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A B S T R A C T

In this study, a total of 30 yeast strains belonging to the genera *Dipodascus*, *Galactomyces*, *Geotrichum*, *Magnusiomyces* and *Saprochaete* were investigated for volatile organic compound production using HS-SPME-GC/MS analysis. The resulting flavour profiles, including 36 esters and 6 alcohols compounds, were statistically evaluated by cluster and PCA analysis. Two main groups of strains were extracted from this analysis, namely a group with a low ability to produce flavour and a group producing mainly alcohols. Two other minor groups of strains including *Saprochaete suaveolens*, *Geotrichum marinum* and *Saprochaete gigas* were diverging significantly from the main groups precisely because they showed a good ability to produce a large diversity of esters. In particular, we found that the *Saprochaete* genus (and their closed relatives) was characterized by a high production of unsaturated esters arising from par-tial catabolism of branched chain amino-acids. These esters were produced by eight phylogenetically related strains of *Saprochaete* genus.

## 1. Introduction

*Geotrichum* and *Saprochaete* yeasts are filamentous yeast like fungi which belong to the Ascomycota division, Saccharomycetes class and Saccharomycetales order (De Hoog & Smith, 2011a, 2011b). *Saprochaete* and *Geotrichum* species are very closely related. This is best illustrated by the fact that some species like *Saprochaete suaveolens* and *Saprochaete clavata* have a separate Geotrichum name (synonym) (De Hoog & Smith, 2004). These microbial eukaryotes are cosmopolitan and widespread and are often found in soil, manure, fruits, dairy products, human skin and digestive tract, insects as well as in other environments such as decaying plants and industrial effluents (Damasceno, Cereda, Pastore, & Oliveira, 2003; Suh & Blackwell, 2006). De Hoog and Smith (2011a) listed 11 species of *Geotrichum* of which one has a teleomorphic state in the genus *Dipodascus* de Lagerh, three in the genus *Galactomyces* Redhead and Malloch and seven for which a sexual state has not been found. *G. ghanense* (Nielsen, Jakobsen, & Jespersen, 2010) was the most recent *Geotrichum* species found in nature. In the *Saprochaete* genus, 13 species were described by De Hoog and Smith (2011b) of which, only three have a teleomorphic state in the genus *Magnusiomyces*. Within this genus, some species like *S. suaveolens*, *S. clavata*, *S. gigas* and *S. ingens* have their syn onym in the *Geotrichum* genus (*G. fragrans*, *G. clavatum*, *G. gigas* and *G. ingens*, respectively). With respect to safety, strains such as *Saprochaete capitata*, *S. clavata* or *G. candidum* are described as human pathogens (Camus et al., 2014; Garcia Ruiz et al., 2013).

*Geotrichum candidum* is one of the most studied species among this group of yeasts. It is well known in food industry for cheese ripening (Marcellino, Beuvier, Grappin, Guéguen, & Benson, 2001) and enzyme production (Ayed, Assas, Sayadi, & Hamdi, 2005; Brabcova et al., 2013; Hang & Woodams, 1990). *Geotrichum can didum*, as well as *S. suaveolens*, *G. klebahnii*, *Gal. geotrichum*, *Gal. reessii*, *D. aggregatus*, *D. albidus D. armillariae*, *M. capitatus and M.*

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*magnusii* are also well known for flavour production (Bonnarme et al., 2001; Buzzini, Martini, Cappelli, Pagnoni, & Davoli, 2003; Damasceno et al., 2003; De Oliveira et al., 2013; Farbood, Morris, & Seitz, 1987; Fischer, Senser, & Grosch, 1983; Jollivet, Chataud, Vayssier, Bensoussan, & Belin, 1994; Neto, Pastore, & Macedo, 2004; Shimizu, Kataoka, Kizaki, & Yasohara, 2004; Sinha, 2007; Wu, Xu, & Chen, 2012).

Flavours are secondary metabolites produced by living cells through some specific pathways such as Ehrlich (Hazelwood, Daran, van Maris, Pronk, & Dickinson, 2008), β oxidation (Maggio Hall & Keller, 2004) and glycolytic pathways (Liu, Holland, & Crow, 2004; Ugliano & Henschke, 2009). Among yeasts, *Saccharomyces cerevisiae* is probably the best know species for its applications in the field of food flavouring (Saerens, Delvaux, Verstrepen, & Thevelein, 2010). Other non conventional yeasts, like *Saprochaete suaveolens*, have also been described as excellent producers of fla vours including unsaturated esters such as ethyl tiglate (ethyl (*E*) 2 methylbut 2 enoate), an interesting top note flavour character ized by a strong fruity odor (Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al., 2015; Grondin, Shum Cheong Sing, Caro, Raherimandimby, et al., 2015). Numerous publications deal with the production of flavours from both an academic and applied per spective, but comparatively few of them uses flavours as a tool to discriminate between species and/or strains. Chemotaxonomy, according to the definition given by Frisvad, Andersen, and Thrane (2008) using flavours as a taxonomic tool was first reported for the filamentous fungi *Penicillium* (Larsen & Frisvad, 1995).

The purpose of this study was to determine the flavour produc tion profiles of the *Saprochaete*, *Geotrichum* and closely related yeasts. Experimental measurement of flavour production profiles using HS SPME CG/SM and processing of the data using descriptive statistic methods were performed to characterise the volatile organic compounds (VOCs) of these yeasts. Multivariate statistical methods were applied to find out whether the flavours production by these yeast species and their genomic classification using the ribosomal internal transcribed spacer (ITS) sequences were correlated.

**Table 1**
Strains of *Saprochaete*, *Geotrichum* and teleomorphs used in this study.

| Strain number | Species | Collection number | GenBank accession number of ITS sequences |
|---|---|---|---|
| S1 | *Dipodascus albidus* | CBS 766.85 | AY788342 |
| S2 | *Dipodascus armilliare* | CBS 834.71 | AY788350.1 |
| S3 | *Galactomyces candidus* | CBS 11176 | KJ579946[a] |
| S4 | *Galactomyces citri-aurantii* | CBS 176.89 | AY788296.1 |
| S5 | *Galactomyces geotrichum* | CBS 774.71 | JN974293.1 |
| S6 | *Galactomyces pseudocandidus* | CBS 10073 | JN974292.1 |
| S7 | *Geotrichum candidum* | CBS 615.84 | KJ608128[a] |
| S8 | *Geotrichum carabidarum* | CBS 9891 | DQ143888.1 |
| S9 | *Geotrichum cucujoidarum* | CBS 9893 | DQ143890 |
| S10 | *Geotrichum eriense* | CBS 694.83 | This study |
| S11 | *Geotrichum europaeum* | CBS 866.68 | AY788351 |
| S12 | *Geotrichum fermentans* | CBS 625.85 | AY788319 |
| S13 | *Geotrichum ghanense* | CBS 11010 | This study |
| S14 | *Geotrichum histeridarum* | CBS 9892 | DQ143889 |
| S15 | *Geotrichum klebahnii* | CBS 179.30 | AY788298 |
| S16 | *Geotrichum marinum* | MUCL 42958 | This study |
| S17 | *Geotrichum phurueaensis* | CBS 11418 | HE663403 |
| S18 | *Geotrichum restrictum* | CBS 111234 | EF126738.1 |
| S19 | *Magnusiomyces ingens* | CBS 101346 | This study |
| S20 | *Magnusiomyces magnusii* | CBS 151.30 | AY788290.1 |
| S21 | *Magnusiomyces ovetensis* | CBS 192.55 | AY788303.1 |
| S22 | *Saprochaete chiloensis* | CBS 8187 | AY788349 |
| S23 | *Saprochaete fungicola* | CBS 625.85 | AY788333.1 |
| S24 | *Saprochaete gigas* | CBS 126.76 | AY838940.1 |
| S25 | *Saprochaete ingens* | CBS 524.90 | AY788326.1 |
| S26 | *Saprochaete japonica* | CBS 100158 | AY788287.1 |
| S27 | *Saprochaete psychrophila* | CBS 765.85 | AY788341.1 |
| S28 | *Saprochaete quercus* | CBS 752.85 | This study |
| S29 | *Saprochaete saccharophila* | CBS 412.95 | AY788316.1 |
| S30 | *Saprochaete suaveolens* | GEC0 | This study |

[a] Sequence of another strains of the same species.

## 2. Materials and methods

### 2.1. Yeast strains

The list of strains used in this study is presented in Table 1. Most of them were purchased from CBS (Utrecht, The Netherlands) and from BCCM (Brussels, Belgium) strain collections. The *Saprochaete suaveolens* strain (GEC 0) used in this study was isolated from Pitaya fruits collected in Reunion Island as described elsewhere (Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al., 2015; Grondin, Shum Cheong Sing, Caro, Raherimandimby, et al., 2015). The rDNA D1/D2 sequence of the isolated strain showed a difference of one nucleotide with the *G. fragrans* reference strain CBS 152.25.

### 2.2. Culture media

Cells were stored in a rich medium contening glycerol in cryo genic vials at 80 °C and refreshed on autoclaved YEPD agar slants containing 20 g/L of glucose (Sigma), 20 g/L of peptone (Becton, Dickinson and Co.), 10 g/L of yeast extract (Biokar Diagnostics) and 15 g/L of agar (Merck). Cells were refreshed at 28 °C for 48 h prior to their utilization.

### 2.3. VOCS analysis

Isolation and characterization of volatile metabolites was per formed using solid phase micro extraction (SPME), followed by gas chromatography mass spectrometry (GC MS) analysis. The VOCS extraction and the chromatographic conditions were described in our previous studies (Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al., 2015; Grondin, Shum Cheong Sing, Caro, Raherimandimby, et al., 2015).

### 2.4. Phylogenetic analysis

The ribosomal internal transcribed spacer (ITS) sequences were retrieved from GenBank (NCBI database) under the accession num bers indicated in Table 1. Where sequences were not available in the database, analysis was performed according to James et al. (2014). Yeast cells were breaked using microwaves (Panasonic, 800 W) for 30 s in 50 μL of water to obtain cells extracts. The ITS region was amplified by PCR directly from whole yeast cell extracts, amplified using primers ITS5 and ITS4 and sequenced using these primers as well as internal primers ITS2 and ITS3. The amplified DNA was checked by 1% agarose gel electrophoresis, purified and concentrated using QIAquick PCR purification spin columns (Qiagen) and sequenced using a Life Technologies 3730XL sequencer at the Genome Analysis Centre (TGAC), Nor wich, UK. Pairwise alignments and phylogenetic analysis were con ducted using Geneious 7.1 software created by Biomatters. A phylogenetic neighbour joining tree was then generated using the distance Tamura Nei model. Confidence values for branch nodes were estimated from bootstrap analyses of 1000 replicates

**Table 2**
Volatile organic compounds (VOCs) produced by the representative yeasts of the genus *Geotrichum*, *Saprochaete* and teleomorphs during growth on YEPD and identified by SPME-GC/MS.

| Volatils compounds | RRI EXP[a] | RRI TH[b] | Yeast producing strains |
|---|---|---|---|
| **Alcohol** | | | |
| 2-Methylpropan-1-ol (isobutanol) | 637 | 628 | *G. pseudocandidus; G. europaeum; S. fungicola* |
| Butan-1-ol | 661 | 662 | *G. candidum; G. carabidarum; G. restrictum; M. magnusii; S. suaveolens* |
| 2-Methylbutanol (active amyl alcohol) | 770 | 739 | *G. pseudocandidus; G. candidum; G. cucujoidarum; G. europaeum; G. histeridarum; G. marinum; G. restrictum; M. magnusii; S. fungicola; S. quercus; S. suaveolens* |
| 3-Methylbutanol (isoamyl alcohol) | 731 | 734 | *D. armilliare; G. candidus; G. citri-aurantii; G. geotrichum; G. pseudocandidus; G. candidum; G. cucujoidarum; G. europaeum; G. fermentans; G. histeridarum; G. marinum; G. phurueaensis; G. restrictum; M. magnusii; S. fungicola; S. gigas; S. japonica; S. quercus; S. suaveolens* |
| 2-Phenylethanol | 1105 | 1114 | *G. candidus; G. citri-aurantii; G. pseudocandidus; G. candidum; G. eriense; G. europaeum; G. fermentans; G. ghanense; G. histeridarum; G. marinum; G. phurueaensis; M. magnusii; M. ovetensis; S. fungicola; S. gigas; S. japonica; S. quercus; S. suaveolens* |
| 2-Ethylhexanol | 1021 | 1030 | *G. histeridarum; M. magnusii; S. fungicola; S. quercus* |
| **Ester** | | | |
| Methyl 2-methylbutanoate | 774 | | *G. marinum* |
| Methyl 3-methylbutanoate (methyl isovalerate) | 774 | | *G. pseudocandidus* |
| Ethyl ethanoate (ethyl acetate) | 620 | 612 | *G. carabidarum; G. europaeum; G. marinum; G. restrictum; S. quercus; S. suaveolens* |
| Ethyl propanoate | 709 | 709 | *G. marinum; S. gigas; S. suaveolens* |
| Ethyl 2-methylpropanoate (ethyl isobutyrate) | 755 | 756 | *G. pseudocandidus; G. europaeum; G. histeridarum; G. marinum; S. gigas; S. suaveolens* |
| Ethyl butanoate | 797 | 800 | *G. pseudocandidus; G. candidum; G. histeridarum; G. marinum; G. restrictum; S. suaveolens* |
| Ethyl but-2-enoate (ethyl crotonate) | 841 | 833 | *G. marinum; S. suaveolens* |
| Ethyl 2-methylbutanoate | 847 | 846 | *G. pseudocandidus; G. candidum; G. europaeum; G. histeridarum; G. marinum; S. suaveolens* |
| Ethyl (*E*)-2-methylbut-2-enoate (ethyl tiglate) | 935 | 936 | *G. geotrichum; G. pseudocandidus; G. europaeum; G. marinum; M. magnusii; S. gigas; S. quercus; S. suaveolens* |
| Ethyl 3-methylbutanoate (ethyl isovalerate) | 850 | 849 | *G. geotrichum; G. pseudocandidus; G. candidum; G. europaeum; G. fermentans; G. marinum; S. gigas; S. suaveolens* |
| Ethyl (*E*)-3-methylbut-2-enoate (ethyl 3-methylcrotonate) | 919 | 920 | *G. marinum; S. gigas; S. suaveolens* |
| Ethyl pentanoate (ethyl valerate) | 895 | | *G. marinum* |
| Ethyl hexanoate (ethyl caproate) | 991 | | *G. marinum* |
| Ethyl hex-2-enoate | 1037 | | *G. marinum* |
| Ethyl octanoate (ethyl caprylate) | 1186 | | *G. carabidarum; G. restrictum; S. quercus* |
| 2-Methylpropyl ethanoate (isobutyl acetate) | 771 | 753 | *S. suaveolens* |
| 2-Methylpropyl 2-methylpropanoate (isobutyl isobutyrate) | 909 | 910 | *G. marinum; S. gigas; S. suaveolens* |
| 2-Methylpropyl butanoate (isobutyl butyrate) | 951 | 950 | *G. marinum; S. suaveolens* |
| 2-Methylpropyl 2-methylbutanoate (isobutyl 2-methylbutanoate) | 995 | 998 | *G. marinum; S. gigas; S. suaveolens* |
| 2-Methylpropyl (*E*)-2-methylbut-2-enoate (isobutyl tiglate) | 1084 | 1086 | *G. europaeum; G. marinum; S. gigas; S. suaveolens* |
| 2-Methylpropyl 3-methylbutanoate (isobutyl isovalerate) | 997 | 1002 | *G. pseudocandidus; G. marinum; S. gigas; S. suaveolens* |
| Butyl 2-methylbutanoate | 1035 | | *S. suaveolens* |
| Butyl (*E*)-2-methylbut-2-enoate (butyl tiglate) | 1125 | 1128 | *S. suaveolens* |
| Butyl 3-methylbutanoate (butyl isovalerate) | 1039 | 1040 | *S. gigas; S. suaveolens* |
| 2-Methylbutyl ethanoate | 903 | 874 | *S. suaveolens* |
| 2-Methylbutyl 2-methylpropanoate | 1009 | | *G. marinum; S. suaveolens* |
| 2-Methylbutyl butanoate | 1052 | 1052 | *G. marinum; S. suaveolens* |
| 3-Methylbutyl ethanoate (isoamyl acetate) | 873 | 871 | *G. candidum; G. histeridarum; G. marinum; S. suaveolens* |
| 3-Methylbutyl propanoate (isoamyl propanoate) | 964 | 964 | *G. marinum; S. suaveolens* |
| 3-Methylbutyl butanoate (isoamyl butanoate) | 1049 | 1050 | *G. marinum; S. suaveolens* |
| 3-Methylbutyl 2-methylbutanoate (isoamyl 2-methylbutanoate) | 1091 | | *G. marinum; S. suaveolens* |
| 3-Methylbutyl (*E*)-2-methylbut-2-enoate (isoamyl tiglate) | 1185 | 1253 | *G. marinum; S. suaveolens* |
| 3-Methylbutyl 3-methylbutanoate (isoamyl isovalerate) | 1096 | 1101 | *G. citri-aurantii; G. fermentans; G. marinum; S. gigas; S. suaveolens* |
| Pentyl propanoate (amyl propanoate) | 964 | 964 | *S. gigas* |
| Pentyl 3-methylbutanoate (amyl isovalerate) | 1098 | 1103 | *G. marinum; S. gigas; S. suaveolens* |
| Octyl ethanoate | 1199 | | *G. carabidarum; G. restrictum* |

[a] Relative Retention index on non-polar column determined experimentally.
[b] Relative Retention index on non-polar column (http://webbook.nist.gov/chemistry/).

**Table 3**
Classification of the VOCs produced by the strains belonging to the genus *Geotrichum, Saprochaete* and teleomorphs.

| Name of species | Concentration of VOCs per chemical class (μg/L) | | | Number of VOCs per chemical class | | | Number of VOCs per type of hypothetical pathway | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [Al] | [Es] | [Ctotal] | Al | Es | Total | GP | MP | PP | BP | Pep | B1P | B2P | EP |
| *Dipodascus albidus* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dipodascus armilliare* | 5 | nd | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Galactomyces candidus* | 85 | Nd | 85 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Galactomyces citri-aurantii* | 48 | 7 | 55 | 2 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Galactomyces geotrichum* | 9 | 39 | 49 | 1 | 2 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 3 |
| *Galactomyces pseudocandidus* | 305 | 37 | 342 | 4 | 7 | 11 | 5 | 1 | 0 | 1 | 0 | 0 | 1 | 11 |
| *Geotrichum candidum* | 437 | 8 | 445 | 4 | 4 | 8 | 4 | 0 | 0 | 2 | 0 | 0 | 0 | 6 |
| *Geotrichum carabidarum* | 46 | 121 | 167 | 1 | 3 | 4 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 0 |
| *Geotrichum cucujoidarum* | 75 | nd | 75 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Geotrichum eriense* | 11 | nd | 11 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Geotrichum europaeum* | 1164 | 165 | 1329 | 4 | 6 | 10 | 5 | 0 | 0 | 0 | 0 | 0 | 2 | 10 |
| *Geotrichum fermentans* | 82 | 11 | 93 | 2 | 2 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| *Geotrichum ghanense* | 14 | nd | 14 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Geotrichum histeridarum* | 247 | 30 | 277 | 4 | 4 | 8 | 4 | 0 | 0 | 1 | 0 | 1 | 0 | 6 |
| *Geotrichum Klebahnii* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Geotrichum marinum* | 375 | 3159 | 3534 | 3 | 27 | 30 | 13 | 1 | 2 | 5 | 1 | 2 | 5 | 29 |
| *Geotrichum phurueaensis* | 109 | Nd | 109 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Geotrichum restrictum* | 244 | 169 | 414 | 3 | 4 | 7 | 4 | 0 | 0 | 2 | 0 | 2 | 0 | 2 |
| *Magnusiomyces ingens* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Magnusiomyces magnusii* | 151 | 1 | 152 | 5 | 1 | 6 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 4 |
| *Magnusiomyces ovetensis* | 13 | nd | 13 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Saprochaete chiloensis* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Saprochaete fungicola* | 238 | nd | 238 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| *Saprochaete gigas* | 48 | 1753 | 1801 | 2 | 13 | 15 | 5 | 0 | 1 | 1 | 1 | 0 | 3 | 19 |
| *Saprochaete ingens* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Saprochaete japonica* | 47 | nd | 47 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Saprochaete psychrophila* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Saprochaete quercus* | 79 | 24 | 103 | 4 | 3 | 7 | 4 | 0 | 0 | 0 | 0 | 2 | 1 | 4 |
| *Saprochaete saccharophila* | nd | nd | nd | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Saprochaete suaveolens* | 268 | 3464 | 3732 | 4 | 28 | 32 | 12 | 0 | 2 | 9 | 1 | 0 | 5 | 33 |

VOCs were classified in three categories (Concentration of VOCs per chemical class, Number of VOCs per chemical class and Number of VOCs per type of hypothetical pathway) for cluster and PCA analysis. [Al]: concentration of alcohols; [Es]: concentration of esters; [CTotal]: Concentration of VOCs produced; nd: not detectable; Al: number of alcohols; Es: number of ester; Total: number of VOcs produced; Number of molecules derived from GP: Glycolysis pathway; PP: Propanoate pathway; BP: Butanoate pathway; Pep: Pentanoate pathway; B1P: β-oxidation pathway; B2P: unsaturated compounds from β-oxidation pathway; EP: Ehrlich pathway.

(Cardinali et al., 2012; Urubschurov, Janczyk, Souffrant, Freyer, & Zeyner, 2011).

### 2.5. Statistical analysis

Principal Component Analysis and cluster analysis (Ward's method) were performed on yeast flavours using XLSTAT (Addin soft) (Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al., 2015; Grondin, Shum Cheong Sing, Caro, Raherimandimby, et al., 2015).

## 3. Results and discussion

### 3.1. Yeast strain selection

Yeasts were selected in accordance to De Hoog and Smith (2011a, 2011b) who described all currently accepted yeast species in these genera. *Saprochaete clavata*, which is categorized as a Class 2 microorganism, was arbitrarily withdrawn because of associated safety problems. VOCs analysis was extended to some strains of *Geotrichum*, which are not described by De Hoog and Smith (2011a, 2011b) namely *G. marinum*, *G. phuruaensis* and *G. ghanense*. Given that anamorphic and teleomorphic species are genetically very close (Liu et al., 2001), we also selected some yeasts which have an anamorphic state in the genera *Geotrichum* and *Saprochaete*, such as *Galactomyces citri aurantii* (*Geotrichum citri aurantii*), *Gal. pseudocandidus* (*Geotrichum pseudocandidum*), *Gal. candidus* (*Geotrichum candidum*), *Magnusiomyces magnusii* (*Saprochaete ludwigii*), *M. capitatus* (*Saprochaete capitata*), *M. ovetensis* (Saprochaete sericea) and *Dipodascus armillariae* (*Geotrichum decipiens*).

### 3.2. VOCS production by different yeast species

Qualitative analysis of VOCs were carried out using HS SPME GC/MS on 30 representative strains belonging to the anamorphic genera *Saprochaete* (9 species), *Geotrichum* (12 species) and related teleomorphic genera *Magnusiomyces* (3 species), *Dipodascus* (2 species) and *Galactomyces* (4 species) after 24 h growth on YEPD (Table 2). A total of 42 different compounds were identified and classified into alcohols and esters (6 and 36 molecules, respectively). Due to their aromatic properties, esters are valuable molecules and are of relevance to industry. These molecules usually impart a characteristic fruity note to fermented beverages such as beer and wine (Schrader, 2007).

Among VOCs, 3 methylbutanol, 2 phenylethanol and 2 methylbutanol, which arise from the degradation of leucine, phenylalanine and isoleucine respectively by the Ehrlich pathway (Hazelwood et al., 2008), were the most frequently encountered compounds and were produced by 19, 18 and 11 different strains, respectively. In contrast, some VOCs such as methyl 2 methylbutanoate and butyl 2 methylbut 2 enoate were produced by only 1 yeast strain (*G. marinum* and *S. suaveolens*, respectively). Strains *S. suaveolens*, *G. marinum*, *S. gigas* and *G. europaeum* showed the best ability to produce qualitatively (more than 10 different compounds) and quantitatively (relative concentration higher than 1000 μg/L) flavour compounds (Table 3). Strain *Gal. pseudocandidus* was also found to produce a large number of VOCs (11 compounds) but quantitatively, the overall production of VOCs was significantly

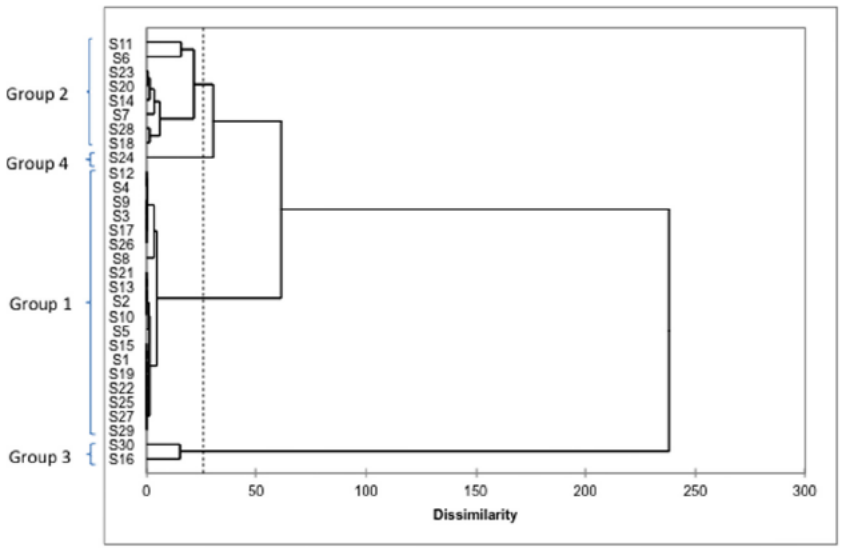


**Fig. 1.** Cluster analysis dendograms from 30 yeasts based on flavour production. Dendrograms were calculated using ward's method and performed using XLSTAT (Addinsoft). Based on entropy, automatic truncation (dotted line) allowed identifying four consistent groups of yeasts among the strains S1 to S30 (Table 1).

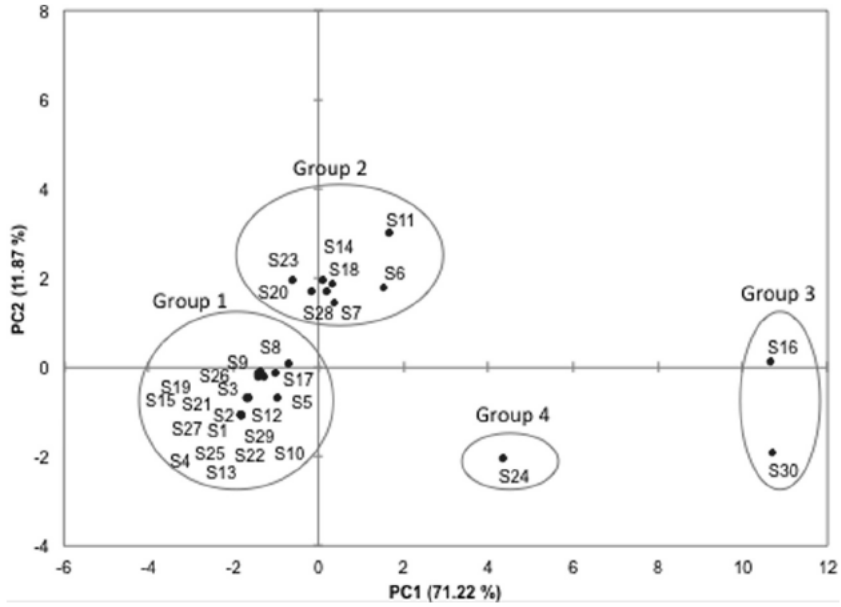


**Fig. 2.** Score plot of PC2 versus PC1 for yeast flavour analyzed by HS-SPME-GC/MS Score plot of PC2 versus PC1 for yeast flavour analyzed by HS–SPME–GC/MS. Principal Component Analysis was performed using XLSTAT (Addinsoft). Variables including the relative concentration of VOCs (μg/L), number of VOCs per chemical classes and per hypothetical metabolic pathway (Table 3). S1 to S30 are the analyzed strains. Group 1 included strains that have a low ability to produce VOCs, group 2 strains that produced mainly alcohol and Group 3 and 4 strains that produced mainly esters.

lower than the above strains (less than 400 μg/L). Comparatively, our results were in good agreement with other published data. For instance, production of 2 methylbutanol, 3 methylbutanol, 2 phenylethanol, ethyl acetate, ethyl propanoate, 3 methylbutyl ethanoate, ethyl 2 methylbut 2 enoate and 2 methylbutyl ethano ate were also detected in *G. candidum*, *S. suaveolens*, *G. klebahnii*, *Gal. geotrichum* and *M. magnusii* (Buzzini et al., 2003; Damasceno et al., 2003; Farbood et al., 1987; Fischer et al., 1983; Jollivet et al., 1994; Sinha, 2007; Wu et al., 2012). However, some results differed from other published data. Buzzini et al. (2003) reported a production of 3 methylbutanol and pentan 1 ol for *G. klebanhii* (S13) during growth in shaked flasks containing 10 g/L yeast extract, 10 g/L $(NH_4)_2HPO_4$ and 20 g/L glucose (pH 5.0) at 25 °C for 72 h. In our study (YEPD standard agar medium), no VOCs could be detected for this strain. This apparent contradiction might either come from differences in the design of the experiment (e.g., liquid vs. solid media, presence of phosphate buffer, …) or from the genetic background of the strains. It should be noted however that one of the five *G. klebanhii* strains isolated by Buzzini et al. (2003) was also unable to produce VOCs. Such a dif ference between strains from the same species was previously reported, for example by Berger, Khan, Molimard, Martin, and Spinnler (1999) who observed differences in the production of sul phur compounds by ten isolates of the same species, *G. candidum*.

As previously shown by Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al. (2015), Grondin, Shum Cheong Sing, Caro, Raherimandimby, et al. (2015), *Saprochaete suaveolens* produced several unsaturated ester compounds such as ethyl but 2 enoate,

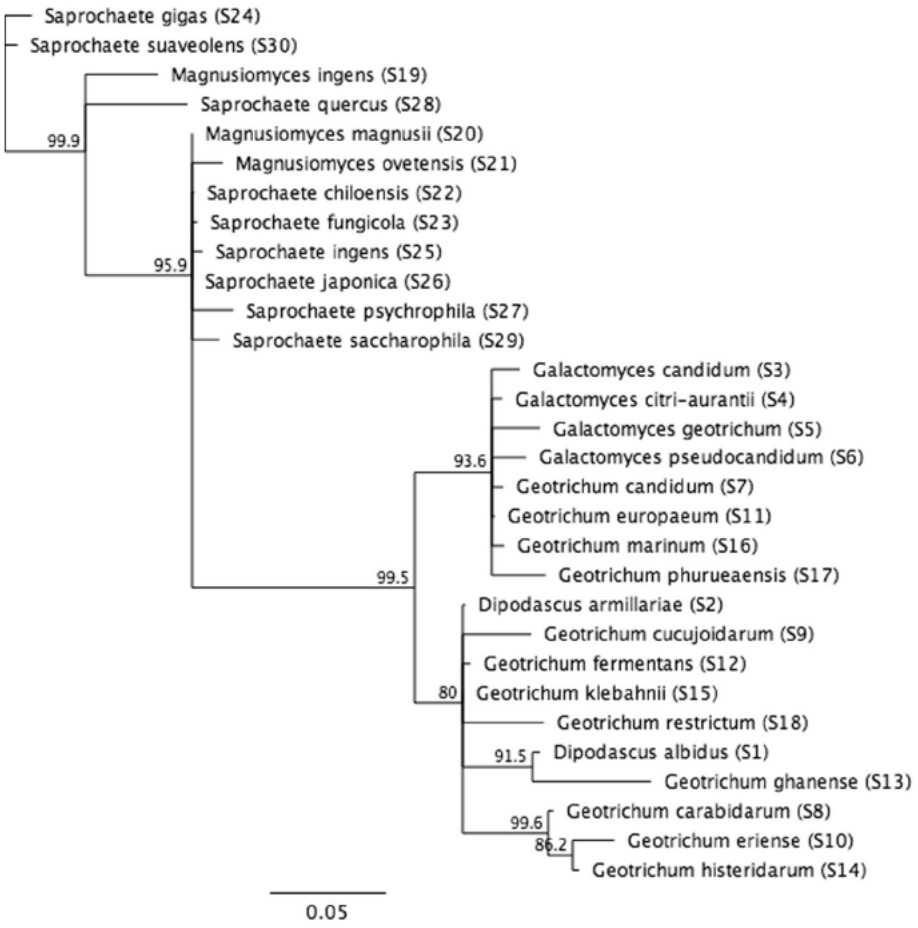


**Fig. 3.** Phylogenetic tree of species of the genus *Saprochaete*, *Geotrichum* and some teleomorphs strains. The tree *was* constructed using Geneious 7.1 (Biomatters) according to neighbour joining method with the ITS1–5.8S–ITS2 sequences collected from NCBI or determined in the laboratory. Bootstrap values (>80%) are from 1000 replicates. Scale Bar represents the number of substitution per site.

ethyl 2 methylbut 2 enoate, ethyl 3 methylbut 2 enoate, 2 methylpropyl 2 methylbut 2 enoate, butyl 2 methylbut 2 enoate and 3 methylbutyl 2 methylbut 2 enoate. Some of them were also produced by other strains, namely *Gal. geotrichum*, *Gal. pseudocandidus*, *G. europaeum*, *G. marinum*, *M. magnusii*, *S. gigas*, *S. quercus* and *S. suaveolens* (Tables 1 and 2). Like *S. suaveolens*, *G. marinum* was found to produce 5 out of the 6 unsaturated esters. Ethyl 2 methylbut 2 enoate (ethyl tiglate) was produced by the 8 strains whereas some unsaturated esters like ethyl hex 2 enoate or butyl 2 methylbut 2 enoate (butyl tiglate) were produced by only one strain (*G. marinum* and *S. suaveolens*, respectively). Since four out of unsaturated esters were derived from 2 methylbutanoyl CoA or tiglyl CoA, we could argue according to Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al. (2015), that these esters arose from the catabolism of isoleucine that partially halted at the dehydration step due to low enoyl CoA hydratase activity.

As reported in Table 2, *G marinum* can produce ethyl butanoate, ethyl but 2 enoate, ethyl hexanoate and ethyl hex 2 enoate, whereas *S. suaveolens* was limited to the production of the first two esters. This result may suggest that either *S. suaveolens* cannot further condense a acetyl CoA to elongate the fatty acid chain, or alternatively, butanoyl CoA is generated by the butanoate pathway that exist in human and some bacteria (http://www.genome.jp/kegg/pathway.html) but to our knowledge had never been reported in fungi. This pathway can lead to either butanoate as well as to butanol. Together with intermediates in catabolism of isoleucine, this pathway may account for the production of butyl 2 methylbutanoate, butyl 2 methylbut 2 enoate and butyl 3 methylbutanoate.

### *3.3. Comparison of the VOC profile of the strains by cluster and multivariate analysis and correlation with a taxonimical analysis*

Multivariate statistical analysis was used in order to group yeast species based on their flavouring characteristics. To this end, each strain was associated with variables described in Table 3. Cluster analysis (Fig. 1) and PCA analysis (Fig. 2) both suggested the occurrence of two main and two minor groups of yeasts. The first group seems to be characterized by strains which have a low ability to produce VOCs and the second by strains that produced mainly alcohols. Three strains, namely *G. marinum*, *S. suaveolens* (group 3) and *S. gigas* (group 4) deviated from these groups because of their ability to produced esters (Fig. 2). Among group 2, *G. europaeum* appeared to be the best producer of alcohols with 1164 μg/L. In other experiments, we found *Debaryomyces nepalensis* to be the best strain for alcohol production (985 μg/L of alcohol was produced when tested in the same experimental conditions; Grondin, Shum Cheong Sing, Caro, Raherimandimby, et al., 2015). With a production of 15, 30 and 32 different VOCs respectively, *S. gigas*, *G. marinum* and *S. suaveolens* were by far the best producers of esters, and more specifically unsaturated esters among all strains analyzed (Table 3). *Geotrichum marinum* has the particularity to produce methyl esters like methyl 2 methylbutanoate and methyl 3 methylbutanoate (Table 2).

The fact that 4 genera (8 species) produced these unsaturated compounds suggested phylogenetic belonging. Then, only *Saprochaete* and related genera *Geotrichum*, *Galactomyces* and *Magnusiomyces* were found to produce these unsaturated esters, and in particular ethyl tiglate which could reach 113 mg/l in *S. suaveolens* culture (Grondin, Shum Cheong Sing, Caro, de Billerbeck, et al., 2015). The presence of unsaturated compounds in eight species of these neighbour genera indicated high metabolic similarities between these yeasts.

In order to establish a genomic link between these strains, a phylogenetic tree was generated using rDNA sequences either retrieved from the NCBI and GenBank databases or determined in our laboratory (Fig. 3). The internal transcribed spacer (ITS) region was selected because it is widely used to identify a broad range of different fungi (Schoch et al., 2012). As we can see, the strains under study seem very closed genetically (0.3 substitution per site between the most distant strains) and could explain the metabolic similarity for VOCS production (Fig. 3).

To summarize, this work was a first approach to study VOCS from the yeast of the genus *Saprochaete*, *Geotrichum* and close relative strains. Statistical analysis allowed us to classify the strains according to their flavour production and four groups of strains were highlighted by this approach. While we identified the *Saprochaete genus* as exhibiting an unusual capacity to produce a large variety of unsaturated esters such as ethyl tiglate, we could not find any relationship between flavours profiles and genomic classification of these yeast strains, suggesting that the metabolic activities underlining the flavour production has been shaped by their ecological niche.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.06.009.

## References

Ayed, L., Assas, N., Sayadi, S., & Hamdi, M. (2005). Involvement of lignin peroxidase in the decolourization of black olive mill wastewaters by *Geotrichum candidum*. *Letters in Applied Microbiology, 40*(1), 7–11.

Berger, C., Khan, J. A., Molimard, P., Martin, N., & Spinnler, H. E. (1999). Production of sulfur flavors by ten strains of *Geotrichum candidum*. *Applied and Environmental Microbiology, 65*(12), 5510–5514.

Bonnarme, P., Arfi, K., Dury, C., Helinck, S., Yvon, M., & Spinnler, H. E. (2001). Sulfur compound production by *Geotrichum candidum* from L-methionine: Importance of the transamination step. *FEMS Microbiology Letters, 205*(2), 247–252.

Brabcova, J., Demianova, Z., Vondrasek, J., Jagr, M., Zarevucka, M., & Palomo, J. M. (2013). Highly selective purification of three lipases from *Geotrichum candidum* 4013 and their characterization and biotechnological applications. *Journal of Molecular Catalysis B: Enzymatic, 98*, 62–72.

Buzzini, P., Martini, A., Cappelli, F., Pagnoni, U. M., & Davoli, P. (2003). A study on volatile organic compounds (VOCs) produced by tropical ascomycetous yeasts. *Antonie van Leeuwenhoek, 84*(4), 301–311.

Camus, V., Thibault, M.-L., David, M., Gargala, G., Compagnon, P., Lamoureux, F., ... Leprêtre, S. (2014). Invasive *Geotrichum clavatum* fungal infection in an acute myeloid leukaemia patient: A case report and review. *Mycopathologia*, 1–6.

Cardinali, G., Antonielli, L., Corte, L., Roscini, L., Bagnetti, A., Pelliccia, C., & Puddu, G. (2012). *Kazachstania ichnusensis* sp. nov., a diploid homothallic ascomycetous yeast from Sardinian lentisk rhizosphere. *International Journal of Systematic and Evolutionary Microbiology, 62*(Pt 3), 722–727.

Damasceno, S., Cereda, M. P., Pastore, G. M., & Oliveira, J. G. (2003). Production of volatile compounds by *Geotrichum fragrans* using cassava wastewater as substrate. *Process Biochemistry, 39*(4), 411–414.

De Hoog, S. G., & Smith, M. T. (2004). Ribosomal gene phylogeny and species delimitation in *Geotrichum* and its teleomorphs. *Studies in Mycology, 50*, 489–515.

De Hoog, S. G., & Smith, M. T. (2011a). Chapter 91 – Geotrichum link. In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The Yeasts* (5th ed., pp. 1279–1286). London: Elsevier.

De Hoog, S. G., & Smith, M. T. (2011b). Chapter 97 – Saprochaete Coker & Shanor ex D.T.S. Wagner & Dawes (1970). In C. P. Kurtzman, J. W. Fell, & T. Boekhout (Eds.), *The yeasts* (5th ed., pp. 1317–1327). London: Elsevier.

De Oliveira, S. M. M., Gomes, S. D., Sene, L., Machado, S. R., Coelho, A. C. B., Cereda, M. P., ... Piechontcoski, J. (2013). Production of 2-phenylethanol by *Geotrichum fragrans*, *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* in cassava wastewater. *Journal of Food, Agriculture & Environment, 11*(2), 158–163.

Farbood, M. I., Morris, J. A., & Seitz, E. W. (1987). Preparation of naturally-occurring C2–C5 alkyl esters of C4–C5 carboxylic acids by means of fermentation of C5–C6 amino acids in the presence of C2–C5 alcohols. US patent US4657862 A.

Fischer, K.-H., Senser, F., & Grosch, W. (1983). Aromabildung durch Mikroorganismen. *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung, 177*(5), 336–338.

Frisvad, J. C., Andersen, B., & Thrane, U. (2008). The use of secondary metabolite profiling in chemotaxonomy of filamentous fungi. *Mycological Research, 112*(2), 231–240.

Garcia-Ruiz, J. C., Lopez-Soria, L., Olazabal, I., Amutio, E., Arrieta-Aguirre, I., Velasco-Benito, V., ... Moragues, M. D. (2013). Invasive infections caused by *Saprochaete capitata* in patients with haematological malignancies: Report of five cases and review of the antifungal therapy. *Revista Iberoamericana de Micología, 30*, 248–255.

Grondin, E., Shum Cheong Sing, A., Caro, Y., de Billerbeck, G. M., François, J. M., & Petit, T. (2015a). Physiological and biochemical characteristics of ethyl tiglate production pathway in the yeast *Saprochaete suaveolens*. *Yeast, 32*(1), 57–66.

Grondin, E., Shum Cheong Sing, A., Caro, Y., Raherimandimby, M., Randrianierenana, A. L., James, S., ... Petit, T. (2015b). A comparative study on the potential of epiphytic yeasts isolated from tropical fruits to produce flavoring compounds. *International Journal of Food Microbiology, 203*, 101–108.

Hang, Y. D., & Woodams, E. E. (1990). Lipase production by *Geotrichum candidum* from sauerkraut brine. *World Journal of Microbiology & Biotechnology, 6*(4), 418–421.

Hazelwood, L. A., Daran, J.-M., van Maris, A. J. A., Pronk, J. T., & Dickinson, J. R. (2008). The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. *Applied and Environmental Microbiology, 74*(8), 2259–2266.

James, S. A., Barriga, E. J. C., Barahona, P. P., Harrington, T. C., Lee, C.-F., Bond, C. J., & Roberts, I. N. (2014). *Wickerhamomyces arborarius f.a.*, sp. *nov.*, an ascomycetous yeast species found in arboreal habitats on three different continents. *International Journal of Systematic and Evolutionary Microbiology, 64*(Pt 3), 1057–1061.

Jollivet, N., Chataud, J., Vayssier, Y., Bensoussan, M., & Belin, J.-M. (1994). Production of volatile compounds in model milk and cheese media by eight strains of *Geotrichum candidum Link*. *Journal of Dairy Research, 61*(02), 241–248.

Larsen, T. O., & Frisvad, J. C. (1995). Chemosystematics of *Penicillium* based on profiles of volatile metabolites. *Mycological Research, 99*(10), 1167–1174.

Liu, S.-Q., Holland, R., & Crow, V. L. (2004). Esters and their biosynthesis in fermented dairy products: A review. *International Dairy Journal, 14*(11), 923–945.

Liu, Z.-Y., Yao, Y.-J., Liang, Z. Q., Liu, A.-Y., Pegler, D. N., & Chase, M. W. (2001). Molecular evidence for the anamorph–teleomorph connection in *Cordyceps sinensis*. *Mycological Research, 105*(07), 827–832.

Maggio-Hall, L. A., & Keller, N. P. (2004). Mitochondrial β-oxidation in *Aspergillus nidulans*. *Molecular Microbiology, 54*(5), 1173–1185.

Marcellino, N., Beuvier, E., Grappin, R., Guéguen, M., & Benson, D. R. (2001). Diversity of *Geotrichum candidum* strains isolated from traditional cheesemaking fabrications in France. *Applied and Environmental Microbiology, 67*(10), 4752–4759.

Neto, R. S., Pastore, G. M., & Macedo, G. A. (2004). Biocatalysis and biotransformation producing γ-decalactone. *Journal of Food Science, 69*(9), 677–680.

Nielsen, D. S., Jakobsen, M., & Jespersen, L. (2010). *Candida halmiae* sp. *nov.*, *Geotrichum ghanense* sp. *nov.* and *Candida awuaii* sp. *nov.*, isolated from Ghanaian cocoa fermentations. *International Journal of Systematic and Evolutionary Microbiology, 60*(6), 1460–1465.

Saerens, S. M. G., Delvaux, F. R., Verstrepen, K. J., & Thevelein, J. M. (2010). Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microbial Biotechnology, 3*(2), 165–177.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., ... Schindel, D. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences, 109*(16), 6241–6246.

Schrader, J. (2007). Microbial flavour production. In R. G. Berger (Ed.), *Flavours and fragrances* (pp. 507–574). Berlin Heidelberg: Springer.

Shimizu, S., Kataoka, M., Kizaki, N., & Yasohara, Y. (2004). Incubating ashbya with 2-chloro-1-(3′-chlorophenyl)ethanone, allowing sterospecific reduction, recovering (*R*)-2-chloro-1-(3′-chlorophenyl)ethanol; raw materials for medicine. US patent US20040219658 A1.

Sinha, N. (2007). *Handbook of food products manufacturing, 2 volume set*. John Wiley & Sons.

Suh, S.-O., & Blackwell, M. (2006). Three new asexual arthroconidial yeasts, *Geotrichum carabidarum* sp. *nov.*, *Geotrichum histeridarum* sp. *nov.*, and *Geotrichum cucujoidarum* sp. *nov.*, isolated from the gut of insects. *Mycological Research, 110*(Pt. 2), 220–228.

Ugliano, M., & Henschke, P. A. (2009). Yeasts and wine flavour. In M. V. Moreno-Arribas & M. C. Polo (Eds.), *Wine chemistry and biochemistry* (pp. 313–392). New York: Springer.

Urubschurov, V., Janczyk, P., Souffrant, W.-B., Freyer, G., & Zeyner, A. (2011). Establishment of intestinal microbiota with focus on yeasts of unweaned and weaned piglets kept under different farm conditions. *FEMS Microbiology Ecology, 77*(3), 493–502.

Wu, Q., Xu, Y., & Chen, L. (2012). Diversity of yeast species during fermentative process contributing to Chinese Maotai-flavour liquor making. *Letters in Applied Microbiology, 55*(4), 301–307.