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Flavour production by *Saprochaete* and *Geotrichum* yeasts and their close relatives

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\(^b\) Département Génie Biologique, IUT, Université de La Réunion, Saint-Pierre, Ile de la Réunion, France
\(^c\) National Collection of Yeast Cultures, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK
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**Abstract**

In this study, a total of 30 yeast strains belonging to the genera *Dipodascus*, *Galactomyces*, *Geotrichum*, *Magnusomyces* 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, Saccharomycetales class and Saccharomycetales order (De Hoog & Smith, 2011). *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 (2011) 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 (2011) of which, only three have a teleomorphic state in the genus *Magnusomyces*. Within this genus, some species like *S. suaveolens*, *S. clavata*, *S. gigas* and *S. ingens* have their synonym in the *Geotrichum* genus (C. fragrans, C. clavatum, C. gigas and G. ingens, respectively). With respect to safety, strains such as *Saprochaete capitata*, *S. clavata* or *C. 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 (Aved Assas, Sayadi, & Hamdi, 2005; Brabcova et al., 2013; Hang & Woodams, 1990). *Geotrichum candidum*, as well as *S. suaveolens*, *G. kiebahnii*, *G. geotrichum*, *G. reessii*, *D. aggregatus*, *D. albidus* and *D. armillariae*, *M. capitatus* and *M.*
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, & Grosh, 1983; Jollivet, Chataud, Vaysi, Bensousan, & Belin, 1994; Neto, Pastor, & 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), € 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 flavours including unsaturated esters such as ethyl tiglate (ethyl (E) 2 methylbut 2 enoate), an interesting top note flavour characterized 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 perspective, 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 production profiles of the Saprochaete, Geotrichum and closely related yeasts. Experimental measurement of flavour production profiles using HS SPME GC/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.

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 tDNA 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 containing glycerol in cryogenic vials at 80 °C and refreasted 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 refreasted at 28 °C for 48 h prior to their utilization.

2.3. VOCS analysis

Isolation and characterization of volatile metabolites was performed 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 numbers 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), Norwich, UK. Fairwise alignments and phylogenetic analysis were conducted 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>
<thead>
<tr>
<th>Table 1</th>
<th>Strains of Saprochaete, Geotrichum and teleomorphs used in this study.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain number</td>
<td>Species</td>
</tr>
<tr>
<td>S1</td>
<td>Dipodascus albidus</td>
</tr>
<tr>
<td>S2</td>
<td>Dipodascus armillare</td>
</tr>
<tr>
<td>S3</td>
<td>Galactomyces candidus</td>
</tr>
<tr>
<td>S4</td>
<td>Galactomyces citr-</td>
</tr>
<tr>
<td>S5</td>
<td>Galactomyces geotrichum</td>
</tr>
<tr>
<td>S6</td>
<td>Galactomyces pseudocandidus</td>
</tr>
<tr>
<td>S7</td>
<td>Geotrichum candidum</td>
</tr>
<tr>
<td>S8</td>
<td>Geotrichum carabidarium</td>
</tr>
<tr>
<td>S9</td>
<td>Geotrichum cucujoidarium</td>
</tr>
<tr>
<td>S10</td>
<td>Geotrichum erymese</td>
</tr>
<tr>
<td>S11</td>
<td>Geotrichum europaea</td>
</tr>
<tr>
<td>S12</td>
<td>Geotrichum fermentans</td>
</tr>
<tr>
<td>S13</td>
<td>Geotrichum ghanense</td>
</tr>
<tr>
<td>S14</td>
<td>Geotrichum histeriderum</td>
</tr>
<tr>
<td>S15</td>
<td>Geotrichum klebahnii</td>
</tr>
<tr>
<td>S16</td>
<td>Geotrichum marinum</td>
</tr>
<tr>
<td>S17</td>
<td>Geotrichum phaeoaeansis</td>
</tr>
<tr>
<td>S18</td>
<td>Geotrichum restrictum</td>
</tr>
<tr>
<td>S19</td>
<td>Magnusomyces ingens</td>
</tr>
<tr>
<td>S20</td>
<td>Magnusomyces magnusii</td>
</tr>
<tr>
<td>S21</td>
<td>Magnusomyces ovetsiensis</td>
</tr>
<tr>
<td>S22</td>
<td>Saprochaete chilensis</td>
</tr>
<tr>
<td>S23</td>
<td>Saprochaete fungicola</td>
</tr>
<tr>
<td>S24</td>
<td>Saprochaete gigas</td>
</tr>
<tr>
<td>S25</td>
<td>Saprochaete ingens</td>
</tr>
<tr>
<td>S26</td>
<td>Saprochaete japonica</td>
</tr>
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<td>S27</td>
<td>Saprochaete psychrophila</td>
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<tr>
<td>S28</td>
<td>Saprochaete quercus</td>
</tr>
<tr>
<td>S29</td>
<td>Saprochaete saccharophila</td>
</tr>
<tr>
<td>S30</td>
<td>Saprochaete suaveolens</td>
</tr>
</tbody>
</table>

* Sequence of another strains of the same species.
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.

<table>
<thead>
<tr>
<th>Volatiles compounds</th>
<th>RRI Th*</th>
<th>Yeast producing strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylpropan-1-ol (isobutanol)</td>
<td>637</td>
<td>628</td>
</tr>
<tr>
<td>Butan-1-ol</td>
<td>661</td>
<td>662</td>
</tr>
<tr>
<td>2-Methylbutanol (active amyl alcohol)</td>
<td>770</td>
<td>739</td>
</tr>
<tr>
<td>3-Methylbutanol (isoamyl alcohol)</td>
<td>731</td>
<td>734</td>
</tr>
<tr>
<td>2-Phenylethanol</td>
<td>1105</td>
<td>1114</td>
</tr>
<tr>
<td>2-Ethylhexanol</td>
<td>1021</td>
<td>1030</td>
</tr>
<tr>
<td>Ester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl 2-methylbutanoate</td>
<td>774</td>
<td>774</td>
</tr>
<tr>
<td>Methyl 3-methylbutanoate (methyl isovalerate)</td>
<td>620</td>
<td>612</td>
</tr>
<tr>
<td>Ethyl ethanoate (ethyl acetate)</td>
<td>709</td>
<td>709</td>
</tr>
<tr>
<td>Ethyl propanoate</td>
<td>755</td>
<td>756</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>797</td>
<td>800</td>
</tr>
<tr>
<td>Ethyl but-2-enoate (ethyl crotonate)</td>
<td>841</td>
<td>833</td>
</tr>
<tr>
<td>Ethyl 2-methylbutanoate (ethyl isovalerate)</td>
<td>847</td>
<td>846</td>
</tr>
<tr>
<td>Ethyl ((E)-2)-methylbut-2-enoate (ethyl tiglate)</td>
<td>935</td>
<td>936</td>
</tr>
<tr>
<td>Ethyl 3-methylbutanoate (ethyl isovalerate)</td>
<td>850</td>
<td>849</td>
</tr>
<tr>
<td>Ethyl ((E)-3)-methylbut-2-enoate (ethyl 3-methylcrotonate)</td>
<td>919</td>
<td>920</td>
</tr>
<tr>
<td>Ethyl pentanoate (ethyl valerate)</td>
<td>895</td>
<td>895</td>
</tr>
<tr>
<td>Ethyl hexanoate (ethyl caproate)</td>
<td>991</td>
<td>991</td>
</tr>
<tr>
<td>Ethyl hex-2-enoate</td>
<td>1037</td>
<td>1037</td>
</tr>
<tr>
<td>Ethyl octanoate (ethyl caprylate)</td>
<td>1186</td>
<td>1186</td>
</tr>
<tr>
<td>2-Methylproplyl ethanoate (isobutyl acetate)</td>
<td>771</td>
<td>753</td>
</tr>
<tr>
<td>2-Methylproplyl 2-methylpropanoate (isobutyl isovalerate)</td>
<td>909</td>
<td>910</td>
</tr>
<tr>
<td>2-Methylproplyl butanoate (isobutyl butyrate)</td>
<td>951</td>
<td>950</td>
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<tr>
<td>2-Methylproplyl 2-methylbutanoate (isobutyl 2-methylbutanoate)</td>
<td>995</td>
<td>998</td>
</tr>
<tr>
<td>2-Methylproplyl ((E)-2)-methylbut-2-enoate (isobutyl tiglate)</td>
<td>1084</td>
<td>1086</td>
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<tr>
<td>2-Methylproplyl 3-methylbutanoate (isobutyl isovalerate)</td>
<td>997</td>
<td>1002</td>
</tr>
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<td>Butyl 2-methylbutanoate</td>
<td>1035</td>
<td>1035</td>
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<tr>
<td>Butyl ((E)-2)-methylbut-2-enoate (butyl tiglate)</td>
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<td>1128</td>
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<td>Butyl 3-methylbutanoate (butyl isovalerate)</td>
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<tr>
<td>2-Methylbutyl ethanoate</td>
<td>903</td>
<td>874</td>
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<td>2-Methylbutyl 2-methylpropanoate</td>
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<td>2-Methylbutyl butanoate</td>
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</tr>
<tr>
<td>3-Methylbutyl ethanoate (isooamyl acetate)</td>
<td>873</td>
<td>871</td>
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<td>3-Methylbutyl propanoate (isooamyl propanoate)</td>
<td>964</td>
<td>964</td>
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<td>3-Methylbutyl butanoate (isooamyl butanoate)</td>
<td>1049</td>
<td>1050</td>
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<td>3-Methylbutyl 2-methylbutanoate (isooamyl 2-methylbutanoate)</td>
<td>1091</td>
<td>1091</td>
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<td>3-Methylbutyl ((E)-2)-methylbut-2-enoate (isooamyl tiglate)</td>
<td>1185</td>
<td>1253</td>
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<tr>
<td>3-Methylbutyl 3-methylbutanoate (isooamyl isovalerate)</td>
<td>1096</td>
<td>1101</td>
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<tr>
<td>Pentyl propanoate (amyl propanoate)</td>
<td>964</td>
<td>964</td>
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<tr>
<td>Pentyl 3-methylbutanoate (amyl isovalerate)</td>
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<td>1103</td>
</tr>
<tr>
<td>Octyl ethanoate</td>
<td>1199</td>
<td>1199</td>
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</tbody>
</table>

* Relative Retention index on non-polar column determined experimentally.
Table 3
Classification of the VOCs produced by the strains belonging to the genus Geotrichum, Saprochaete and teleomorphs.

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Concentration of VOCs per chemical class (µg/L)</th>
<th>Number of VOCs per chemical class</th>
<th>Number of VOCs per type of hypothetical pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Al] [Es] [Ctotal]</td>
<td>Al Es Total</td>
<td>GP MP PP BP Pep B1P B2P EP</td>
</tr>
<tr>
<td>Dipodascus albidus</td>
<td>nd nd nd</td>
<td>0 0 0</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Dipodascus armillare</td>
<td>5 5 5</td>
<td>2 0 2</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Galactomyces candidus</td>
<td>85 Nd 85</td>
<td>2 0 2</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Galactomyces citri-aurantii</td>
<td>48 7 5</td>
<td>2 1 3</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Galactomyces geotrichum</td>
<td>9 39 49</td>
<td>1 2 3</td>
<td>2 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Galactomyces pseudocandidus</td>
<td>305 37 342</td>
<td>4 7 11</td>
<td>5 1 0 1 0 0 1 11</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
<td>437 8 48</td>
<td>4 4 8</td>
<td>0 0 2 0 0 0 0 6</td>
</tr>
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<td>Geotrichum carabidurum</td>
<td>46 121 167</td>
<td>1 3 4</td>
<td>0 0 0 1 0 1 0 0</td>
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<td>Geotrichum cucujidurum</td>
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<td>2 0 2</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
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<td>Geotrichum eirene</td>
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<td>0 0 1</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Geotrichum europaeus</td>
<td>1164 165 1329</td>
<td>4 6 10</td>
<td>5 0 0 0 0 0 0 2 10</td>
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<td>Geotrichum fermentans</td>
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<td>2 2 4</td>
<td>1 0 0 0 0 0 0 0 5</td>
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<td>Geotrichum ghanaense</td>
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<td>1 0 0</td>
<td>0 0 0 0 0 0 0 0 1</td>
</tr>
<tr>
<td>Geotrichum histeriderum</td>
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<td>4 4 8</td>
<td>4 0 0 1 0 1 0 6</td>
</tr>
<tr>
<td>Geotrichum Klebahnii</td>
<td>nd nd nd</td>
<td>0 0 0</td>
<td>0 0 0 0 0 0 0 0</td>
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<td>Geotrichum marinum</td>
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<td>3 27 30</td>
<td>13 1 2 5 1 2 5 29</td>
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<td>2 0 2</td>
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<td>Geotrichum restrictum</td>
<td>244 169 414</td>
<td>3 4 7</td>
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<td>Magnusiomyces ingens</td>
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<td>Magnusiomyces magnusii</td>
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<td>5 1 6</td>
<td>1 0 0 1 0 1 1 4</td>
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<td>13 nd 13</td>
<td>1 0 0</td>
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<td>Saprochaete chiloensis</td>
<td>nd nd nd</td>
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<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Saprochaete fungicola</td>
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<td>0 0 0 0 0 0 0 1 0 0 4</td>
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<td>2 13 15</td>
<td>5 0 1 1 1 0 3 19</td>
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<td>0 0 0</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
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<td>Saprochaete psychrophila</td>
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<td>0 0 0</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
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<td>Saprochaete quercus</td>
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<td>4 3 7</td>
<td>4 0 0 0 0 0 2 1 4</td>
</tr>
<tr>
<td>Saprochaete saccharophila</td>
<td>nd nd nd</td>
<td>0 0 0</td>
<td>0 0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Saprochaete suaveoleus</td>
<td>268 3464 3732</td>
<td>4 28 32</td>
<td>12 0 2 9 1 0 5 33</td>
</tr>
</tbody>
</table>

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 (Addinsoft) (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. phurueaensis and G. ghanaense. 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 auranntii (Geotrichum citri auranntii), Gal. pseudocandidus (Geotrichum pseudocandidum), Gal. candidus (Geotrichum candidum), Magnusiomyces magnusii (Saprochaete ludwigii), M. capitatus (Saprochaete capitata), M. ovetensis (Saprochaete sericea) and Dipodascus armillariarum (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 molecular cues 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. suaveoleus, respectively). Strains S. suaveoleus, 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. Dendograms 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 ethanoate were also detected in G. candidum, S. suaveolens, G. klebahnii, G. geotrichum and M. magnusii (Buzzini et al., 2003; Damasceno et al., 2003; Farbod et al., 1987; Foster 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 of for G. klebahnii (S13) during growth in shaked flasks containing 10 g/L yeast extract, 10 g/L (NH4)2HPO4 and 20 g/L glucose (pH 5.0) at 25°C for 72 h. In our study (YEPA 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. klebahnii strains isolated by Buzzini et al. (2003) was also unable to produce VOCs. Such a difference 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 sulphur 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,
3.3. Comparison of the VOC profile of the strains by cluster and multivariate analysis and correlation with a taxonomical 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. marinus*, *S. suaveolens* (group 3) and *S. gigas* (group 4) deviated from these groups because of their ability to produce esters (Fig. 2). Among group 2, *G. europeae* 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, Raherimanimbly, et al., 2015). With a production of 15, 30 and 32 different VOCs respectively, *S. gigas*, *G. marinus* and *S. suaveolens* were by far the best producers of esters, and more specifically unsaturated esters among all strains analyzed (Table 3). Geotrichum marinus 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 Magnopisomyces 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 (Schuch et al., 2012). As we can see, the strains under study seem very closely 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 underlying 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


