillus ochraceus* (CCDCA 10612) fungal strains were isolated from grape juice and coffee, respectively, and obtained from the Culture Collection of Microorganisms from the Food Sciences Department (CCDCA), Federal University of Lavras, Brazil. These strains were in their preserved form on filter paper discs stored at −80°C. To reactive them, malt extract agar (MEA) (20 g malt extract/L; 1 g bacteriological peptone/L; 20 g glucose/L; 20 g agar/L) were used and incubated at 25°C for 7 days.

The selection of yeasts strains was based in their ability to produce VOCs with antifungal activity [10, 14]. In the total, seven *Saccharomyces cerevisiae* strains (CCMA 0159; CCMA 1299; CCMA 1302; CCMA 1306; CCMA 1313; CCMA 1315; CCMA 1317) and one *Rhodotorula mucilaginosa* (CCMA 1305) were selected. All yeast strains belong to the Culture Collection of Agricultural Microbiology (CCMA) from the Biology Department, Federal University of Lavras, Brazil. The isolated was preserved at −80°C and reactivated by incubation on yeast extract peptone glucose agar (10 g yeast extract/L; 20 g peptone/L; 10 g glucose/L; and 15 g agar/L) at 28°C for 24 h.

### 2.2 | Culture conditions

The culture conditions consist on pure culture of fungal strains (F1 and F2), pure culture of yeast strains (Y1 to Y8), *A. carbonarius* CCDCA 10608 interacting with yeast strains culture (F1Y1 to F1Y8), and *A. ochraceus* CCDCA 10612 interacting with yeast strain culture (F2Y1 to F2Y8). These last two are shown in Table 1.

Yeast cultures were standardized in suspensions of 8 log cells/mL, and fungal cultures with 6 log spores/mL. Fifteen microliters vials with septa were prepared containing 2 mL of MEA medium. Pure cultures of fungal strains were inoculated with 5 μL of spore suspension. Pure cultures of yeast strains were inoculated with 25 μL of cell suspension.

TABLE 1 Cultures carried out to identify microbial VOCs in pure cultivation and in fungi and yeast interactions.

| Cultures | Code |
|---|---|
| *Aspergillus carbonarius* CCDCA 10608 | F1 |
| *Aspergillus ochraceus* CCDCA 10612 | F2 |
| *Saccharomyces cerevisiae* CCMA 0159 | Y1 |
| *S. cerevisiae* CCMA 1299 | Y2 |
| *S. cerevisiae* CCMA 1302 | Y3 |
| *S. cerevisiae* CCMA 1306 | Y4 |
| *S. cerevisiae* CCMA 1313 | Y5 |
| *S. cerevisiae* CCMA 1315 | Y6 |
| *S. cerevisiae* CCMA 1317 | Y7 |
| *Rhodotorula mucilaginosa* CCMA 1305 | Y8 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 0159 | F1Y1 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1299 | F1Y2 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1302 | F1Y3 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1306 | F1Y4 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1313 | F1Y5 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1315 | F1Y6 |
| *A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1317 | F1Y7 |
| *A. carbonarius* CCDCA 10608 + *R. mucilaginosa* CCMA 1305 | F1Y8 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 0159 | F2Y1 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1299 | F2Y2 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1302 | F2Y3 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1306 | F2Y4 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1313 | F2Y5 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1315 | F2Y6 |
| *A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1317 | F2Y7 |
| *A. ochraceus* CCDCA 10612 + *R. mucilaginosa* CCMA 1305 | F2Y8 |

Also, yeast/fungi interaction cultures were inoculated with 5 and 25 μL of spores and cell suspension, respectively. All cultures were incubated for 7 days at 25°C. The control treatment consisted on uninoculated MEA medium. Cultures were performed in triplicate.

## 2.3 | Identification of VOCs by gas chromatography–mass spectrometry

VOCs analysis were performed according to published methodology [15], with modifications. A fiber of headspace solid-phase microextraction (HS-SPME) (Supelco Inc.) coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm was used to adsorb the VOCs. Vials were incubated for 5 min at 38°C, then SPME fibers were exposed through the headspace for 15 min and injected into the chromatograph.

A chromatograph GCMS-QP2010 (Shimadzu) with a fused silica capillary column model Rtx-5MS (Restek) (30 m × 0.25 mm × 0.25 μm) (5% diphenyl, 95% dimethylpolysiloxane) stationary phase, were used. The injector was operated in "*splitless*" mode, and helium gas was used as the carrier gas at a flow rate of 5.8 mL/min. Injector and detector temperatures were maintained at 250°C. The GC oven temperature was programmed as follows: 40°C for 5 min, ramp up to 100°C at 3°C/min, then to 250°C at 20°C/min, and finally hold at 250°C for 10 min. The range of detection in the mass spectrometer was between 35 and 350 $m/z$ with an ionizing energy of 70 eV.

Volatile compounds were identified by comparing their spectra with those of the National Institute of Standards and Technology Library 11. A series of alkanes ($C_{10}$–$C_{40}$) was used to calculate retention index (RI) for each congener and were compared with RI data, found in the literature.

## 2.4 | Statistical analysis

The variables number of compounds identified per treatment and number of compounds identified per group, were analyzed using Kruskal–Wallis one-way analysis of variance on ranks ($p \leq 0.001$). The means were compared by the pairwise multiple comparison procedures (Tukey test) ($p < 0.05$). Poisson regression was used for variable numbers of compounds within each group, using the generalized linear model, where each compound was evaluated for significance within the model ($p < 0.05$). To evaluate model accuracy, robust standard errors for parameter estimates were calculated. In addition, $p$ values were calculated using the best deviation of the adjustment test. All analysis were performed using R programming language [16].

# 3 | RESULTS

Fifty-four VOCs, belonging to the groups: alcohols (9), esters (20), ketones (4), aromatic compounds (2), acids (3), aldehydes (4), furans (1), phenols (4), nitrogen compounds (3), and others (4), were identified (Figure 1). A statistical significance was observed in the number of VOCs produced by each group. Alcohols and esters represented the highest number of VOCs, differing significantly from the others. A higher number of

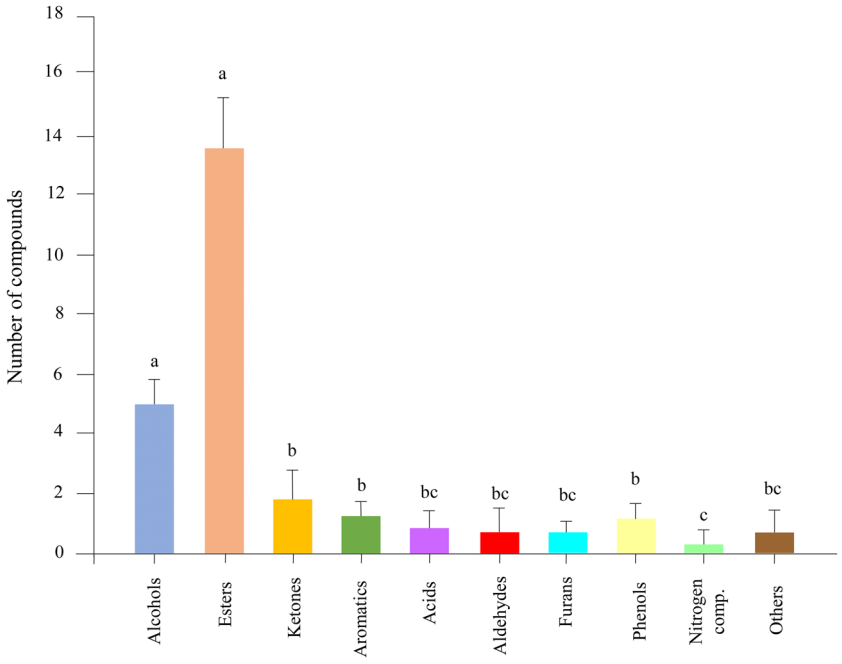


**FIGURE 1** Number of volatile organic compounds in each functional group identified by headspace solid-phase microextraction/gas chromatography–mass spectrometry from all cultures. Distinct letters indicate significant differences according to Tukey's test ($p < 0.05$).

ketones, aromatic compounds, and phenols VOCs were produced compared to nitrogenous compounds. The other groups did not differ in terms of number of VOCs (Figure 1).

Significant differences of VOCs were observed within each group of compounds, indicating that their presence was predominant in most cultures (Figure 2). Within the alcohol group, ethanol, 2-methyl-1-butanol, 2-methyl-1-propanol, 2-phenylethanol, and 3-methyl-1-butanol, they were present in all 26 treatments. Other alcohol compounds, including 2-nonanol and 2-heptanol were detected in 11 and 8 treatments, respectively (Figure 2). 2-Heptanol was identified in all cultures in which *A. carbonarius* was present, except in coculture with *R. mucilaginosa* (FIY8). These experiments indicate that *R. mucilaginosa* can alter patterns of VOC production, as confirmed by the formation of two distinct groups (Figure 3). The different strains of *Saccharomyces* did not show marked differences in alcohol production.

Ten VOCs were identified in all cultures. Fourteen esters were found at significant levels (Figure 2) and were present in 20 cultures. Some compounds from other classes also differed significantly between cultures, including the compounds 2-heptanone and 2-pentanone (ketone class), and styrene (aromatics class). Others, including acetic acid (organic acids), 3-methylbutanal (aldehyde class), and 2,4-di-*tert*-butylphenol (phenol class) were identified in all cultures.

Some compounds were found in only a few cultures (Figure 2). Monoculture of *A. carbonarius* (F1) accounted for 50.9% (28 compounds) of total identified compounds. *cis*-Thujopsene was identified only in the cultures with F1. Monocultures of *A. ochraceus* (F2) accounted for the total of 34.5% (19 compounds) identified compounds. 2-Methylbutanal, a compound of the aldehyde class, was identified only in this culture (F2) and in F1Y1 combination cultures. 4-Butan-2-yl-2,6-di-*tert*-butylphenol and methyl (Z)-N-hydroxybenzenecarboximidate, two phenol class compounds, were identified in F1Y2, and F2Y2, respectively. Regarding interactions between fungi and yeasts, it can be observed that the yeast coculture with *A. carbonarius* (F1) yielded more compounds [17] (F1Y8) to 32 compounds (F1Y2) than the coculture with *A. ochraceus* (F2), [23 (F2Y8) to 29 compounds (F2Y2)].

Monocultures of *S. cerevisiae* (Y1 to Y7) yielded between 41.8% and 50.9% (23–28 compounds) of total identified compounds. Monoculture of *R. mucilaginosa* (Y8) resulted in 25 compounds (45.5%). Four compounds were identified only in yeast cultures: 2,3-butanediol in Y3, Y5, and Y7; 2-hydroxiethyl propanoate only in Y1; ethyl heptanoate in Y6; and 1,3-pentadiene in Y7 and Y8.

To visualize the similarities and differences between cultures in production of volatile compounds, a dendrogram was constructed (Figure 3). The dendrogram suggests the occurrence of two distinct groups. The first group requires the use of *A. carbonarius* (F1), in pure culture or in mixed cultures with yeasts, except *R. mucilaginosa* (F1Y8), that behaves differently, qualifying for the second group. The second group includes cultures composed only of yeasts, or the fungus *A. ochraceus* (F2) and their respective cocultures. Group two also includes coculture of fungus *A. carbonarius* with the yeast *R. mucilaginosa* (F1Y8). This analysis shows the strong influence of the yeast *R. mucilaginosa* (Y8) and fungal strain on VOC production.

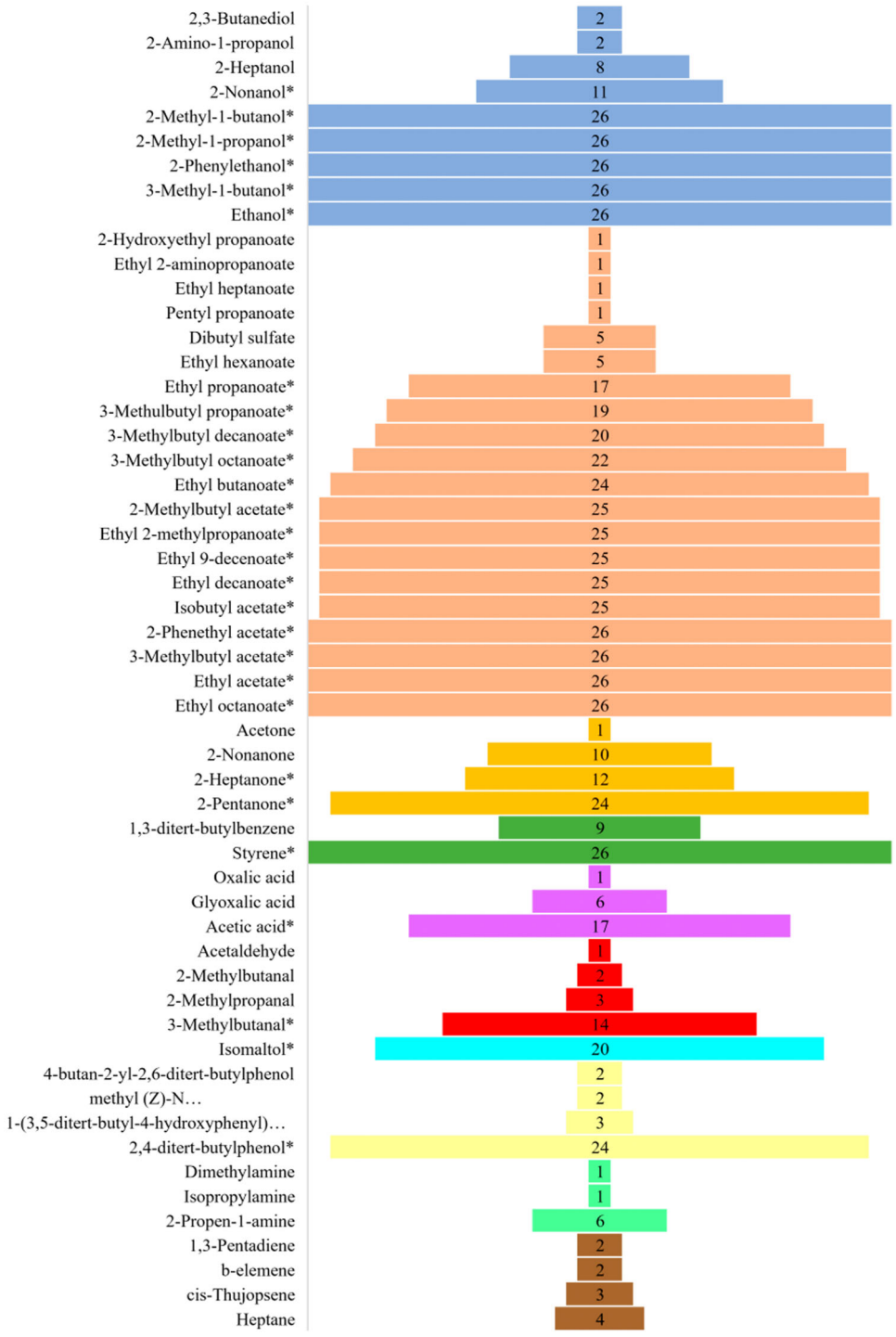


**FIGURE 2** Compounds and number of cultures that presented the compound identified by headspace solid-phase microextraction/gas chromatography–mass spectrometry. Asterisks indicate significant differences according to Tukey's test ($p < 0.05$). Classes of compounds: ( ) alcohols; ( ) esters; ( ) ketones; ( ) aromatic; ( ) acids; ( ) aldehydes; ( ) furans; ( ) phenols; ( ) nitrogen compounds; ( ) others.

Eight compounds were only identified in competitive yeast/fungi cocultures (Table 2). Figure 4 shows the chemical structures of these compounds. Some nitrogen compounds, such as 2-amino-1-propanol, isopropylamine, dimethylamine (DMA), and ethyl-2-aminopropanoate have been identified. 2-Amino-1-propanol was produced in two culture conditions: F1Y4 and F2Y2. Interestingly, the F1Y4 coculture also produced an amino compound called isopropylamine. Therefore, production of these two compounds may be linked because they are produced by the same F1Y4 coculture. Among the nitrogen compounds, DMA was identified in this coculture. Pentyl propanoate is a

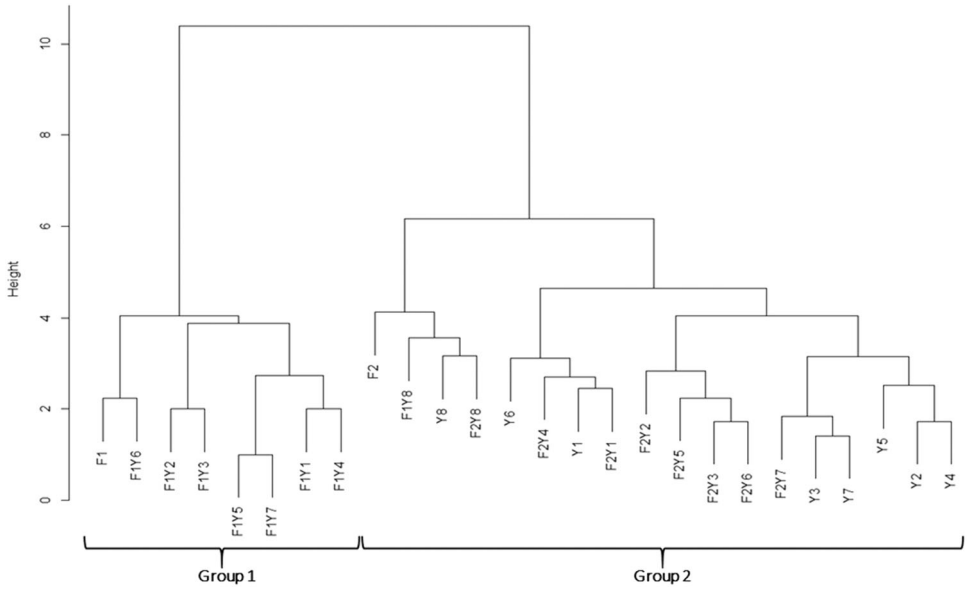


**FIGURE 3** Cluster analysis of cultures based on produced volatile organic compounds.

**TABLE 2** Volatile organic compounds (VOCs) identified only on interactions of mixed culture, properties of compounds, and biotechnological and cellular functions.

| No. | Culture[a] | Compound | Molecular formula | Molecular weight (g/mol) | LRI | Area (×10$^4$) | Biotechnological and cellular function | References |
|---|---|---|---|---|---|---|---|---|
| 1 | F1Y4; F2Y2 | 2-Amino-1-propanol | $C_3H_9NO$ | 75.11 | 741 | 5.1234; 6.3960 | Pharmaceutical and biological industries, drugs; antimicrobial and antifungal activities | [18, 19] |
| 2 | F1Y4 | Isopropylamine | $C_3H_9N$ | 59.11 | 498 | 3.2588 | Solvent and herbicide | [19, 20] |
| 3 | F2Y5 | Dimethylamine (DMA) | $C_2H_7N$ | 45.08 | 419 | 336.9209 | Carcinogenic and teratogenic effect | [21] |
| 4 | F1Y2 | Pentyl propanoate | $C_8H_{16}O_2$ | 144.21 | 984 | 1.2228 | Flavoring ingredient | – |
| 5 | F2Y4 | Ethyl-2-aminopropanoate | $C_5H_{11}NO_2$ | 117.15 | 864 | 748.7876 | – | |
| 7 | F1Y8 | Acetone | $C_3H_6O$ | 58.08 | 455 | 27.7143 | Solvent, plastic synthesis, vanish remover and paint thinner, pesticide and herbicide | [22, 23] |
| 8 | F2Y1 | Oxalic acid | $C_2H_2O_4$ | 90.03 | 933 | 1249.5914 | Complex minerals in soil | [17, 24] |
| 9 | F1Y2; F1Y3 | β-Elemene | $C_{15}H_{24}$ | 204.35 | 1398 | 0.2427; 0.2856 | Plant-environment interactions, defending predators, pathogens, and competitors | [25–27] |

Abbreviation: LRI, linear retention index.

[a]F1Y2 (*A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1299); FIY3 (*A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1302); FIY4 (*A. carbonarius* CCDCA 10608 + *S. cerevisiae* CCMA 1306); F1Y8 (*A. carbonarius* CCDCA 10608 + *R. mucilaginosa* CCMA 1305); F2Y1 (*A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 0159); F2Y2 (*A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1299); F2Y4 (*A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1306); F2Y5 (*A. ochraceus* CCDCA 10612 + *S. cerevisiae* CCMA 1313).

branched ester produced by the F1Y2 coculture. Ethyl-2-aminopropanoate, also a branched ester identified only in coculture, was produced by F2Y4. Acetone was identified in the F1Y8 coculture. Oxalic acid was produced by the F2Y1 coculture. Finally, the compound β-elemene was identified in F1Y2 and F1Y3 cocultures. Another sesquiterpene identified in this study was cis-thujopsene, produced by *A. carbonarius* (F1), FIY8 and FIY6.

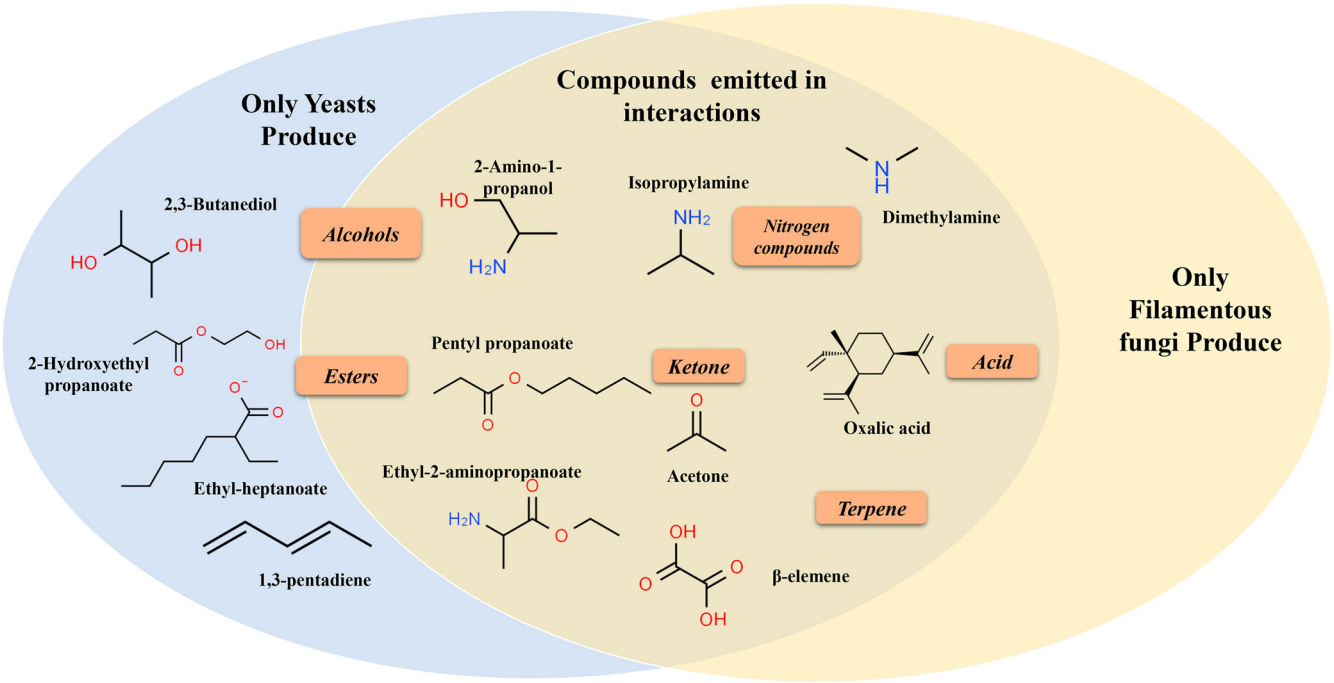


FIGURE 4 Chemical structure of volatile microbial organic compounds detected only by yeasts (blue circle, four compounds), only by filamentous fungi (circle in orange, no compounds), and detected on interactions of mixed culture (intersection of circles, eight compounds). Some ones were results of reaction between compounds from microbial metabolism.

## 4 | DISCUSSION

Most of these microbial VOCs are considered secondary products of primary and secondary metabolism. Alcohols are produced naturally by microbial metabolic pathways and are widely known. They are mainly formed by oxidation of various intermediates of glucose catabolism [6], which is why they predominate among the other classes of compounds. The presence of ethanol, 2-methyl-1-butanol, 2-methyl-1-propanol, 2-phenylethanol, and 3-methyl-1-butanol in all cultures was expected, because these compounds are frequently produced as part of yeast and fungal metabolism [28].

It is particularly important to emphasize that medium composition directly affects volatile compound production. Therefore, it is expected that some compounds would be produced by all tested culture conditions. However, many of these compounds display some inhibitory action toward growth and spore production in filamentous fungi of the *Aspergillus* genus, thus highlighting their importance in biotechnological applications such as biocontrol [10].

In this study, some nitrogen compounds, including 2-amino-1-propanol, isopropylamine, DMA, and ethyl-2-aminopropanoate were identified only in cocultures. 2-Amino-1-propanol, also called alaninol, contains an amine and an alcohol group. This compound was produced under two culture conditions: F1Y4 and F2Y2. Amino alcohol derivatives have been used as catalysts, and as coupling partners in the synthesis of many compounds. The study of amino alcohols is of pharmaceutical and biological interest since several important drugs, including β-blockers, belong to this structural category [18]. They are also studied for their antimicrobial and antifungal activities and for modulating the physicochemical properties of drug molecules. Alaninol is an important intermediate in the synthesis of ofloxacin and it is clinically used as a quinolone-class antimicrobial agent [19]. In some organisms, isopropylamine degradation is initiated by monooxygenation to produce alaninol (2-amino-1-propanol), which can later be oxidized to alanine, or deaminated to propionaldehyde. Isopropylamine is used as a solvent and raw material, for the manufacture of various chemicals. This compound occurs as a constituent of the herbicides atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine] and propachlor (2-chloro-N-isopropylacetanilide), from which it is released during microbial degradation [19, 29].

DMA, found in F2Y5, is a biogenic amine that belongs to a class of alkylamines. DMA formation can also occur through amino acid decarboxylation or amination, and through transamination of aldehydes and ketones [29]. Biochemical processes of autotrophic and heterotrophic nitrification and denitrification can result in the formation of nitrosamines by numerous microorganisms, such as soil fungi of *Aspergillus*, *Fusarium*, *Penicillium*, *Candida*, and *Cephalos* genera.

It is believed that microorganisms convert nitrate to nitrite, break down proteins to yield secondary amines, and create a suitable slightly acidic environment [30]. In the F2Y5 coculture, it is believed that the yeast *S. cerevisiae* may have produced a conducive environment by production of acids, thereby causing stress and consequently inducing production of DMA.

Pentyl propanoate found in F1Y2 is a product of fungal metabolism derived from 1-pentanol. It is an important flavoring ingredient, formed by condensation of pentanol and propanoic acid. However, none of these compounds have been identified. The fruity smell of esters makes them unique, with wide applications in the flavor, fragrance, and solvent industries [22].

Acetone compound can be derived from metabolism of carbohydrates and compounds including isopropanol, 2-propanol, and propanoate (https://www.kegg.jp/kegg/kegg2.html2021). Acetone is an important raw material in the chemical industry. Worldwide production of acetone relies primarily on fossil resources and is associated with phenol production (cumene process). It is also used as a varnish remover and paint thinner, and in production of pesticides and herbicides [23]. The production of this compound via a biological route is more sustainable. More studies are needed to affirm that this production would be advantageous in terms of economics and productivity.

Organic acids produced by fungi and their roles in ecology and the environment have been thoroughly studied for some years. Oxalic acid biosynthesis in *Aspergillus niger* has been well documented and occurs by hydrolysis of oxaloacetate to oxalate and acetate, catalyzed by cytosolic oxaloacetase. It can also be generated via citric acid degradation through the glyoxylate cycle [17]. Among the organic acids produced by fungi, oxalate is especially important in microbial ecology due to its ability to form metal complexes with calcium, aluminum, and other metals, resulting in mineral dissolution from rocks, causing damage that can produce pores and fissures [24].

β-Elemene is also known as (1*R*, 2*R*, 4*S*)-1-ethenyl-1-methyl-2,4-bis(prop-1-en-2-yl) cyclohexane. Elemenes are a group of closely related natural chemical compounds found in a wide variety of plants, that play important roles in plant-environment interactions, defending against predators, pathogens, and competitors [25]. Some endophytic fungi, including *Nodulisporium* sp. produce sesquiterpenes, with β-elemene being the most abundant, and playing roles in storage and in inhibiting a wide range of plant pathogens. In this sense, it can be said that these compounds act in communication between fungi, insects, and plants, warding off some insects and attracting others, to defend against enemies [26]. β-Elemene can be produced by other species of fungi, including *Penicillium clavigerum*, *Penicillium roqueforti*, *Inonotus obliquus*, and *Piptoporus betulinus* [27, 31].

In nature, organisms rarely exist in isolation. Instead, they form complex relationships that develop through evolution and adaptation to diverse ecosystems [32]. This complex web of interactions defines how a community is assembled and maintained spatially and temporally [33]. Our biggest challenge is to unravel the biological and ecological functions of these microbial volatiles. Growing evidence supports that, microbial volatiles can act as info-chemicals in interactions between microbes [8]. We can also explore their relationships and the production of these compounds for biotechnological applications.

Fifty-four VOCs, belonging to different groups such as alcohols, esters, ketones, aromatic compounds, organic acids, aldehydes, furans, phenols, and nitrogen compounds, were identified as result of different competitive biological interactions between strains of *A. carbonarius* and *A. ochraceus*, and different strains of yeasts of *S. cerevisiae* and *R. mucilaginosa* species or isolated cultures of these microorganisms. The strain level influenced the production of some VOC's compounds. Eight compounds were produced only in coculture. Five of these were produced in cocultures including *A. carbonarius* (F1). These co-cultures produced compounds, including 2-amino-1-propanol, isopropylamine, pentyl propanoate, acetone, and β-elemene, with important biotechnological applications.

Future analysis of organismal interactions, discovery of natural and biotechnological functions of these compounds, and insights into the roles they play in cell–cell communication are crucial for the continuity of discoveries in microbial ecology and biotechnology. In addition, the compounds disclosed here provided insights into microbial interactions, whether beneficial or deleterious, and their application in different areas.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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