

VOC Profile of endophytic fungi is altered by nature of lignocellulosic biomass feedstock

Eizadora T. Yu¹, Gary Strobel², Craig Taatjes³ and Masood Z. Hadi^{4,5}

¹Systems Biology Department, Sandia National Laboratories, Livermore, CA, 94551;

²Department of Plant Sciences, Montana State University, Bozeman, MO, 59717;

³Combustion Chemistry Department, Sandia National Laboratories, Livermore, CA, 94551;

⁴Biomass Conversion Technologies, Sandia National Laboratories, Livermore, CA, 94551;

⁵Synthetic Biology Program, Space BioSciences Division, Ames Research Center, National Aeronautics and Space Administration, Moffett Field, California, USA

AUTHOR EMAIL ADDRESS: Masood.Hadi@NASA.Gov

CORRESPONDING AUTHOR: Masood Z. Hadi

Synthetic Biology Program, Space BioSciences Division, Ames Research Center, National Space and Aeronautics Administration, Mail Stop 239-15, Bldg N239, Rm 213, Moffett Field, CA 94035 Phone: 650-604-1072

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VOC, terpene, headspace SPME-GC-MS, biomass, endophytic fungus, corn stover, eucalyptus, switchgrass

Abbreviations

VOC (volatile organic compounds), SPME (Solid Phase Microextraction), GC-MS (gas chromatography-mass spectrometry)

Abstract (100-150w)

Biofuel production from cellulosic feedstocks is a grand challenge that is being pursued on a number of fronts. One promising area is engineering of organisms that can directly convert biomass into more technologically useful forms. The technologies for next generation biofuels must not only design efficient processes to convert lignocellulosic biomass to liquid fuels but must also ensure that produced fuels are compatible with current and future engines. Endophytic fungi have been shown to produce a range of moderate-molecular weight hydrocarbons (volatile organic compounds – VOC) directly from cellulosic biomass without any pretreatment. These organisms have the capacity to alter their VOC profile as a function of carbon source.

We cultured a number of these fungi on potential biofuel biomass (corn stove, eucalyptus and switchgrass) as the sole carbon source and their growth rates were compared to potato dextrose agar. We then performed a VOC profile of the biomass-supported fungal cultures. Our findings are an important first step in when developing industrial hosts (microbes or fungi) with both biomass deconstruction and fuel synthesis pathways. Follow-up genomic and proteomics are needed to link genomics based information to function which will allow us to identify and understand response thresholds and determine how populations can be controlled for more sustainable outcomes.

Introduction

Diminishing fossil fuels reserves and global climate change are the catalyst for research and development into fuel alternatives derived from renewable resources. A promising strategy for biofuel production involves the direct conversion of lignocellulose from energy crops or agricultural wastes by microbial biotechnology to value-added fuels. Microorganisms that possess necessary pathways for cellulose degradation and biofuel production are continuously being discovered and optimized through synthetic biology (1-4). In recent years, novel endophytic fungi isolated from rainforests have been demonstrated to produce metabolites touted to be compatible with existing fuels (5-10). Several of these fungal isolates produce short- to mid-chain aliphatic hydrocarbons, alcohols, and isoprenoids, which could be potential replacements for diesel fuels or fuel additives(11). Furthermore, because of their ecologically unique lifestyle (e.g., efficient plant colonization), endophytes possess the necessary chemi-enzymatic machinery to breakdown and utilize lignocellulosic biomass in situ. Therefore, these endophytes are attractive bioprospecting candidates as they not only encode lignocellulose deconstruction pathways but also hydrocarbon/isoprenoid biosynthesis pathways- both of which are highly desirable in bioenergy applications.

Hydrocarbon production by *Hypoxylon sp.* and *Daldinia sp.* isolates have only been demonstrated when the fungi is cultured on standard laboratory media (e.g. potato dextrose, oatmeal, and cellulose)(8, 10), which are much simpler carbohydrates compared to lignocellulosic plant biomass. However it is not well understood how hydrocarbon production will be affected by the nature of biomass feedstock available to the fungi. The aim of this study is to assess the effect of lignocellulosic biomass feedstocks on VOC production by candidate fungal isolates.

Material and Methods

Chemicals and Standards

2-Phenylethanol (>99%), 1-Pentanol(>99.8%), 2-ethyl-1-hexanol (>99/5%), Cyclohexyl isothiocyanate (98%); 1-methyl-1,4-cyclohexadiene(>96%), 3-pentanone(>99%), 2,6-Di-tert-butyl-1,4-benzoquinone(98%), and all terpene and alkane standard solution (mixture of C8-C20 in hexane) are the highest grade available from Sigma-Aldrich (Saint Louis, MI). Potato dextrose and M9 media were purchased from Difco Laboratories.

Organisms and Culture Conditions

This study is focused on several endophytic fungi isolates (listed in Table 1) obtained from the Mycological Collection of the Department of Plant Sciences at Montana State University. Fungal cultures were grown on potato dextrose agar (PDA, 39g/L) at room temperature and passaged every 7 days. For investigating fungal growth in biomass feedstock (BF), fungal isolates were grown in agar plates containing M9 media supplemented with 2mM MgSO₄, 0.1mM CaCl₂, trace elements, and 0.25-0.5% feedstock (corn stover, CRN; switchgrass, SWG; or arbog eucalyptus, EUC) milled using a Thomas-Wiley Mini Mill with a 40-mesh screen (Model 3383-L10, Arthur H. Thomas Co., Philadelphia, PA) and sterilized via gamma irradiation. PDA and BF containing plates were inoculated with 5 mm plugs (placed in the center of plate) containing fungal mycelium and allowed to grow at 20°C for several weeks. Growth measurements were taken from four replicate plates for PDA and each feedstock biomass (at 0.25% and 0.5% w/v biomass concentration). Radial growth measurements were taken every 3 days, marking four points along the circumference of fungal mycelia, until growth reached the edge of the plate. An average growth was determined by taking 16 points from four individual plates. VOC analysis, fungi were grown in 60 mL of potato dextrose (PD) broth or feedstock-supplemented M9 media (as described above) in 250 ml culture flasks sealed with screw caps and incubated at 20°C on orbital platform shakers (~100 rpm) for time periods of up to 25 days.

VOC Extraction and GC-MS analysis

The volatiles were extracted from the culture headspace after 7 days (for PD cultures) or 15 days (for BF cultures). Briefly, a small hole was drilled in the cap for insertion of a Solid Phase Micro Extraction (SPME) fiber holder (Supelco) containing a pre-conditioned Stableflex SPME fiber with 50/30 μm DVB/CAR/PDMS coating. Fiber was exposed to headspace for 5 minutes (up to 15 minutes for the BF cultures) and analyzed immediately. The volatiles bound to the fiber were desorbed for 3 minutes in a split-/splitless injector (splitless mode, 250°C) of a Varian 3800 gas chromatograph coupled with a Varian Saturn 2000 ion trap mass spectrometer. The column was a DB5-HT (30m x .25 mm x 0.25 μm). Helium was used as carrier gas at constant flow rate 1 ml/min. The oven program was 30°C (hold 3 min), 5°C/min to 220°C, 10°C/min to 250°C (hold 5 min). The MSD parameters were EI at 70eV, mass range was 30-500 Da, and the scan speed was 2 scans/sec. Headspace SPME-GC-MS was performed on at least three replicate cultures for each fungi-feedstock combination.

MS Data Analysis

GC-MS data deconvolution was performed using the Automated Mass Spectral Deconvolution and Identification System (AMDIS) spectral deconvolution software package (v. 2.70, NIST Gaithersburg)(12). AMDIS deconvolution settings were as follows: resolution (medium), sensitivity (low), shape requirement (medium), and component width at 10. Spectral components were searched against the NIST 2011 mass spectral library, and only components with mass spectra match factors > 70% were reported as tentatively identified compounds. Compounds with peak areas > 1% of the total peak area in the chromatogram are reported. Sørensen similarity indices (based on the presence/absence of VOC in culture headspace) was measured as a means to estimate the differences among samples from different fungal species and carbon/feedstock source (13).

Results and Discussion

Cultivation of fungi in minimally-treated biomass feedstocks

Biomass pretreatment is essential in order to disrupt the bonding network between cellulose, hemi-cellulose and lignin thereby increasing hydrolysis rates and eventual sugar yields. Current methods relying on thermochemical processes although efficient, tend to be very harsh and expensive. Alternative pretreatment methods are being explored to reduce cost and environmental impact (14-15). In nature, these endophytes obtain nutrition from the host plant they colonize, and thus, should have the necessary biochemical machinery to obtain sugars directly from lignocellulosic biomass. Initially, we wanted to determine whether the fungal isolates can make use of minimally-treated lignocellulosic biomass to support their growth. We followed the growth of two *Hypoxylon* sp. and two *Daldinia* sp. (Table 1) on M9 agar plates supplied with milled corn stover, switchgrass or arbog eucalyptus as the sole carbon source. All three biomass feedstock supported the growth of all four isolates, albeit at slower growth rates and reached less fungal biomass density as compared to control PDA plates (Figure 1). We observed noticeable differences in the organism's ability to propagate in the feedstock media. The Hypoxylons (CI-4A, CO27-A) and EC-38 mycelium exhibited the fastest growth in corn stover. CI-4A and CO27-A growth was slowest on switchgrass while growth was delayed the most for eucalyptus in EC-38. On the other hand, *D. eschscholzii* (EC-12) did not have any feedstock preference and achieved comparable growth rates. Similar growth trends are observed when fungus was cultivated in liquid cultures. Slowest growth was expected for eucalyptus-fed fungal cultures since it is the more recalcitrant plant material (29% lignin), however, we only observed this for EC-38. FT-IR analyses on the feedstocks used in this study show that corn stover and switchgrass have comparable lignin and cellulose content (~18% lignin, ~38% cellulose), however, growth of Hypoxylon isolates was favored in corn stover. Further examination of this feedstock preference of the fungal isolates is in progress.

VOC profiles of fungus grown in PD and biomass feedstock

Previous reports show prolific VOC production by endophytic fungi when grown in potato dextrose media (8, 10). These reports were obtained from headspace SPME-GC-MS analysis of fungal cultures cultivated in agar plates or tube slants. Growing the fungi in the BF tube slants was not feasible, as the feedstock sunk to the bottom of the culture tubes and was inaccessible to the fungi plug on top of the agar slants. For this study, we switched to liquid cultures to obtain higher fungal biomass densities and VOC production. We performed headspace SPME-GC-MS on all four fungal cultures in PD broth (positive control) and on uninoculated BF media (negative control) prior to profiling VOC in headspace of BF-fed fungal cultures. When cultivated in potato dextrose media, all four fungi produced primarily terpene compounds, with CI-4A generating monoterpenoids (C10 terpenes) and the CO-27A and Daldinia isolates producing sesquiterpenoids (C15 terpenes) (Figure 2; list of compounds in Table 2, and Supplementary Table 1-3). Significant alcohol production in PD-fed EC-12 was also observed (Figure 2), consistent with earlier reports.

However, growing the fungi in feedstocks significantly changed the VOC profile for all four isolates. Calculated Sørensen similarity indices show ~ 40% similarity between the total VOCs produced in PD and the other BF media (Figure 3). VOC profiles from BF-fed fungal cultures were comparable (similarity indices of 60-74%) between the different fungi. We also performed comparisons of the VOC profile in terms of compound functional groups (e.g. terpenes, ketones, aromatics, alcohols, etc), to look at the changes in more detail. The similarity between VOCs from BF-fed fungi can be attributed primarily to the terpenoid compounds. In fact, the terpenoid metabolite production of EC38 and CO-27A do not appear to be affected much by the nature of BF media, as indicated by their Sorensen indices (>73% similarity; Figure 4). Ketones in the VOC of BF-fed EC38 and BF-fed CO27A cluster together and are distinct from the ketone profile of BF-fed EC12 and BF-fed C14A (Figure 4). Notably, C14A VOC profile was affected the most by changing feedstocks-just by switching from potato dextrose to BF media (24% similarity), and even between biomass feedstock (38% similarity). Not only did we observe differences

in the types of terpenes detected in the headspace, but maybe more importantly, we observed a decrease in the percentage of terpenes in the headspace of the fungal cultures (Figure 2). For example, in PD-fed CI-4A cultures, we consistently detected 1,8-cineole and cyclic ketones as predominant peaks in the chromatogram. The 1,8-cineole peak is dramatically decreased in CRN- and EUC-fed C1-4A cultures. In fact, most of the monoterpenes detected in PD-fed CI-4A cultures was not observed at all in any of the BF-fed C1-4A cultures, and there appeared to be a shift to limited sesquiterpene production.

We also detected compounds in the headspace of BF-fed cultures that were not originally observed (or is a minor component, < 1% of total peak area) in the PD-fed cultures. In particular, methoxy-phenyl oxime and isothiocyanate and thiol compounds, which were minimal components in PD-fed cultures, were significant constituents in the headspace of BF-fed cultures. The biological significance of these compounds for the fungi is unknown, but may have a connection with the fact that these compounds are effective oxygen and radical scavengers, and have been demonstrated in other systems to be potent against fungal pathogens (16-17). Ketones were detected in CRN- and SWG-fed isolates, and were particularly abundant in SWG-fed CO27A (Table 2). Elevated levels of 3,7-dimethyl-1,6-octadien-3-ol or (aka linalool), was detected in the headspace of all switchgrass-fed cultures(18). There was also a prevalence of aromatic compounds, mostly benzene derivatives (Figure 2) in BF-fed CO27 cultures. However, it is more likely that these benzene derivatives are not produced by the fungi but rather, possible by-products of lignin degradation (19).

Conclusions

Consolidating lignocellulosic processing and fuel synthesis capabilities in a single microbe is an ideal platform for biofuel production. *Hypoxylon* and *Daldinia* endophytes have demonstrated the ability to produce hydrocarbons when cultivated in prototypical media. In this study, we have shown that while the endophytes can be cultivated on lignocellulosic biomass, it also altered and diminished their capacity

to produce hydrocarbons. This effect is most significant in *Hypoxylon* C14A, where 1,8-cineole production was significantly reduced in BF-fed cultures. Further research is needed (e.g. metabolic engineering) to optimize growth in lignocellulosic biomass and modulate VOC production in these endophytic fungi. These findings are important when developing industrial hosts (microbes or fungi) with both biomass deconstruction and fuel synthesis pathways.

Acknowledgement

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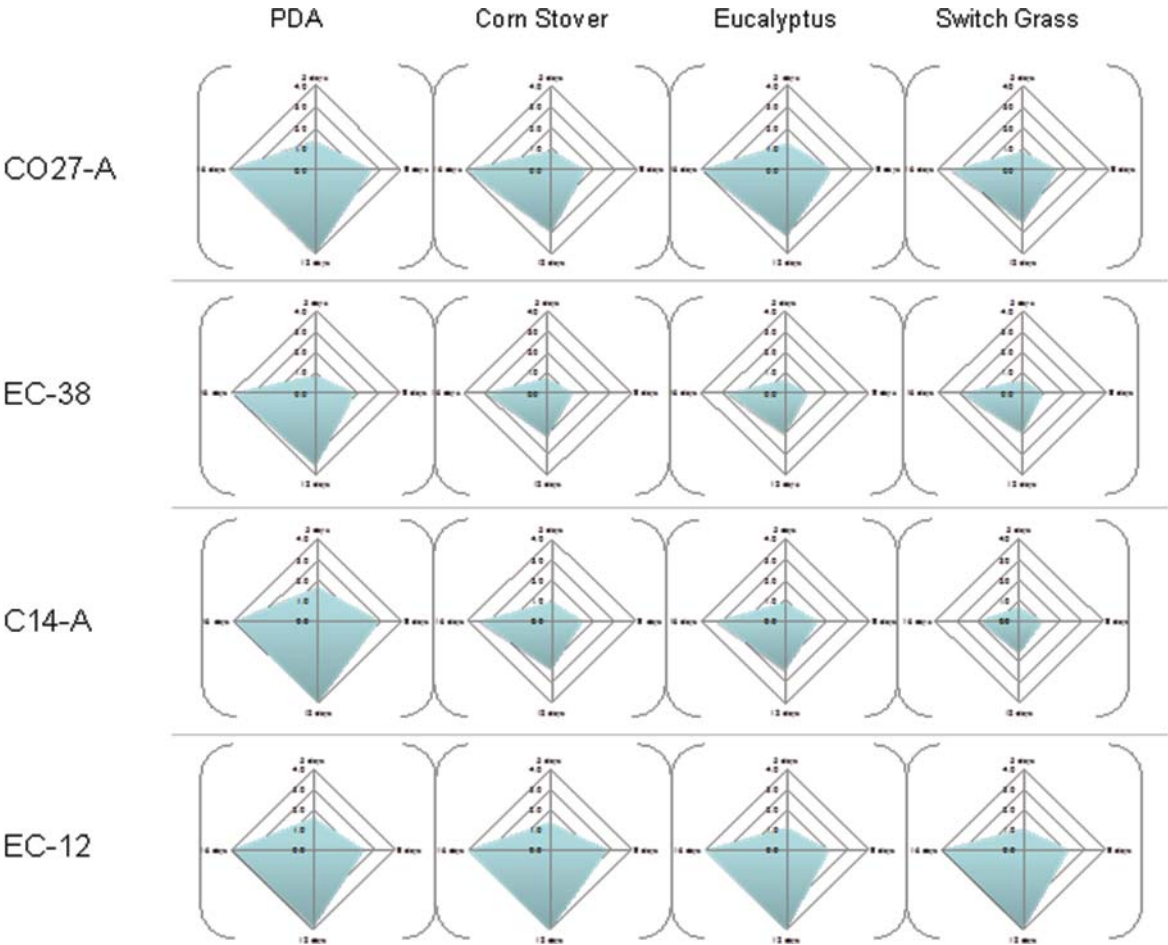
Table 1. List of endophytic fungal isolates in this study.

Fungal isolate	Genus*	Host Plant
CI-4A**	Hypoxylon	Persea indica
CO27-A	Hypoxylon	Rhizophora sp.
EC-12**	Daldinia	Myroxyton balsamum
EC-38	Daldinia/Nodulisporium	Neea floribunda

* The taxonomic/phylogenetic classification of fungal isolates was performed by ITS sequence comparisons against reference database.

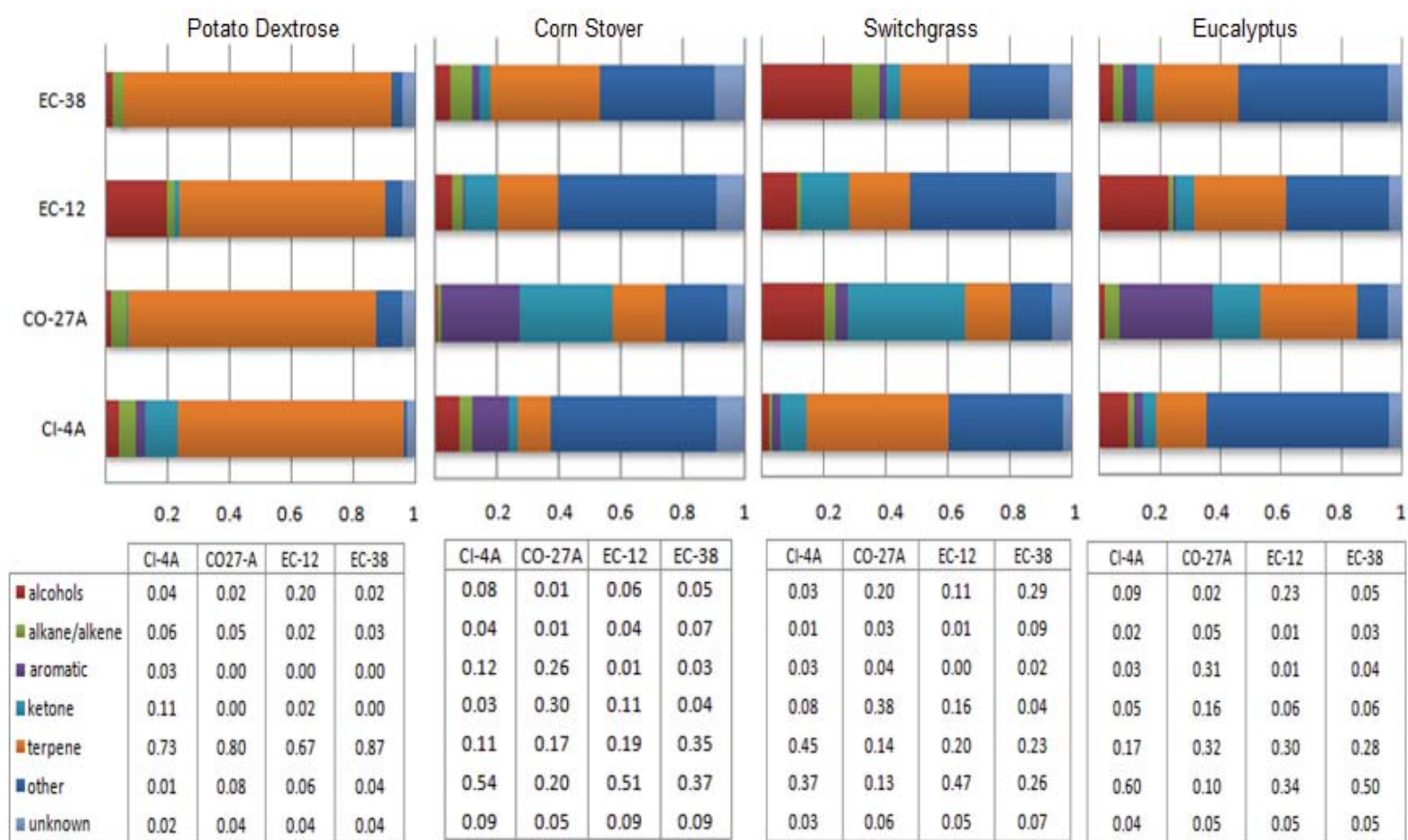
** Detailed bioactivity and GC-MS analysis of the volatile metabolites for CI-4A and EC-12 have been previously described(8, 10).

Figure 1. Radial growth measurements of four fungi on various carbon sources.



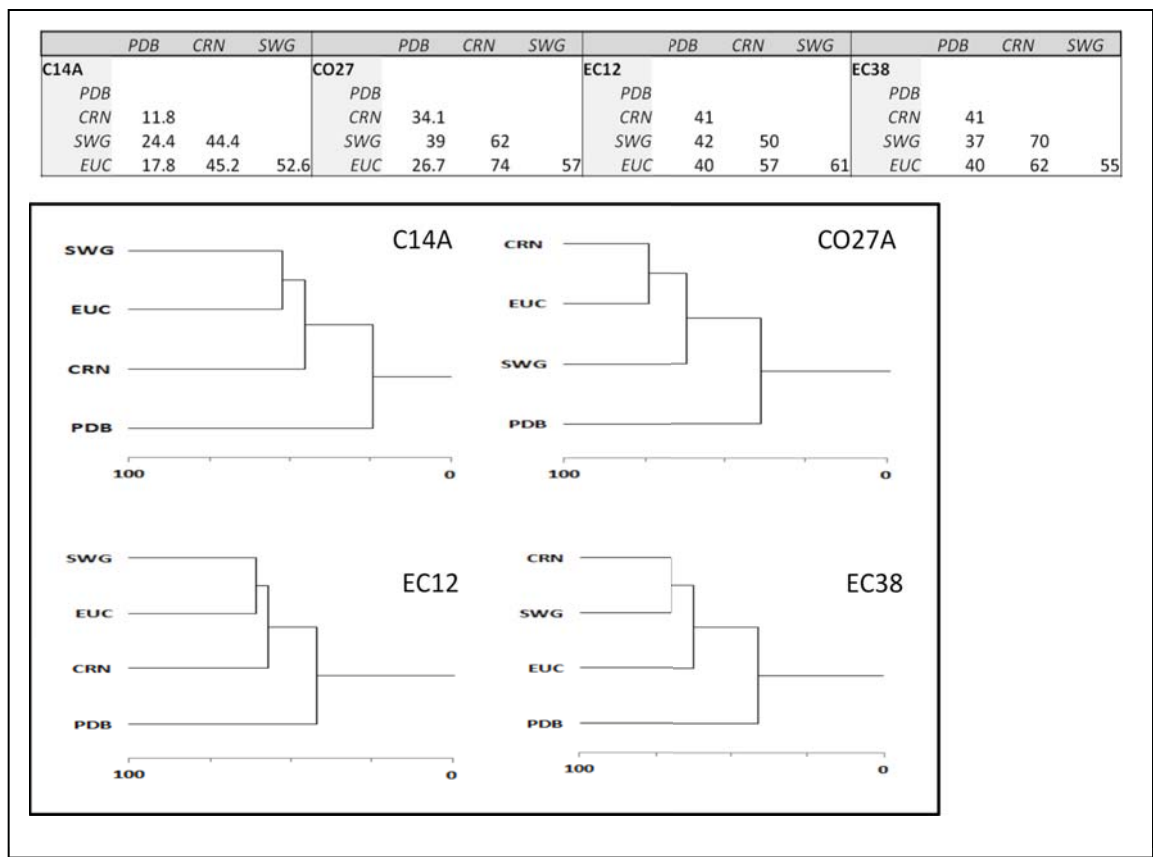
Growth measurements were taken every 3 days until growth reached the edge of plate. Data shown is average from 4 replicate plates for each feedstock.

Figure 2. VOC profile of endophytic fungi cultivated on various carbon sources.



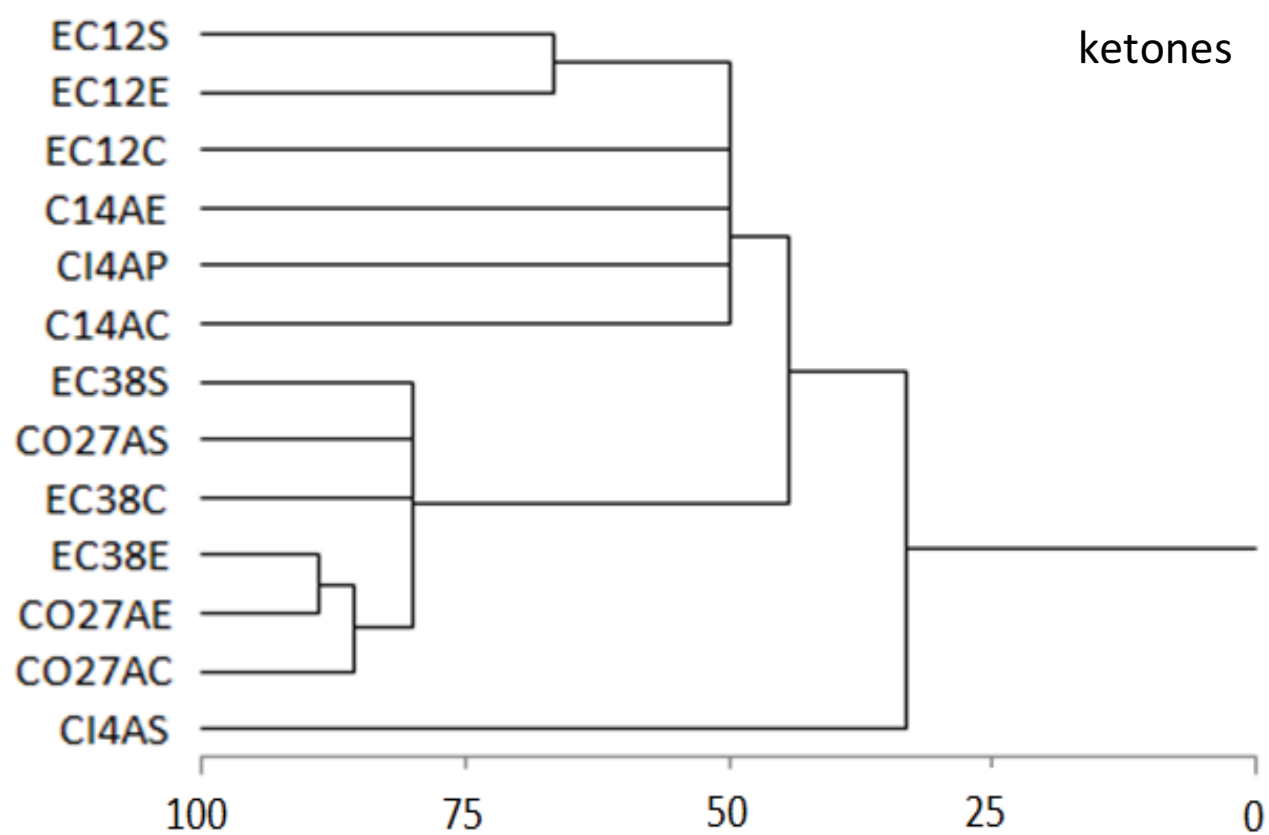
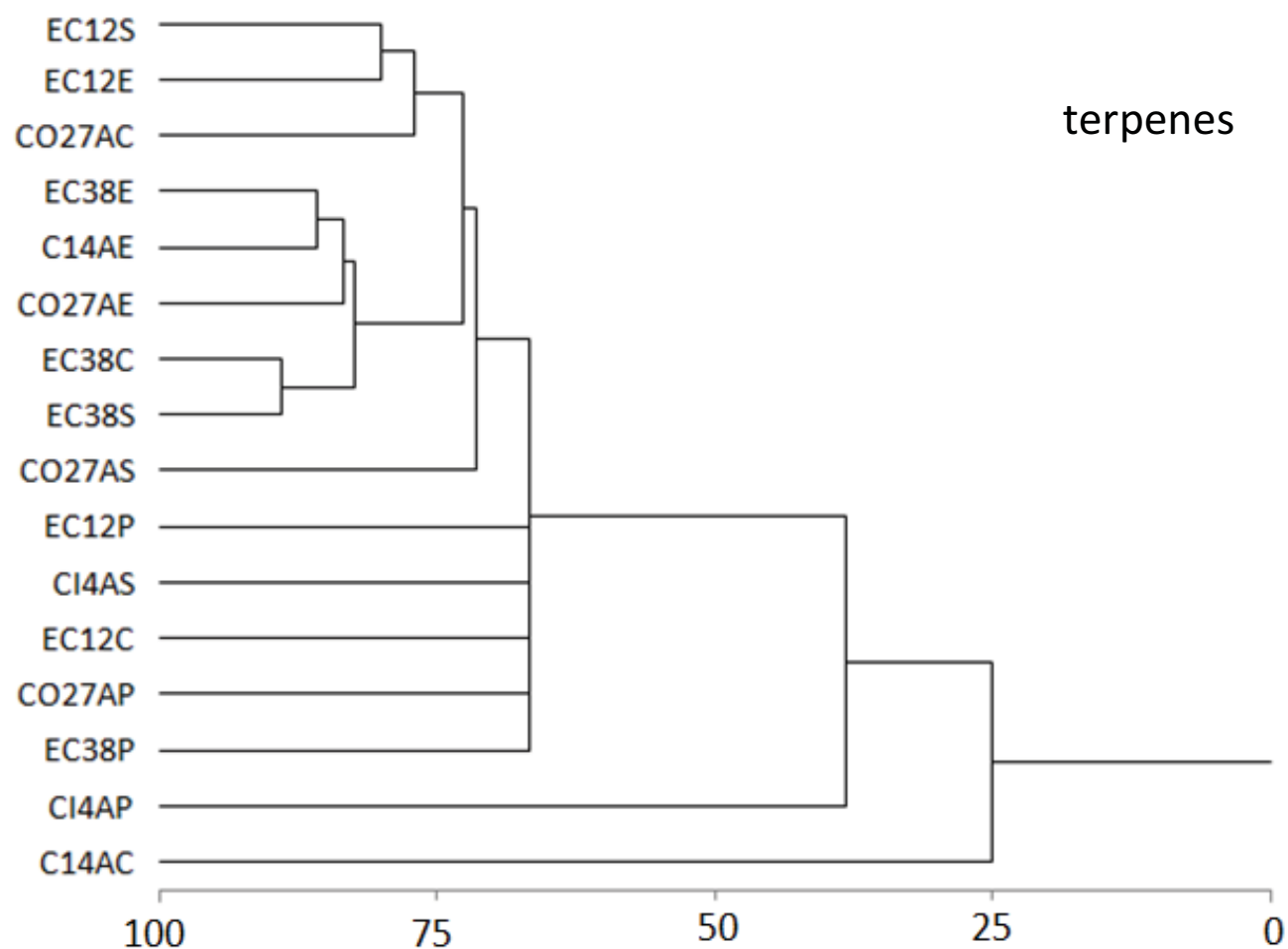
Compounds in the VOC profile are classified based on functional groups (see color legend) and relative abundances are reported as the sum of peak areas for each class of compounds vs total area of the peaks in chromatogram. Data shown is representative of at least three, independent

Figure 3. Similarity matrix indices of the VOC profile of four fungi on various carbon sources.



Values are reported as percentage Sørensen similarity indices (top). Dendrogram showing the clustering based on detection of individual compounds in fungal headspace by SPME-GC-MS (bottom).

Figure 4. Comparison of terpene and ketone compounds in the headspace VOCs of fungal cultures



Dendogram was constructed using the nearest neighbor clustering method based on Sorensen similarity indices of the four fungi (C14A, CO27A, EC12 and EC38) cultivated on potato dextrose (P), corn stover (C), switchgrass (S) and eucalyptus (E).

Table 2. Compounds identified in the headspace of CO-27A cultures in PD and biomass feedstocks.

Ret. time (min)	Tentative Compound	Molecular Formula	Match*	% Area PDB	% Area CRN	% Area SWG	% Area EUC
28.4	Guaia-1(10),11-diene	C15H24	87	31.5	2.9	2.6	7.7
27.9	Caryophyllene	C15H24	83	13.8	nd	<1	nd
32.0	γ -Gurjunene	C15H24	86	12.6	5	4.8	16.7
10.6	<i>Oxime-, methoxy-phenyl-</i>	<i>C8H9NO2</i>	85	7.3	1.5	1.6	2.6
25.5	β -Elemene	C15H24	87	5.9	4.8	4	1.6
28.1	Chamigrene	C15H24	87	4.8	1.5	1.3	1.8
15.0	Eucalyptol	C10H18O	88	4.8	nd	nd	nd
26.7	α -Guaiene	C15H24	88	2.4	2.2	2.4	1.1
28.6	Aromandendrene	C15H24	75	2	nd	nd	nd
17.6	Phenylethyl Alcohol	C8H10O	77	1.6	nd	nd	nd
28.9	Calamenene	C15H22	75	1.3	<1	<1	nd
11.2	Anisole	C7H8O	78	1.3	nd	nd	nd
28.2	δ -Selinene	C15H24	76	1.2	nd	nd	nd
21.3	<i>Cyclohexane, isothiocyanato-</i>	<i>C7H11NS</i>	75	<1	1.7	<1	10.2
24.0	Benzene, 3-cyclohexen-1-yl-	C12H14	75	<1	nd	nd	<1
27.6	β -Cadinene	C15H24	83	1	<1	nd	nd
28.7	δ -Cadinene	C15H24	81	<1	1	<1	nd
15.1	α -Pinene	C10H16	76	nd	nd	3.4	nd
27.3	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	77	nd	nd	nd	3
18.2	2-Heptenal, 2-propyl	C10H18O	75	nd	5.1	-	4.8
17.6	Benzene, 1-ethyl-4-methoxy-	C9H12O	88	nd	9.2	nd	1.4
16.8	3-Heptanone, 5-ethyl-4-methyl-	C10H20O	82	nd	13.3	29.5	2.6
16.5	3-Pentanone	C5H12O	75	nd	2.9	10.1	7.4
18.9	Benzene, 1-ethenyl-4-methoxy-	C9H10O	85	nd	27.1	6.6	nd
21.1	Benzothiazole	C7H5NS	84	nd	1.3	<1	2.2
14.7	Benzene, 1-methoxy-4-methyl-	C8H10O	80	nd	2.2	nd	2
16.2	Ethanone, 2,2-dihydroxy-1-phenyl-	C8H8O3	75	nd	1	nd	<1
17.4	2,5-Dimethylanisole	C9H12O	85	nd	3.9	nd	4.2
21.5	Benzene, 1-(1,1-dimethylethyl)-4-methoxy-	C11H16O	83	nd	1	nd	<1
18.6	Benzene, 1,2-dimethoxy-	C8H10O2	77	nd	<1	<1	5.2
17.2	1,6-Octadien-3-ol, 3,7-dimethyl-	C10H18O	84	nd	nd	15	nd
10.4	Cyclohexanone	C6H10O	78	nd	nd	nd	1.5
19.5	o-Isopropylanisole	C10H14O	84	nd	nd	nd	1
23.6	Benzene, 4-ethyl-1,2-dimethoxy-	C10H14O2	89	nd	nd	nd	1.1
25.8	Methyleugenol	C11H14O2	89	nd	nd	nd	1.3
33.2	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	C18H20	77	nd	nd	nd	2.4

*VOC profiles from potato dextrose broth (PDB), corn stover (CRN), switchgrass (SWG) and arbog eucalyptus (EUC). % Area is taken as AUC (area under curve) of peak of interest versus total AUC for entire chromatogram. Some compounds are not detected (nd) for certain conditions. Compounds are observed in some uninoculated media (italicized).

Supplementary Table 1. List of tentative compounds identified in the headspace of CI-4A cultures cultivated with PD and biomass feedstocks.

Ret. time (min)	Tentative Compound	Molecular Formula	Match*	% Area PDB	% Area a CRN	% Area SWG	% Area EUC
15.2	Eucalyptol	C10H18O	84	47.4	2.7	14.6	7.6
24.0	2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)-	C10H14O	76	19.2	4	<1	nd
15.5	Cyclohexane, 1,2,4-tris(methylene)-	C9H12	84	3.2	-	nd	<1
13.6	β -pinene	C10H16O	83	3.2	nd	nd	nd
13.3	1,3,6-Cyclooctatriene	C8H10	81	3	nd	nd	nd
17.7	Phenylethyl Alcohol	C8H10O	84	2.2	nd	nd	nd
15.9	γ -Terpinene	C10H16	85	1.8	nd	nd	nd
22.8	Umbellulone	C10H14O	75	1.8	nd	nd	nd
17.8	1-Decen-3-yne	C10H16	75	1.7	nd	nd	nd
11.4	β -Thujene	C10H16	79	1.4	nd	nd	nd
15.8	Cyclohexene, 1-(1-propynyl)-	C9H12	80	1	nd	nd	nd
20.1	α -Terpineol	C10H18O	84	1	nd	<1	nd
21.3	<i>Cyclohexane, isothiocyanato-</i>	<i>C7H11NS</i>	<i>80</i>	<1	32.4	17.9	54.6
28.1	Chamigrene	C15H24	81	<1	1	1.2	1
28.4	Guaia-1(10),11-diene	C15H24	85	<1	3	3.9	3.9
32.0	γ -Gurjunene	C15H24	75	<1	2	10.1	7.4
10.7	<i>Oxime-, methoxy-phenyl-</i>	<i>C8H9NO2</i>	<i>81</i>	nd	17.2	1.7	10.9
17.6	Benzene, 1-ethyl-4-methoxy-	C9H12O	91	nd	8.2	nd	nd
14.9	1-Hexanol, 2-ethyl-	C8H18O	84	nd	7.4	nd	<1
18.9	Benzene, 1-ethenyl-4-methoxy-	C9H10O	90	nd	1.9	1.7	nd
21.1	Benzothiazole	C7H5NS	90	nd	2.2	<1	1.3
5.7	2-Pentenal, 2-methyl-	C6H10O	75	nd	2	17.9	nd
26.0	Valencene	C15H24	75	nd	1	nd	nd
13.5	3-Octanone	C8H16O	85	nd	nd	6.5	2.8
17.2	1,6-Octadien-3-ol, 3,7-dimethyl-	C10H18O	83	nd	nd	3	nd
25.5	β -Elemenene	C15H24	75	nd	nd	2.6	2.2
26.7	α -Guaiene	C15H24	89	nd	nd	1	1
24.3	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.0.1(1,7)]decan-5-one	C13H20O2	80	nd	nd	2	1.4
18.6	Benzene, 1,2-dimethoxy-	C8H10O2	91	nd	nd	nd	1.1

*VOC profiles from potato dextrose broth (PD), corn stover (CRN), switchgrass (SWG) and arbog eucalyptus (EUC). % Area is taken as AUC (area under curve) of peak of interest versus total AUC for entire chromatogram. Some compounds are not detected (nd) for certain conditions. Compounds are observed in some uninoculated media (italicized).

Supplementary Table 2. List of tentative compounds identified in the headspace of EC-12 cultures cultivated with PD and biomass feedstocks.

Ret. time (min)	Tentative Compound	Molecular Formula	Match*	% Area PD	% Area CRN	% Area SWG	% Area EUC
5.4	1-Butanol, 3-methyl- or 1-Pentanol	C5H12O	80	23.5	nd	nd	nd
10.6	<i>Oxime-, methoxy-phenyl-</i>	<i>C8H9NO2</i>	84	4.7	30.2	<1	2.7
17.6	Phenylethyl Alcohol	C8H10O	90	12	nd	nd	nd
25.3	Alloaromadendrene	C15H24	78	0.9	nd	1	nd
25.5	β -Elemene	C15H24	86	8.8	nd	11.1	11.4
26.3	Caryophyllene	C15H24	84	0.7	-	1	<1
26.7	α -Guaiene	C15H24	88	4.1	nd	4.4	5.9
27.7	2H-1-Benzopyran-2-one, 4,7-dihydroxy-	C9H6O4	75	3.8	nd	nd	nd
28.1	Valencene	C15H24	88	2.9	3	nd	3.6
28.2	Chamigrene	C15H24	83	1.5	-	nd	nd
28.3	Guaia-1(10),11-diene	C15H24	86	15.7	nd	10.1	15
32.0	γ -Gurjunene	C15H24	83	15.5	nd	22.3	18.8
19.3	6-Butyl-1,4-cycloheptadiene	C11H18	82	nd	3.4	nd	nd
23.9	γ -Elemene	C15H24	78	nd	1	nd	nd
28.4	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	87	nd	5.1	nd	nd
28.8	trans-calamenene	C15H22	70	nd	2.9	nd	nd
28.7	β -Cadinene	C15H24	76	nd	2.9	nd	nd
25.1	α -Copaene	C15H24	75	nd	2.9	nd	nd
27.3	Aromandendrene	C15H24	77	nd	2.7	<1	nd
25.3	Eremophilene	C15H24	75	nd	1	nd	nd
26.2	4a(2H)-Naphthalenol, octahydro-4,8a-dimethyl-	C12H22O	75	nd	1.6	nd	nd
18.8	2,4,6-Cycloheptatrien-1-one	C7H6O	75	nd	1	nd	nd
14.9	1-Hexanol, 2-ethyl-	C8H18O	82	nd	nd	2.6	1
21.3	Cyclohexane, isothiocyanato-	C7H11NS	78	nd	nd	3.7	19.4
21.9	2(5H)-Furanone, 4-methyl-5,5-bis(2-methyl-2-propenyl)-	C13H18O2	78	nd	nd	7.9	7
17.2	1,6-Octadien-3-ol, 3,7-dimethyl-	C10H18O	80	nd	nd	16.4	nd
13.5	3-Heptanone, 6-methyl	C8H16O	58	nd	nd	7	nd
28.1	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	C15H24	88	nd	nd	3.6	2.2
27.3	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	C14H20O2	83	nd	nd	1	1.3
21.1	Benzothiazole	C7H5NS	94	nd	nd	nd	1
19.9	Creosol	C8H10O2	70	nd	nd	nd	1
13.3	Phenol	C6H6O	83	nd	nd	nd	2.2

*VOC profiles from potato dextrose broth (PD), corn stover (CRN), switchgrass (SWG) and arbog eucalyptus (EUC). % Area is taken as AUC (area under curve) of peak of interest versus total AUC for entire chromatogram. Some compounds are not detected (nd) for certain conditions. Compounds are observed in some uninoculated media (italicized).

Supplementary Table 3. List of tentative compounds identified in the headspace of EC-38 cultures cultivated with PD and biomass feedstocks.

Ret. time (min)	Tentative Compound	Molecular Formula	Match*	% Area PD	% Area CRN	% Area SWG	% Area EUC
28.4	Guaia-1(10),11-diene	C15H24	86	35	7.7	5.3	9.7
32.1	γ -Gurjunene	C15H24	83	13.3	22.2	10.3	nd
15.0	Eucalyptol	C10H18O	86	11.6	2.6	nd	<1
28.1	Chamigrene	C15H24	82	9.2	3	1.6	2.8
25.5	β -Elemene	C15H24	87	4.2	7.2	2.4	2.7
28.3	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	C15H24	75	3.5	nd	nd	nd
26.7	α -Guaiene	C15H24	89	3	2.7	3.2	2
5.4	1-Pentanol or 1-Butanol, 3-methyl-	C5H12O	75	2.7	nd	nd	nd
14.9	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	C10H16	78	2.1	nd	nd	nd
13.6	β -Pinene	C10H16	86	1.8	nd	nd	nd
17.6	Phenylethyl Alcohol	C8H10O	92	1	nd	nd	nd
10.6	<i>Oxime-, methoxy-phenyl-</i>	<i>C8H9NO2</i>	83	1	19.2	11.6	1
26.9	Himachala-2,4-diene	C15H24	79	1	nd	nd	nd
28.8	Calamenene	C15H22	75	<1	1	<1	1.9
28.7	β -Cadinene	C15H24	86	<1	1.3	<1	1.7
21.3	Cyclohexane, isothiocyanato-	C7H11NS	85	nd	1	<1	7.2
14.9	<i>1-Hexanol, 2-ethyl-</i>	<i>C8H18O</i>	75	nd	3.6	2.5	nd
16.5	3-Pentanone	C5H10O	89	nd	2.9	2.9	<1
16.8	3-Heptanone, 5-ethyl-4-methyl-	C9H18O	91	nd	3	4.4	2.5
18.6	Benzene, 1,2-dimethoxy-	C8H10O2	75	nd	<1	nd	4
18.9	Benzene, 1-ethenyl-4-methoxy-	C9H10O	86	nd	2.1	1.4	nd
6.2	1,3,5-Cycloheptatriene	C7H8	85	nd	2	<i>nd</i>	nd
17.2	1,6-Octadien-3-ol, 3,7-dimethyl-	C10H18O	87	nd	nd	31.2	nd
33.1	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	C18H20	75	nd	nd	nd	1
25.8	Methyleugenol	C11H14O2	84	nd	nd	nd	1
23.6	Benzene, 4-ethyl-1,2-dimethoxy-	C10H14O2	89	nd	nd	nd	1.2
21.1	Benzothiazole	C7H5NS	93	nd	nd	nd	1.4
18.5	Cyclohexanol, 1-methyl-4-(1-methylethyl)-, trans-	C10H20O	78	nd	nd	nd	4
16.2	Ethanone, 2,2-dihydroxy-1-phenyl-	C8H8O3	75	nd	nd	nd	1.2
13.3	Phenol	C6H6O	80	nd	nd	nd	7.8

*VOC profiles from potato dextrose broth (PD), corn stover (CRN), switchgrass (SWG) and arbog eucalyptus (EUC). % Area is taken as AUC (area under curve) of peak of interest versus total AUC for entire chromatogram. Some compounds are not detected (nd) for certain conditions. Compounds are observed in some uninoculated media (*italicized*).