ХИМИЯ РАСТИТЕЛЬНОГО СЫРЬЯ. 2024. №3. С. 273–279.
KHIMIYA RASTITEL'NOGO SYR'YA, 2024, no. 3, pp. 273–279.
DOI: 10.14258/jcprm.20240312536

UDC 630.844.2

IDENTIFICATION OF MARKERS FOR MYCELIA OF WHITE-ROT AND BROWN-ROT FUNGI BY PY-GC-MS AND VOC ANALYSIS

© ***O.A. Shapchenkova*, A.A. Aniskina, N.V. Pashenova, S.R. Loskutov***

V.N. Sukachev Institute of Forest SB RAS – separate subdivision of the Federal Research Center «Krasnoyarsk Science Center SB RAS», Akademgorodok, 50/28, Krasnoyarsk, 660036, Russia, shapchenkova@mail.ru

Pathogenic fungi, along with fires and insect pests, are among the most important factors affecting coniferous forests in Siberia. Conifers are attacked by fungi that cause stains and decay of wood. The decrease in quality and timber loss due to stain and decay caused by fungi can be significant. The early detection of fungi in wood allows taking preventive measures to reduce the potential threats caused by fungi in the forests. The mycelia of brown-rot (*Fomitopsis betulina, Phaeolus schweinitzii*) and white-rot (*Trametes versicolor*, *Phellinus chrysoloma*) fungi and mycelia extracts were studied using methods of pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) and GC/MS, respectively, to find out whether the chemical compounds can be useful as fungal markers. Py-GC/MS of mycelia showed pyrolysis products of glucans, chitin, chitosan, proteins, and lipids. Volatile organic compounds (VOCs) of mycelia extracts were represented by 14–28 individual volatile compounds: *Phaeolus schweinitzii> Phellinus chrysoloma> Fomitopsis betulina> Trametes versicolor*. The presence of N-bearing compounds, 1-octene, 1-decanol among pyrolysis products of wood and some fungal VOCs emitted from wood may be indicative of fungi. The usefulness of the markers detected needs to be further confirmed by examining wood infected by these fungi.

Keywords: fungi, mycelia, Py-GC/MS, GC/MS, pyrolysates, VOCs.

For citing: Shapchenkova O.A., Aniskina A.A., Pashenova N.V., Loskutov S.R. *Khimiya Rastitel'nogo Syr'ya*, 2024, no. 3, pp. 273–279. (in Russ.). DOI: 10.14258/jcprm.20240312536.

Introduction

Coniferous forests in Siberia are of great ecological and economic importance. At the same time, conifers are significantly affected by fungal pathogens. Fungal diseases cause massive decline and death of stands and significant damage to timber. Losses of merchantable volume may reach up to 75%. Weakening and decline of infected trees usually results in bark beetle and wood-borer infestations [1]. Therefore, detection of fungal pathogens at early stage of wood damage allows taking preventive measures to reduce the potential threats caused by fungi in the forests.

Fungi of Basidiomycota are largely responsible for degradation of wood in the forests. Wood-decaying *Basidiomycota* fungi of the systematic class *Agaricomycetes* have adopted unique strategies for decomposition of carbohydrates (hemicelluloses, cellulose) and lignin. White rot fungi are able to degrade all wood components by expression of carbohydrate active enzymes and various oxidoreductases. *Agaricomycetes* brown rot fungi can decompose hemicelluloses and cellulose but cause only minor structural changes in lignin [2].

Upon fungal colonization of wood by hyphal extension, decomposition of carbohydrates and lignin may result in release of volatile organic compounds (VOCs) from wood or generated as fungal metabolites [2]. Fungi produce over 300 of volatile organic compounds comprising aliphatic and aromatic hydrocarbons, acids, esters, alcohols, ketones, aldehydes, phenols, thiols, which play role in the initiation and regulation of fungal interactions [3]. The specific profile produced by each species or microbial consortium is influenced by environmental factors [4]. Several studies showed that fungal VOCs can be used as markers for the presence of fungi [5–7]. Guo et al. [3] confirmed that the distinct VOC patterns can predict trophic modes, (non)symbiotic lifestyle, substrate-use and host type of fungi.

* Corresponding author.

Pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS) has the advantages of being a fast, sensitive, one-step procedure and the ability to identify many structurally related compounds. Therefore, it has been successfully used to study the structure of complex organic macromolecular materials [8]. Approach to characterize microorganisms (bacteria, fungi) with analytical pyrolysis was used by Schwarziger [9], Melucci et al. [10], Wan et al. [11]. Application of analytical pyrolysis for microbial characterization is based on detecting chemical marker compounds that are biological products from the microbial structure and unique in a group of organisms [12].

The aim of our work was to identify fungal markers from pyrograms of mycelia and VOCs profiles of wood-decaying *Basidiomycota* fungi (*Fomitopsis betulina* (Bull.) B.K. Cui, M.L. Han & Y.C. Dai, *Phaeolus schweinitzii* (Fr.) Pat., *Phellinus chrysoloma* (Fr.) Donk, *Trametes versicolor* (L.) Lloyd) common in boreal forests of Siberia.

Materials and methods

Sample preparation. Four cultures of *Basidiomycota* (*Basidiomycetes*) class *Agaricomycetes*: the order *Polyporales species* F. betulina, *P. schweinitzii*, *T. versicolor* and *P. chrysoloma* from the order *Hymenochaetales* were used. The cultures were isolated by staff of V.N. Sukachev Institute of Forest from woods and are maintained in the collection on beer wort agar (2° sugar content according to Balling).

The growth of fungal biomass was carried out on liquid beer wort (sugar content 2°B) in a stationary culture. Each culture was grown in 4 replicates. Cultivation was carried out in the dark at a constant temperature of 24 °C for 45 days. Biomass was collected and washed five times with distilled water by filtration through technical capron (*P. chrysoloma, T. versicolor*) or by centrifugation at 5000 rpm for 10 min (*F. betulina, P. schweinitzii*). The collected biomass was frozen at (-18 °C). Four replicates were combined into a common sample for each culture. Mycelia samples were crushed in liquid nitrogen to get a homogeneous mass then dried at 50 °C. The dried samples were again pulverized in a mortar, avoiding heating, to a fine powder. The powered samples have been analysed by Py-GC/MS.

We used solvent-based extraction method. The resulting solution contains dissolved fungal volatile metabolites. Ca 10 mg of each powered mycelium sample was extracted with 50 ml of pentane (≥99%, Panreac**)** over a week. Then, each of the extracts was further subjected to gas chromatography–mass spectrometry (GC/MS) analysis to identify VOCs of studied fungi.

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS). The pyrolysis products of mycelia were determined using EGA/PY-3030D pyrolyzer (Frontier Laboratories) and GCMS-QP2020 (Shimadzu, Japan). Pyrolysis was carried out at 600 °C, sample mass ~70–95 μg. A capillary column Ultra ALLOY-5 with a length of 30 m, inner diameter 0.25 mm, thickness of the liquid phase layer (5% biphenyl, 95% dimethylpolysiloxane) 0.25 μm was used with a temperature program from 50 °C (held for 10 min) to 240 °C at 5 °C/min, from 240 to 300 °C (held for 5 min) at 10 °C/min. The injector temperature was 250 °C. Injection mode was split (1 : 50 split ratio) and gas carrier was helium at 1.0 mL/min flow rate. The mass spectrometer ion source was set to 250 °C and the interface to 300 °C, scan range was from 40 to 550 m/z. The identification of pyrolysis products was based on match with the NIST mass spectra library (2017) and literature data. The compounds identified with more than 90% quality (as estimated by the software comparing library and measured mass spectra) are presented.

Gas chromatography-mass spectrometry (GC/MS). GC-MS analysis was carried out using «Agilent 5975C-7890A» equipped with an HP-5ms column (5%-phenyl-methylpolysiloxane; length 30 m; inner diameter 0.25 mm; film thickness 0.25 μm). The injection volume was 0.2 μL. The carrier gas was helium with a constant flow rate of 1 mL/min. The temperature of GC oven was initially held at 50 °C for 2 min. Thereafter, the temperature was increased from 50 to 200 °C at the rate of 4 °C/min, then to 220 °C at the rate of 10 °C/min, holding for 10 min. The temperature of injector was 280 °C. Volatile organic compounds were identified using the National Institute of Standards and Technology Mass Specral Library (NIST05a. L), Automated Mass Spectral Deconvolution and Identification System (AMDIS), and by comparison of the Kovats retention indices. The compounds with 90% or greater spectral matching accuracy are reported.

Results and discussion

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS). The brown-rot (*F. betulina, P. schweinitzii***)** and white-rot (*T. versicolor*, *P. chrysoloma***)** fungi are common representatives of wood-decaying fungi communities in boreal forests of Siberia [1]. *P. schweinitzii* is a plant pathogen that causes butt rot in living trees; a saprobe on dead

trees, stumps and logs. *P. chrysoloma* causes white pocket rot in both living and dead coniferous trees. *T. versicolor* and *F. betulina* grow mainly as saprophytes on dead trees and occasionally as parasites of living trees [13–16].

The fungal cell wall consists of glucans, chitin, and glycoproteins. Glucans are the major structural polysaccharides comprising around 50–60% of the cell wall by dry mass. Chitin is considered as the second most abundant polymer in the fungal cell wall. The amount of chitin in fungi is variable, ranging from 1–2% to 10–20%. Glycoproteins constitute 20–30%. Lipids are found in fungi as major constituents of the membrane systems and minor components in the cell wall [17, 18]. It has been reported that Basidiomycetes can contain not only chitin but also chitosan in their cell walls [19]. Mario et al. [20] isolated chitinous material from the mycelium of seven *Basidiomycetes* species and found that the yield of chitin ranged from 8.5 to 19.6% dry weight and the yield of chitosan was about 1%.

Pyrograms of mycelia of *F. betulina*, P. *schweinitzii, P. chrysoloma, T. versicolor* fungi produced compounds resulting from the thermal decomposition of polysaccharides (glucans, chitin), proteins, lipids. The relative abundance of pyrolysis products provides information about chemical composition of fungi. The list of compounds identified on the pyrograms is shown in Table 1. The identified compounds accounted for 82–91% of the total area of the recorded peaks. Pyrolysis products were further grouped based on the precursors, which are the main constituent components of fungi [21].

All pyrograms are dominated by compounds with a polysaccharide origin. The peaks derived from polysaccharides are issued mainly from glucans and chitin. The dominant pyrolysate in all pyrograms was levoglucosan (17–51%). Levoglucosan (1,6-anhydro-β-D-glucopyranose) is a characteristic anhydrosugar produced by pyrolysis of glucans [22]. Other compounds such as furfural, 1,4:3,6-dianhydro-alpha-d-glucopyranose, and 1,6-anhydro-beta.-d-glucofuranose were also present in appreciable amounts. *T. versicolor* was characterized by the highest content of levoglucosan. Therefore, very likely *T. versicolor* contains the highest amount of glucans among the studied fungi.

Most of polysaccharide-derived products listed in Table 1 can be also generated during wood pyrolysis. They originate from cellulose and hemicelluloses [23]. Thus, polysaccharide-derived products originating from mycelia of fungi cannot be well distinguished from those derived from wood with pyrolysis and cannot be useful to detect fungal infections in wood.

Protein pyrolysis products are represented by isobutyronitrile, 3-methylbutanenitrile, phenols, styrene and others. N-bearing compounds such as pyrrole, pyridine and their derivatives may originate from pyrolysis of both proteins and chitin, chitosan. The pyrogram of *T. versicolor* showed both the smallest number and proportions of individual compounds of strictly protein origin. In contrast, a significant contribution of 4-methylphenol, phenol, styrene has been found after pyrolysis *P. chrysoloma* mycelium. The pyrolysis products such as toluene, styrene, phenol, 4-methylphenol, indole, pyridine, pyrrole are indicative of amino acids as tryptophan, phenylalanine, tyrosine, proline, hydroxyproline, glutamine, alanine [21]. It is worth noting that phenols and styrene are also formed during wood pyrolysis [23]. Generally, proteins can have not been considered to be important components of wood. The concentration of total nitrogen in the wood is 0.03–0.1% by dry weight [24]. Therefore, N-bearing compounds can be used as markers for the presence of fungi.

Pyrolysates formed during pyrolysis of chitin and chitosan, in addition to pyrrole, pyridine and their derivatives, are represented by compounds such as methyl 2-(acetylamino)-2-deoxy-alpha-D-galactopyranoside, 2-pyridinecarboxaldehyde and methylpyrazine. Many compounds characteristic of chitin, chitosan were not found for *T. versicolor* whereas they were abundant for *P. chrysoloma*. The compounds 1-octene and 1-decanol are derived from the degradation of fatty acids and lipids [25]. They were found in the pyrolysates of the fungi *F. betulina*, *P. schweinitzii*, *P. chrysoloma* but not *T. versicolor*. Pyrolysis products of chitin and lipids may be indicative markers of fungi in wood.

Gas chromatography-mass spectrometry (GC/MS). The chromatograms of the mycelia extracts showed from 14 to 28 individual volatile compounds (peaks): *P. schweinitzii>P. chrysoloma>F. betulina>T. versicolor*. Identified compounds are listed in Table 2. They counted 69.8–99.8% of the total area of all recorded peaks. The volatile compounds were classified into following types of compounds: monoterpenes, triterpenes, aromatic monoterpenoids, alkanes, fatty acids, fatty acid esters, polycyclic aromatic hydrocarbons (PAHs), Si-containing compounds, phenols, bicyclic heterocycles, steroids.

The composition of VOCs from the mycelium extract of *P. schweinitzii* was dominated by fatty acids (oleic acid, palmitic acid) and linoleic acid ethyl ester together representing ca. 57% of the total peak area. (E)-9-octadecenoic acid ethyl ester was characteristic for *P. schweinitzii*. Fatty acids and their derivatives as components of fungal VOCs have been reported in literature [26]. Johnathan [27] found that the volatile compounds from the fatty acid group were the most predominant in hexane extract of *Lignosus rhinocerus* "Tiger milk mushroom".

Table 1. Relative abundance (percent peak areas) of micelia pyrolysis products

Compound	Origin	*F. betulina*	*P. schweinitzii*	*P. chrysoloma*	*T. versicolor*
1	*2*	*3*	*4*	*5*	*6*
Glucans, chitin and/or chitosan					
Acetaldehyde	Gl, Ch	5.24	2.70	4.35	2.78
1-Propen-2-ol, acetate	Gl	6.33	4.03	10.19	5.32
1,3-Cyclopentadiene	Gl	0.13	0.14	0.26	0.20
Propanal, 2-methyl-	Gl	0.24	0.34	0.03	0.02
Furan, 2,3-dihydro-	Gl	1.83	0.49	1.15	0.91
Acetic acid	Gl, Ch	4.09	2.28	2.68	1.63
Furan, 2-methyl	Gl	1.39	0.68	0.31	0.83
Butanal, 3-methyl-	Gl, Pr	1.55	0.76	0.86	0.72
2-Propanone, 1-hydroxy-	Gl, Ch	1.19	1.06	2.30	1.27
Cyclopentane, ethyl-	Gl	0.46	0.34	0.09	–
Furan, 2,5-dimethyl-	Gl	1.35	0.99	0.63	0.48
2-Vinylfuran	Gl	0.28	0.25	0.23	0.05
Acetic acid, (acetoxy)-	Gl	–	–	0.77	0.27
Propanoic acid, 2-oxo-, methyl ester	Gl, Ch	0.34	0.35	1.13	0.36
Furan, 2-ethyl-5-methyl-	Gl	0.17	–	–	0.05
3-Furaldehyde	Gl	–	0.12	0.27	0.11
Furfural	Gl	1.08	1.2	1.51	1.27
3-Furanmethanol	Gl	0.51	0.31	0.83	0.40
2(3H)-Furanone, 5-methyl-	Gl	0.09	0.11	0.09	–
4-Cyclopentene-1,3-dione	Gl	–	–	0.14	0.05
4-Cyclopentene-1,3-dione	Gl	0.37	0.36	0.17	0.11
2-Cyclopenten-1-one, 2-methyl	Gl	0.14	0.11	0.10	0.05
Hexanal, 3-methyl-	Gl?	0.22	0.26	1.06	0.67
1,2-Cyclopentanedione	Gl, Ch	1.19	1.36	2.08	1.10
2-Furanmethanol, 5-methyl-	Gl	–	0.31	–	–
2,5-Furandione, dihydro-3-methylene-	Gl	0.19	0.08	0.13	0.10
2-Furancarboxaldehyde, 5-methyl-	Gl	1.00	0.89	0.30	0.56
Oxazolidine, 2,2-dietyl-3-methyl-	Gl	0.27	0.37	0.46	0.37
1,2-Cyclopentanedione, 3-methyl-	Gl, Ch	0.43	0.4	0.62	0.33
Furaneol	Gl	0.65	0.66	0.47	0.54
Maltol	Gl	–	0.24	0.16	0.20
4H-Pyran-4-one, 5-hydroxy-2-methyl-	Gl	0.22	0.22	0.21	0.26
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Gl	0.29	0.37	0.11	0.17
4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	Gl	1.24	–	–	1.19
1,4:3,6-Dianhydro-alpha-d-glucopyranose	Gl	1.36	1.59	1.30	0.93
Benzofuran, 2,3-dihydro-	Gl	–	2.99	–	
2,3-Anhydro-d-mannosan	Gl	0.23	0.39	0.38	0.21
Levoglucosan	Gl	16.91	29.95	16.50	50.63
Alpha-D-Glucopyranose, 4-O-beta-D-galactopyranosyl- (alpha-d-Lactose)	Gl	–	0.49	0.80	1.11
1,6-Anhydro-beta.-D-glucofuranose	Gl	1.11	1.93	–	2.78
Alpha-D-Galactopyranoside, methyl 2-(acetylamino)-2-deoxy-	Ch	1.29	2.04	4.17	–
Proteins, chitin and/or chitosan					
Isobutyronitrile (2-methylpropanenitrile)	Pr	0.57	0.08	0.15	–
Butanenitrile, 3-methyl	Pr	–	–	0.10	–
N,N,-Dimethylaminoethanol	Pr, Ch?	0.58	1.01	–	–
Pyridine	Pr, Ch	0.17	0.24	0.56	0.08
Pyrrole	Pr, Ch	0.44	0.35	0.21	0.15
Toluene	Pr, Gl	1.72	2.90	1.05	0.17
N-methyl-L-proline, butyl ester	Pr	0.71	1.12	1.38	0.82
Pyridine, 2-methyl-	Pr, Ch	0.17	0.19	0.18	–
Pyrazine, methyl-	Ch	0.19	0.29	0.26	–
1H-Pyrrole, 2-methyl-	Pr, Ch	0.48	0.50	0.28	–
1H-Pyrrole, 3-methyl-	Pr	0.13	0.20	0.20	–
Styrene	Pr	0.18	0.49	0.82	0.01

End of table 1

1	*2*	*3*	*4*	*5*	*6*
Pyridine, 3,4-dimethyl-	Pr	–	0.05	–	–
2-Pyridinecarboxaldehyde	Ch	–	0.17	0.32	–
4-Methyl-2-oxo-(1H)-pyrimidine	Pr	0.66	0.77	0.87	0.40
Phenol	Pr, Gl	0.23	0.33	1.98	0.08
2-Methylphenol	Pr	–	0.08	0.34	–
4-Methylphenol	Pr	0.84	0.64	2.25	0.30
1H-Pyrrole, 2,5-dihydro-(Pyrroline)	Pr	–	–	1.02	–
Phenol, 2,5-dimethyl-	Pr	–	0.26	0.37	–
Phenol, 2-ethyl-	Pr	–	–	0.38	–
Indole	Pr	0.27	0.50	0.29	–
2(1H)-Pyridinone, 1-methyl-	Ch?	–	0.71	0.85	0.12
Lipids					
1-Octene	Lp	0.30	0.21	0.20	–
1-Decanol	Lp	0.20	0.66	0.12	–
D-Limonene	Lp?	–	0.14	–	–
2-Propenoic acid, 2-methyl-, octyl ester	Lp	0.48	0.41	0.30	–
Nonspecific					
Carbon dioxide		20.64	12.72	14.27	10.41

Note: Gl – glucans, Pr – proteins, Lp – lipids, Ch – chitin, chitosan, – means not detected

Table 2. VOCs detected in the mycelia extracts

Compound name	Time, min	Peak area, %				Types
		F. betulina	*P. schweinitzii*	*P. chrysoloma*	*T. versicolor*	
a-Pinene	7.52	–	–	–	0.937	Monoterpenes
Cyclotetrasiloxane, octamethyl-	9.74	2.964	0.543	5.859	0.107	Si-containing compounds
Undecane	11.7	4.382	0.732	5.122	1.405	Alkanes
Undecane, 4,7-dimethyl-	13.28	0.275	0.267	1.342	0.444	Alkanes
Cyclopentasiloxane, decamethyl-	15.28	0.396	0.092	1.469	–	Si-containing compounds
Benzothiazole	17.30	–	0.181	–	1.187	Bicyclic heterocycles
Sulfurous acid, hexyl octyl ester	19.49	1.692	0.298	2.0	0.583	Esters
Thymol	19.95	28.98	3.553	33.674	2.819	Aromatic monoterpenoids
Cyclohexasiloxane, dodecamethyl-	21.19	–	0.288	2.44	–	Si-containing compounds
Cycloheptasiloxane, tetradecamethyl-	26.60	–	0.457	3.028	–	Si-containing compounds
Phenol, 2,4-bis(1,1-dimethylethyl)-	26.92	2.308	0.485	2.976	0.799	Phenols
Fluorene	28.83	–	0.119	4.12	–	PAHs
Phenanthrene	34.22	15.526	0.567	3.005	0.759	PAHs
n-Hexadecanoic acid (palmitic acid)	38.94	12.943	9.841	17.222	0.717	Fatty acids
Oleic Acid	42.47	0.331	35.248	–	–	Fatty acids
Linoleic acid ethyl ester	42.82	–	12.315	2.592	–	Fatty acid esters
(E)-9-Octadecenoic acid ethyl ester	42.93	–	4.859	–	–	Fatty acid esters
3,5-Cyclo-6,8(14),22-ergostatriene	49.77	–	–	–	13.007	Steroids
Squalene	50.88	–	–	–	76.991	Triterpenes

The mycelium extract of *P. chrysoloma* yielded relatively high amounts of thymol, palmitic acid, Si-containing compounds, and undecane together accounting for ca. 69% of the VOCs peak area. The high relative content of thymol was unexpected. Thymol is a monoterpene phenol usually found in the essential oil of plants. The antibacterial and antifungal activities of thymol have been reported [28]. It is likely that the high abundance of thymol in VOCs from the mycelium extract of *P. chrysoloma* may be due to the fact that fungi can exhibit antagonistic activities against other microorganisms. In previous studies, Si-containing compounds and undecane in the volatile composition of fungi were reported [29]. Si-containing compound as octamethylcyclotetrasiloxane demonstrated antibacterial and antifungal activities [30]. Undecane was described to have nematicidal activity [31].

The predominate volatiles detected in the mycelium extract of *F. betulina* were thymol, phenanthrene, palmitic acid together represented ca. 57% of the total peak area. PAHs, including phenanthrene, have been previously detected in VOCs of hexane extracts from mycelia of *Trametes pubescens* and *Flammulina velutipes* [32]. PAH such as naphthalene is known as an effective insect repellent. Daisy et al. [33] suggested that role of naphthalene in

endophytic fungus *Muscodor vitigenus* may be related to its ability to inhibit some types of fungal proliferation, thus providing the endophyte with the capability to ward off competitors in its natural environment. The fact that PAHs are synthesized by some fungi also explains their ability to eliminate these compounds from the environment [32].

The presence of high relative contents of triterpene squalene (77%) and 3,5-cyclo-6,8(14),22-ergostatriene (13%) in VOC composition was characteristic for *T. versicolor*. Among studied fungi, only *T. versicolor* produced monoterpene (α-pinene). It is worthwhile to note that *T. versicolor* showed the lowest content of palmitic acid. The volatile composition of *T. versicolor* mycelium has been studied by Guo et al. [3]. Carbonyl compounds, alkenes, fatty acids, alcohols, monoterpenoids, sesquiterpenoids and others were detected. In our study, the mycelium extract of *T. versicolor* did not show a large diversity of compounds compared to the literature data. Squalene is a key intermediate for the production of triterpenoids in plants, bacteria and fungi. Squalene has been reported for the mycelia extracts of *Trametes pubescens* and *Flammulina velutipes* [32]. 3,5-Cyclo-6,8(14),22-ergostatriene is a steroid. Steroids occupy an important place among fungal constituents. The vast majority of them are ergosterol metabolites. Ergosterol is the main sterol of fungi involved in the regulation of membrane fluidity and structure [34].

Other notable VOCs of the mycelia extracts were phenol, 2,4-bis(1,1-dimethylethyl)- and benzothiazole. *F. betulina* and *P. chrysoloma* showed significantly higher abundance of phenol, 2,4-bis(1,1-dimethylethyl)- compared to *P. schweinitzii* and *T. versicolor*. Benzothiazole was detected only for *T. versicolor* (1.2 %) and *P. schweinitzii* (0.2%). Phenol-2,4-bis (1,1-dimethylethyl)- is a common toxic secondary metabolite produced by various groups of organisms including fungi [35].

Wood releases a low amount of VOCs, among which the most common are terpenes, aliphatic aldehydes and organic acids. Softwoods emit the highest concentrations of wood VOCs consisting mainly of terpenes (70–90%) and hexanal, acetic acid (10–25%). Emissions of VOCs from hardwoods are much lower (ca. 50 times) and include hexanal, pentanal, acetic acid, other VOCs, but not volatile terpenes [36]. Considering this fact, we suggest that fatty acids (palmitic acid, oleic acid), linoleic acid ethyl ester, Si-containing compounds (octamethyltetrasiloxane), undecane, phenanthrene, 3,5-cyclo-6,8(14),22-ergostatriene can be considered as marker compounds the early detection of fungi in wood.

Conclusion

In the present work, we characterized mycelium pyrolysis products and VOC profiles of the mycelia extracts from *F. betulina, P. schweinitzi, T. versicolor*, *P. chrysoloma*. The obtained results indicated differences in chemical composition of wood-decaying fungi common in Siberia. We suggested that such pyrolysates as pyridine, pyrrole and their derivatives, methyl-2-(acetylamino)-2-deoxy-alpha-D-galactopyranoside, 2-pyridinecarboxaldehyde, methylpyrazine, 2-methylpropanenitrile, 3-methylbutanenitrile, 1-octene, 1-decanol, and VOCs such as fatty acids (palmitic acid, oleic acid), linoleic acid ethyl ester, Si-containing compounds (octamethyltetrasiloxane), undecane, phenanthrene, 3,5-cyclo-6,8(14),22-ergostatriene can be considered as marker compounds for early detection of fungi in wood. This is the first step and the usefulness of the markers detected needs to be confirmed by examining wood infected by these fungi.

Funding

This study was performed in the frame of the state project (FWES-2024-0028).

Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

References

1. Kuz'michev E.P., Sokolova E.S., Kulikova E.G. *Common fungal diseases of Russian forests*. USA, 2001, 137 p.
2. Mali T., Mäki M., Hellén H., Heinonsalo J., Bäck J., Lundell T. *FEMS Microbiol. Ecol*., 2019, vol. 95, no. 9, 135. DOI: 10.1093/femsec/fiz135.
3. Guo Y., Jud W., Weikl F., Ghirardo A., Junker R.R., Polle A., Benz J. P., Pritsch K., Schnitzler J.-P., Rosenkranz M. *Commun. Biol.*, 2021, vol. 4, 673. DOI: 10.1038/s42003-021-02198-8.
4. Hung R., Lee S., Bennett J.W. *Appl. Microbiol. Biotechnol*., 2015, vol. 99, pp. 3395–3405. DOI: 10.1007/s00253-015-6494-4.
5. Polizzi V., Adams A., Malysheva S.V., De Saeger S., Van Peteghem C., Moretti A., Picco A.M., De Kimpe N. *Fungal Biol*., 2012, vol. 116, no. 9, pp. 941–953. DOI: 10.1016/j.funbio.2012.06.001.

6. Yang F., Dong W., Zhang X., Li Y., Zhou S., Zhu G., Xiao C. *Chil. J. Agric. Res.,* 2019, vol.79, no.4, pp. 596–608. DOI: 10.4067/S0718-58392019000400596.
7. Lima T. da C., Santos R.S., Silva S.Y.S., Santos D. de A., Silva S. da C., Gomes A. de A., Oliveira M. C. F., Alves K.F., Pinto L., Oliveira M.N. *Food Chem.*, 2021, vol. 362, 130150. DOI: 10.1016/j.foodchem.2021.130150.
8. Zhao J., Peng P., Song J., Ma S., Sheng G., Fu J., Yuan D. *AAQR*, 2012, vol. 12, no. 1, pp. 83–92. DOI: 10.4209/aaqr.2011.06.0086.
9. Schwarzinger C. *J. Anal. Appl. Pyrolysis*, 2005, vol. 74, no. 1–2, pp. 26–32. DOI: 10.1016/j.jaap.2004.11.025.
10. Melucci D., Fedi S., Locatelli M., Locatelli C., Montalbani S., Cappelletti M. *Curr. Drug Targets,* 2013, vol. 14, no. 9, pp. 1023–1033. DOI: 10.2174/1389450111314090011.
11. Wan Y.Y., Zhu Y.J., Jiang L., Luo N. *Microorganisms*, *2022,* vol. 10*,* 2333. DOI: 10.3390/microorganisms10122333.
12. Morgan S.L., Watt B.E., Ueda K. *Analytical microbiology methods: chromatography and mass spectrometry*. New York, 1990, pp. 179–200. DOI: 10.1007/978-1-4899-3564-9_12.
13. Schmidt O. *Wood and tree fungi: biology, damage, protection, and use*. Germany, 2006, 334 p.
14. Sárközy A. *Isolation and structure elucidation of bioactive metabolites from poroid fungi of Hymenochaetaceae and Meripilaceae: Ph.D. Thesis*. Hungary, Szeged, 2021. 57 p.
15. Bergdahl D.R. *Plant Dis*., 1985, vol. 69, pp. 887–890.
16. Pleszczyńska M., Lemieszek M.K., Siwulski M., Siwulski M.,·Wiater A., Rzeski W., Szczodrak J. *World J. Microbiol. Biotechnol*., 2017, vol. 33, 83. DOI:10.1007/s11274-017-2247-0.
17. Garcia-Rubio R., de Oliveira H.C., Rivera J., Trevijano-Contador N. *Front Microbiol.*, 2020, vol. 10, 2993. DOI: 10.3389/fmicb.2019.02993.
18. Beccaccioli M., Reverberi M., Scala V. *Front. Biosci. (Landmark Ed)*, 2019, vol. 24, no. 1, pp. 172–185. DOI: 10.2741/4712. PMID: 30468650.
19. Abo Elsoud M.M., El Kady E.M. *Bull. Natl. Res. Cent*., 2019, vol. 43, 59. DOI: 10.1186/s42269-019-0105-y.
20. Di Mario F., Rapanà P., Tomati U., Galli E. *Int. J. Biol. Macromol*., 2008, vol. 43, no. 1, pp. 8–12. DOI: 10.1016/j.ijbiomac.2007.10.005.
21. Rouches E., Dignac M.-F., Zhou S., Carrère H. *J. Anal. Appl. Pyrolysis*, 2017, vol. 123, pp. 409–418. DOI: 10.1016/j.jaap.2016.10.012
22. Sugiura M., Nakahara M., Yamada C., Arakawa T., Kitaoka M., Fushinobu S. *J. Biol. Chem. J. Biol. Chem*., 2018, vol. 293, no. 45, pp. 17375–17386. DOI: 10.1074/jbc.RA118.004963.
23. Łucejko J.J., Modugno F., Ribechini E., del Río J.C. *Anal. Chim. Acta*, 2009, vol. 654, pp. 26–34. DOI: 10.1016/j.aca.2009.07.007.
24. Cowling E.B., Merrill W. *Can. J. Bot*., 1966, vol. 44, no 11, pp. 1539–1554. DOI: 10.1139/b66-167.
25. Fabbri D., Sangiorgi F., Vassura I. *Anal. Chim. Acta*, 2005, vol. 530, no. 2, pp. 253–261. DOI: 10.1016/j.aca.2004.09.020.
26. Bojke A., Tkaczuk C., Stepnowski P., Gołębiowski M. *Microbiol. Res.*, 2018, vol. 214, pp. 129–136. DOI: 10.1016/j.micres.2018.06.011.
27. Johnathan M., Nurul A.A., Ezumi M.F., Gan S.H. *Res. J. Pharm. Biol. Chem. Sci*., 2016, vol. 7, no. 4, pp. 5–16.
28. Marchese A., Orhan I.E., Daglia M., Barbieri R., Lorenzo A.D., Nabavi S.F., Gortzi O., Izadi M., Nabavi S.M. *Food Chem*., 2016, vol. 210, pp. 402–414. DOI: 10.1016/j.foodchem.2016.04.111.
29. Joo J.H., Hussein K.A. *Front. Plant Sci.*, 2022, vol. 13, 897668. DOI: 10.3389/fpls.2022.897668.
30. Kong W.-L., Li P.-S., Wu X.-Q., Wu T.-Y., Sun X.-R. *Microorganisms*, 2020, vol. 8, 590. DOI: 10.3390/microorganisms8040590.
31. Kassam R., Yadav J., Chawla G., Kundu A., Hada A., Jaiswal N., Bollinedi H., Kamil D., Devi P., Rao U. *Front. Microbiol.*, 2021, vol. 12, 790223. DOI: 10.3389/fmicb.2021.790223.
32. Kurashov E.A., Ananieva E.P., Krylova Yu.V. *Mikologiya i fitopatalogiya*, 2012, vol. 46, no. 2, pp. 145–152. (in Russ.).
33. Daisy B.H., Strobel G.A., Castillo U., Ezra D., Sears J., Weaver D.K., Runyon J.B. *Microbiology*, 2002, vol. 148, pp. 3737–3741.
34. Zhabinskii V.N., Drasar P., Khripach V.A. *Molecules*, 2022, vol. 27, 2103. DOI: 10.3390/molecules27072103.
35. Zhao F., Wang P., Lucardi R.D., Su Z., Li S. *Toxin,* 2020, vol. 12*,* 35. DOI: 10.3390/toxins12010035.
36. Pohleven J., Burnard M.D., Kutnar A. *Wood and Fiber Sci*., 2019, vol. 51, no. 3, pp. 231–254. DOI: 10.22382/wfs-2019-023.

Received February 2, 2023

Revised February 19, 2024

Accepted August 26, 2024

Information about authors

Shapchenkova Olga Alexandrovna – PhD, Senior researcher, shapchenkova@mail.ru

Aniskina Antonina Alexandrovna – Research assistant, aniskina_a@ksc.krasn.ru

Pashenova Nataliya Veniaminova – PhD, Senior researcher, pasnat@ksc.krasn.ru

Loskutov Sergey Redzhinaldovich – Doctor of Chemical Sciences, Fellow of the IAWS, lsr@ksc.krasn.ru