Chapter

Metabolomic Changes in Wood Inhabiting Filamentous Fungi during Ontogenesis

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Abstract

GC–MS-based metabolomic profiling of different strains of basidiomycetes *Lignomyces vetlinianus, Daedaleopsis tricolor* and *Sparassis crispa* were studied. On different stages of growth in the methanol extracts of mycelium the different compounds including amino acids, organic acid of TCA cycle, sugars, fatty acids, sugar alcohols, and sugar acids were detected. Changes in the metabolite network occurring with age of the mycelium of *L. vetlinianus* and *D. tricolor* are discussed. The exponential phase of mycelium growth is characterized by pronounced differences during of growth, which manifests itself both in the analysis of specific compounds and in the modeling of the statistical model of the metabolic network. The metabolomic network in the stationary growth phase is less susceptible to changes over time, and is also characterized by a lower dispersion of samples from one aging group. For some compounds, including biotechnologically significant ones, targeted analysis by GC–MS was performed. 4, 6-dimethoxy-phthalide (4, 6-dimetoxy-1 (3H) -isobenzofuranone) was isolated from the mycelium of *Lignomyces vetlinianus*, accumulating in the mycelium in the form of large aggregates. The accumulation of sparassol and other orsellinic acid derivatives in *Sparassis crispa* culture under various conditions is described.

Keywords: metabolomic profiling, metabolite network, gas chromatography–mass spectrometry, wood inhabiting fungi, ontogenesis, phthalide

1. Introduction

The interactions and interconversions of small biomolecules that form the metabolic network play an important, if not decisive, role in the development and ontogenetic processes of living organisms [1]. It is well known that in addition to the wide variability of the content of each metabolite in any biological object in the course of its vital activity, the transition from one stage of ontogenesis to the next is associated with a number of systemic changes in the qualitative and quantitative composition of metabolites and their ratios [2, 3], i.e. "Metabolic state" (metabolome). Thus, in the last decade in physiological research, the study of the dynamics of the metabolome of the system with the help of metabolic profiling taking into account the time factor has become increasingly important. The results of research in the field of metabolomics largely depend on the instrumental approach,

detection, methods of quantitative assessment and localization of metabolites [4]. To implement this approach, it is necessary to analyze complex mixtures using methods such as NMR (nuclear magnetic resonance), GC–MS (gas chromatography–mass spectrometry), LC–MS (liquid chromatography-mass spectrometry), CE-MS (capillary electrophoresis-mass spectrometry) [5, 6]. The application of a modern analytical platform in biology has two main results: metabolomic profiling and the dynamics of metabolic networks. The general purpose of metabolomic profiling is to document the set of metabolites [7]. This task has an independent meaning, since characterizes the molecular resources of the system, i.e. its potential in the context of the concept of biodiversity. For physiology, of particular interest is not so much the annotated metabolome as the dynamics of its correlation structure in the process of development and vital activity. Our laboratory has long been using classical methods of multivariate statistics ("-omic" approaches) with a categorical load in the form of a time scale.

Metabolome analysis represents a tool that finds common application in all aspects investigations, and hence it represents a focal point in studies of fungal physiology [8]. Filamentous fungi have a very active and diverse metabolism resulting in the production of a broad range of metabolites. Fungal chemodiversity is important source of the new compounds for biotechnology and medicine. This makes fungi very promising and interesting object for metabolomic studies, both for the study of the fundamental regularities of the metabolic network, and for the targeted analysis of biotechnologically significant compounds. Below a brief introductions of basidial fungi being an object of this investigation is presented.

Common in Russia bracket fungus *Daedaleopsis tricolor* (Bull.) Bondartsev & Singer (*Polyporaceae*, *Polyporales*, *Agaricomycetes*) is often considered as a color variation of *D. confragosa* (Bolton) J. Schröt. It causes a white rot of dead deciduous and coniferous trees (**Figure 1**). It is known as medicinal fungus producing bioactive compounds with antibiotic [9], antiviral [10], antihypertensive [11] and anti-tumor [12] effects.

Figure 1.
Fruiting bodies of Daedaleopsis tricolor *in nature.*

Pleurotoid xylotrophic fungus originally have been described from Poland as *Pleurotus vetlinianus* Domański but in 2008 was found in Russia (**Figure 2**). Its taxonomy was settled as *Lignomyces vetlinianus* (Domański) R.H. Petersen &

Figure 2.
Fruiting bodies of Lignomyces vetlinianus *in nature.*

Figure 3.
Fruiting bodies of Sparassis crispa *in nature.*

Zmitr. (Resupinataceae, Agaricales, Agaricomycetes), and culture characters were studied [13]. It was noticed then that some strains growing in Petri plates produced agglomerates of light colored crystals formed on colony mats, which were later identified as phthalides [14]. Since this species is not studied well, its physiological and biochemical properties are of quite interest.

Xylobiontic aphyllophoroid basidiomycete *Sparassis crispa* (Wulfen) Fr. (*Sparassidaceae*, *Polyporales*, *Agaricomycetes*) grows in the southern part of the Holarctic, parasitizing on the roots of coniferous trees (**Figure 3**). It belongs to rare species. *S. crispa* has the ability to produce various biologically active substances. Many compounds synthesized by *S. crispa* are well studied, as a rule, these are substances with therapeutic properties [15, 16]. Nevertheless, the physiological and biochemical characteristics of *S. crispa*, which determine its ecological specificity, have not been sufficiently studied, which makes it possible to consider this species as an interesting object for ecological and physiological studies.

The aim of this study is to investigate the dynamic changes in the metabolomic profile of presented species of fungi during their cultures growth using GC–MS analysis.

2. Materials and methods

2.1 Cultivation and cultural studies

The following strains from the Komarov Botanical Institute Basidiomycetes Culture Collection were used for the study: *Daedaleopsis tricolor* LE-BIN 2266, *Sparassis crispa* LE-BIN 043, LE-BIN 2902, LE-BIN 2967 and *Lignomyces vetlinianus* LE-BIN 2335, LE-BIN 2339, LE-BIN 3253.

For culture characters, the strains were cultivated in Petri plates 90 or 60 mm in diam. on beer-wort agar (BWA) (4% beer-wort, Severnie pivovarni, Russia; 2% agar, Difco) at 25 °C. Linear growth was measured every other day for *D. tricolor* and every week for *S. crispa* and *L. vetlinianus* strains (n = 4). Macromorphology of *D. tricolor* was described at 6 weeks, of *S. crispa* and *L. vetlinianus* – at 8 weeks. Micromorphology was studied under Zeiss Axio Imager A1, Axio Scope A1 and Stemi 2000-CS (Zeiss, Germany) using transmitted light.

For metabolomic analysis the strains were also grown in Petri plates: *S. crispa* and *D. tricolor* on BWA, whereas *L. vetlinianus* on malt extract agar (1,5% MEA, Oxoid, England) at 25 °C. Mycelium samples for the analysis of *D. tricolor* were taken on the 3rd, 7th, 14th, 21st and 35th days of growth. Analysis of *L. vetlinianus* mycelium on the 7-th, 14-th, 21-st, 35-th and 50-th days of growth was performed. *S. crispa* strains were analyzed on the 7-th, 14-th, 28-th and 60-th days. Mycelium was scraped from Petri plates and was dried for biomass evaluation.

Quenching and extraction of mycelium and fruiting bodies were carried out based on method described by Gummer with co-authors [17].

2.2 *Quenching*

Quenching of cellular metabolism was performed by 50% of cold methanol (-30°C).

2.3 Extraction

After quenching wet mycelium were ground to powder by a liquid N_2. Samples were extracted with 1.5 mL cold methanol (−25 °C) and chloroform (independently), vigorously mixed and centrifuged (10 min, 400 x g) at room temperature.

The supernatant was transferred to a new vial and the precipitated pellet was re-extracted with 1.5 mL of cold methanol. All extracts were drying by a rotary evaporator.

For a more detailed study of the accumulation of individual compounds in cultures, extraction by chloroform was applied. Crystals collected from the lids of the Petri dish and the surface of the *S. crispa* mycelium and aggregates from the *L. vetlinianus* mycelium were dissolved in chloroform and analyzed by GC–MS.

2.4 GC-MS analysis

GC–MS –based metabolomic analysis was carried out according to the standard derivatization scheme using bis—3-methyl-silyl-3-F-acetamide (BSTFA, Sigma). Silylated samples were analyzed using an Agilent 6850 gas chromatograph interfaced with 5975C mass selective detector. An HP5-MS capillary column (30 m x 0.25 mm inner diameter; film thickness of 0.25 um) was used with helium as a carrier gas at a constant rate of 1 ml/min. The temperatures of the injector and MS source were maintained at 320 °C and 230 °C, respectively. The column temperature program consisted of injection at 70 °C with an increase of 4 °C/min up to 320 °C followed by an isothermal hold at 320 °C for 15 min. Tricosane (10 ug) was used as an internal standard for the quantification of analytical results in semiquant mode, excluding sensitivity coefficients. The samples (0.5 ul) were injected in splitless mode using direct Ultra Inert Liner (Restek). The mass spectrometer was operated in the electron impact mode with an ionization energy of 70 eV. The scan mass range was set from 50 to 1000 Da at 1.27 scans per second. The data were processed and quantified with the AMDIS software (http://www.amdis.net). Compounds were identified through comparison with the retention characteristics and mass spectra of authentic standards, reported mass spectra, and the mass spectral library of the GC–MS data system (NIST 2010). The sum of the extracted ion chromatograms of the ions associated with a compound was used for quantification by UniChrom software (http://www.unichrom.com).

2.5 Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2016 and Metaboanalyst.

3. Results and discussion

3.1 Growth and cultural characteristics of the fungi

D. tricolor LE-BIN 2266 grew faster than strains of the other studied species. Average growth rate on BWA – 5,5 ± 0.5 mm/d. The dynamics of biomass change is shown on the **Figure 4**. On BWA the advancing zones of colonies even, hyphae surface or raised and fringed. Colony mat wooly with long aerial interwoven hyphae around inoculum then more or less radially ordered (**Figure 5**), white, with age becoming yellowish with brown droplets of exudate and crustose hazel or brown areas. Fruiting in culture sometimes observed. Aerial hypha 1–5 μm wide, branched with regular clamps, skeletal hypha long rare branched thick walled, 2–3 μm wide (**Figure 6**). Laccase is positive.

L. vetlinianus strains showed rather slow growth rate that varied depending on strain and used medium [13, 14]. In our study, average growth rate on BWA was 1.0 ± 0.3 mm/d, 2.0 ± 0.9 mm/d and 2.9 ± 0.6 mm/d for the strains LE-BIN 2335,

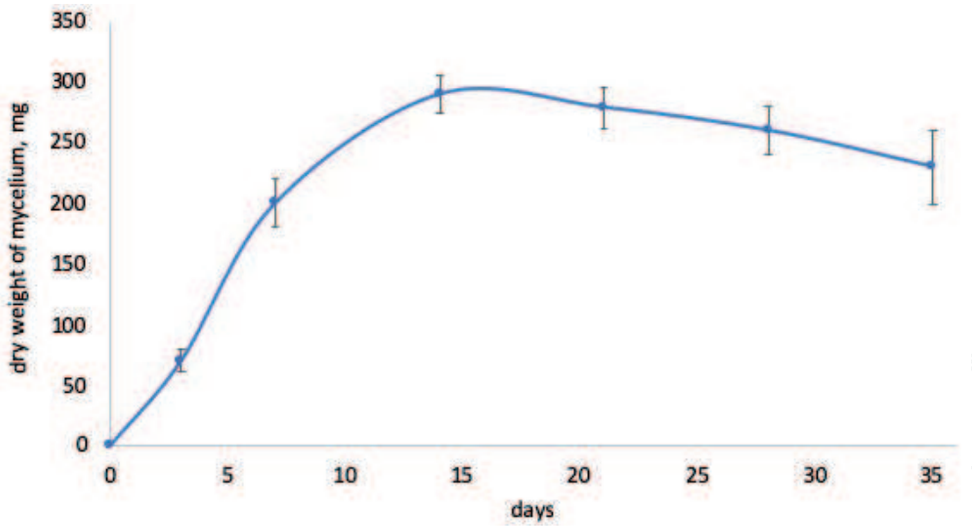


Figure 4.
Dynamic of biomass accumulation of D. tricolor *LE-BIN 2266.*

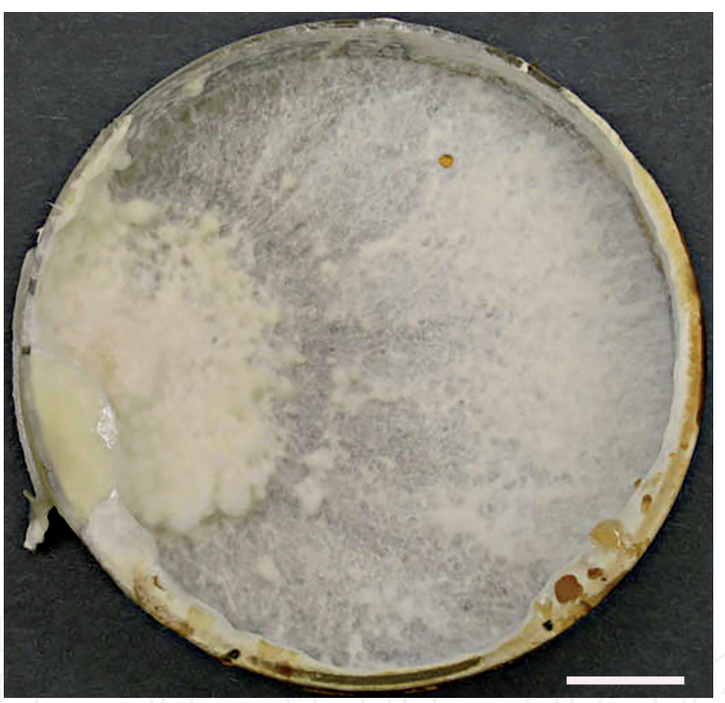


Figure 5.
Colony of D. tricolor *LE-BIN 2266 on BWA, 6 weeks. Bar = 10 mm.*

LE-BIN 2339 and LE-BIN 3253 respectively. The dynamics of biomass change is shown on the **Figure 7**. Characters of colonies were also variable depending on strain and used medium. On BWA the advancing zones of colonies even, hyphae surface or submerged. Colony mat wooly consisting of thin spread on the surface zonate aerial mycelium with more or less expressed radial fibrous hyphal bands (**Figure 8**), with age abundant fruiting [13] and conglomerates of crystals appear on the colonies (**Figures 9** and **10**). Aerial hypha monometic, 1.5–5 μm wide, branched with regular clamps (**Figure 11**). Laccase is positive.

S. crispa strains grew the most slowly in comparison with the other cultures in this study. In our experiments average growth rate of the strain LE-BIN 2902 on BWA was 0.5 ± 0.1 mm/d. The dynamics of biomass accumulation for involved in the experiment three strains is shown on the **Figure 12**.

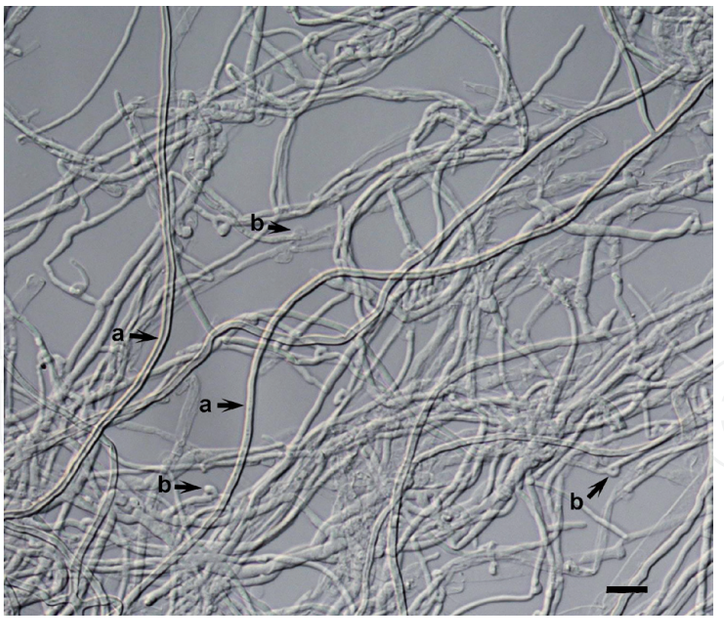


Figure 6.
Micromorphology of D. tricolor *LE-BIN 2266. Arrows indicate: a – Skeletal hypha; b – Clamp connections. Bar = 10 μm.*

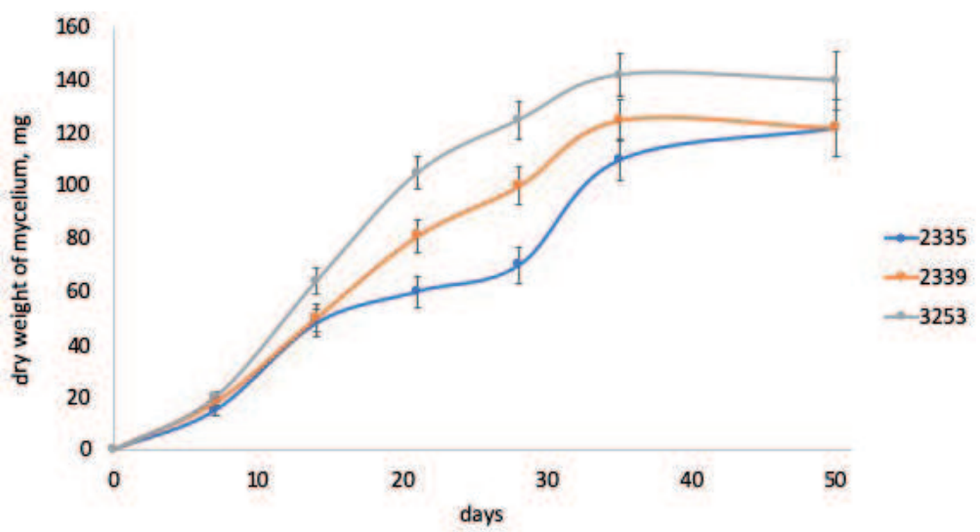


Figure 7.
Dynamic of biomass accumulation of L. vetlinianus *LE-BIN strains.*

Colony of LE-BIN 2902 on BWA is presented on **Figure 13**. The advancing zone of the colony is even, or slightly waved, marginal hyphae distant, appressed and submerged. Colony mat first downy later becoming wooly with aerial interwoven hyphae, white, creamy to grayish. Odor is absent.

Transparent crystals of various size and form can be observed on mycelium or on edges and lids of plastic plates (**Figure 14**). Over time, the number and size of such crystals increased. Aerial hypha irregular 2.0–5.0 μm wide with clamps and numerous swellings up to 10–15 μm in diam. Often arranged in chains (**Figure 15**). Single and grouped crystals were observed under the microscope. Laccase is negative.

3.2 Chemical diversity of metabolites detected by GS-MS in fungal mycelium

The results of metabolomic analysis showed that the GC–MS method allows to detect of many low molecular weight metabolites, among which are identified:

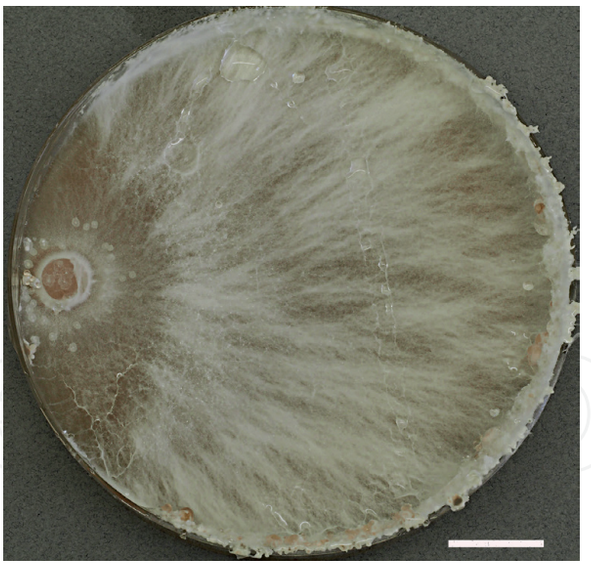


Figure 8.
Colony of L. vetlinianus *LE-BIN 2339 on BWA, 8 weeks. Bar = 10 mm.*

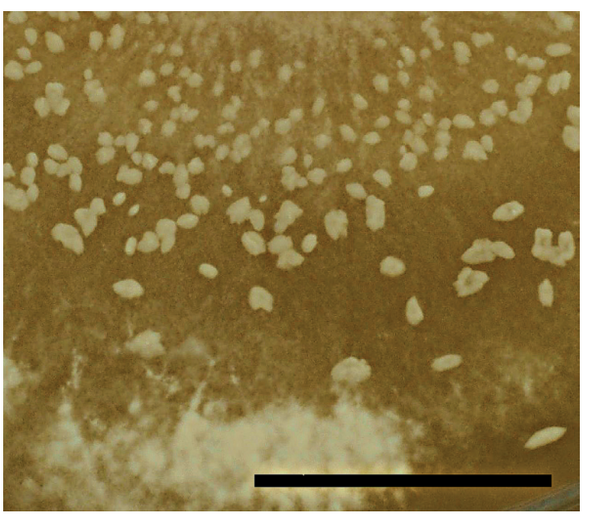


Figure 9.
Conglomerates of crystals in mycelium of L. vetlinianus *LE-BIN 2335. Bar = 10 mm.*

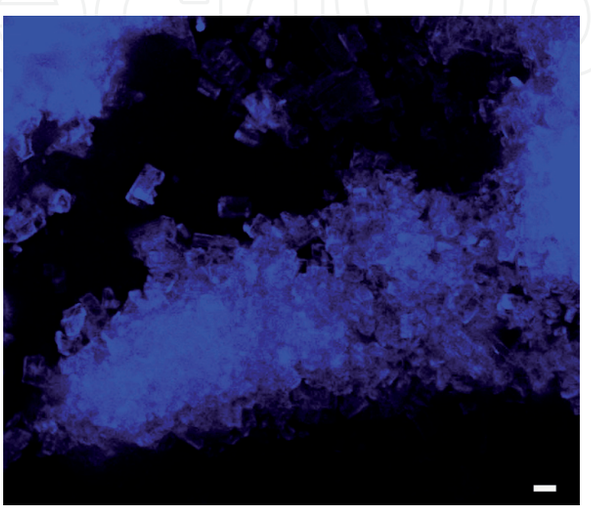


Figure 10.
Conglomerates of crystals in mycelium of L. vetlinianus *LE-BIN 2335 under the microscope in UV light. Bar = 10 μm.*

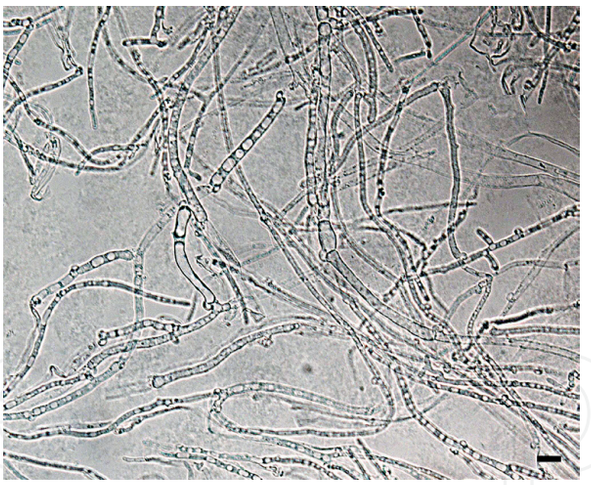


Figure 11.
Micromorphology of L. vetlinianus *LE-BIN 2335. Bar = 10 μm.*

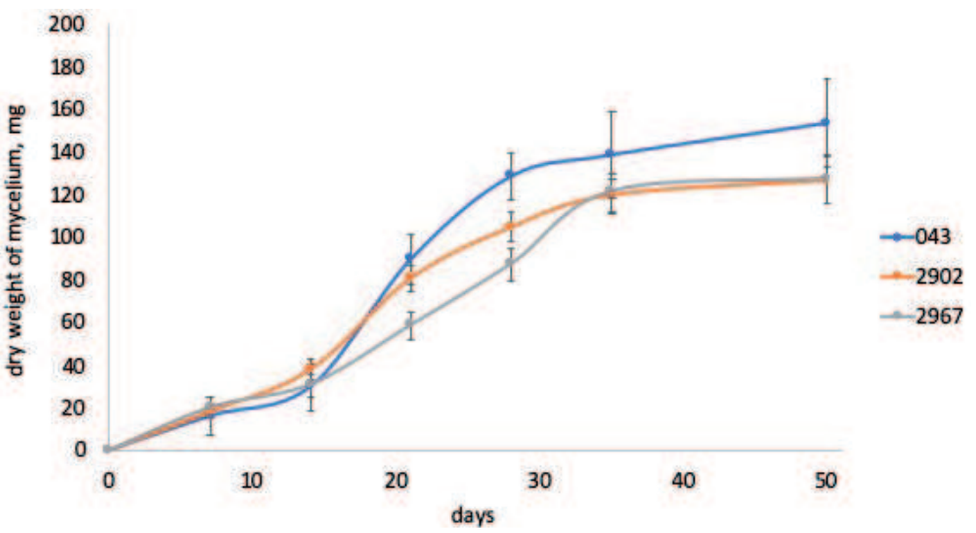


Figure 12.
Dynamic of biomass accumulation of S. crispa *LE-BIN strains.*

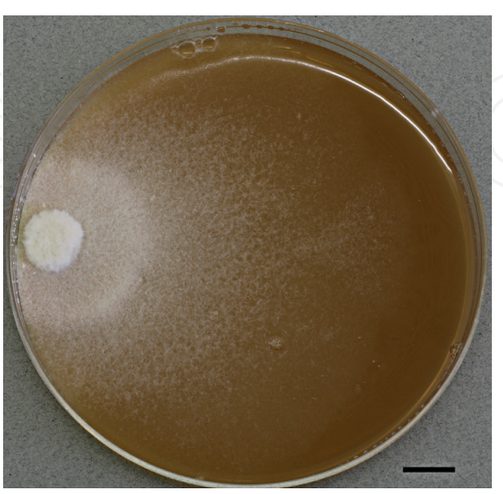


Figure 13.
Colony of S. crispa *LE-BIN 2902 on WBA, 8 weeks. Bar = 10 mm.*

amino acids, TCA (tricarboxylic acid) cycle, sugar-acids, fatty acids, monosaccharides, di- and trisaccharides, poliols, hydroxypropionic acid, cyclic metabolites, including phenolic compounds, glyceric acid, pyruvic acid, nicotinic acid,

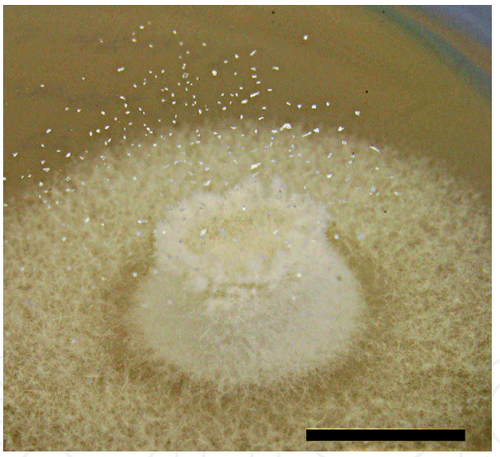


Figure 14.
Crystals on the lid of petri plate with S. crispa. *Bar = 10 mm.*

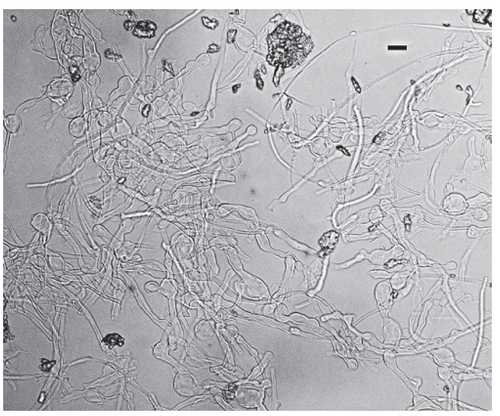


Figure 15.
Micromorphology of S. crispa *LE-BIN 2902. Bar = 10 μm.*

phosphate, methyl phosphate, glyceraldehyde, glycerol-3-P, phosphoglyceric acid (PGA).

The qualitative composition of most of the metabolites was generally similar for the three studied species of fungi. *S. crisra* mycelium contained very high quantitative of sugar that made it difficult to analyze a number of other compounds in the metabolomic profile. For this reason we do not present a detailed matrix of metabolites and statistical models for this species. The main differences between species, bur show the main differences between species only by cyclic compounds.

Specific phenolic compounds (sparassol, methyl ester of sparassol and methyl ester of orsellinic acid) were found in the *S. crispa* mycelium, as well as compounds similar to orsellinates in structure with molecular weights of 298 and 356. In some *S. crispa* cultures, on the mycelium surface and on the inner surface of Petri dish lids were found numerous clear crystals (**Figure 14**). These crystals were identified by GC–MS as methyl 2-hydroxy-4-methoxy-6-methyl benzoate (sparassol). The most intense sparassol formation was observed in the LE-BIN 2902 strain.

The mycelium of *L. vetlinianus* distinguished by the accumulation of furoic acid, cyclohexanone-3-carboxylic acid, 3-oxo-1-cyclohexene-1-carboxylic acid, and 4, 6-dimetoxy-1 (3H) -isobenzofuranone. The fastest growing strain LE-BIN 3253 also contained cyclopentene-3-carboxylic acid, in contrast to the other two *Lignomyces* strains. Numerous crystals-like aggregates glowing in UV were noticed in the plates with *L. vetlinianus* cultures (**Figures 9**, **10**). They were picked up from the plates and identified by GS-MC as clusters of 4,6-dimethoxy-phthalide or 4, 6-dimetoxy-1 (3H) -isobenzofuranone, also found in mycelium.

3.3 Changes in the metabolomic profile of fungi during ontogenesis

The analysis of metabolites was carried out on the following stages of mycelium growth: the onset of growth, the formation of differentiated mycelium, the log phase, the stationary growth phase, and aging.

General patterns of changes in the metabolic network in the ontogeny of fungal mycelium were visualized using the method of principal components (PC). The dynamics of changes in the metabolomic profile of fungi had its own characteristics both at the level of the species and the strain. *D. tricolor* was characterized by the separation of the initial growth stage (day 3) and the exponential growth stage (day 7) (**Figure 16a**). All other points formed a single cluster.

A similar pattern was observed for the statistical model of the dynamics of the *L. vetlinianus* LE-BIN 2339 metabolomite network in ontogenesis (**Figure 16b**). The stages of the exponential growth phase (7, 14, and 21) were distributed in the space, and 35th and 50th days were grouped together.

The distribution of points characterizing the development of *L. vetlinianus* LE-BIN 2335 strain (**Figure 16c**) can be described as a cyclical trend, in which the

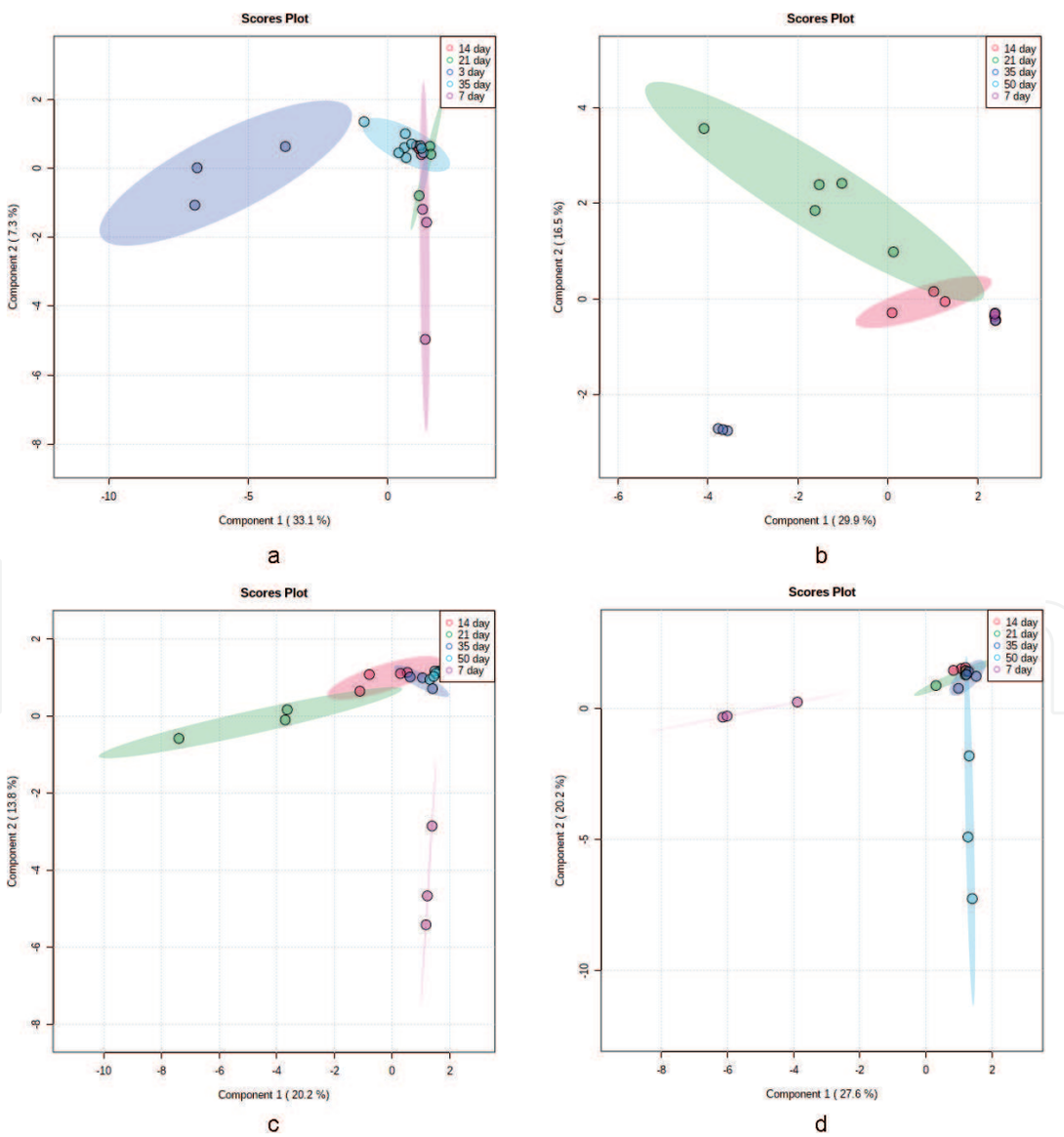


Figure 16.
Principal component analysis (PCA) of the metabolomic profile of fungi in ontogenesis: a – D. tricolor *LE-BIN 2266; b* - L. vetlinianus *LE-BIN 2339; c* - L. vetlinianus *LE-BIN 2335; d* - L. vetlinianus *LE-BIN 3253.*

7th and 50th days of growth formed a single cluster, and other stages of growth were distributed sequentially.

The distribution pattern of the points visualizing the mycelium metabolome in the fast-growing *L. vetlinianus* LE-BIN 3253 strain (**Figure 16d**) differed by an almost unexpressed discrepancy at 14, 21, and 35 days of growth. At the same time, the distribution of points describing the beginning of growth (7th day) and aging (50th days) was very different. Judging by the dynamics of changes in the mycelium biomass, the stationary phase of growth is reached only on day 35, as in other *L. vetlinianus* strains; nevertheless, the beginning of exponential growth, i.e. 7th day is distinguished by the most contrasting metabolic differences.

Thus, the isolation of the initial stage of exponential growth was general for all studied fungi. For all strains, with the exception of *L. vetlinianus* LE-BIN 3253 points characterizing the exponential growth phase on different days were distributed into separate clusters. Joint grouping of 14–21 and 35 days of growth *L. vetlinianus* LE-BIN 3253 into a single cluster is probably associated with striking changes in the metabolic network on the 7th and 50th days.

In systems biology, approaches are being developed to model the behavior of biological systems consisting of a large number of interacting variables, the dynamics of which is determined by numerous linear or non-linear relationships [18]. The method based on nonlinear time series analysis provides a global view of the dynamics of biological systems. A point in multidimensional space represents the state of a system with n-set of variables. Temporary changes in the system, formed by many points, converge to specific regions of space. The importance of this approach lies in providing a global view of the dynamics of a biological object, the experimental study of which is carried out using "-omic" approaches and assumes the factor of time [19].

According to the our results, the metabolomic analysis by GC–MS with subsequent processing of the metabolomic matrix by multivariate statistics methods makes it possible to reveal the biochemical changes of fungal mycelium during ontogenesis. The exponential phase of mycelium development is characterized by pronounced differences during of growth, which manifests itself both in the analysis of specific compounds and in the modeling of the statistical model of the metabolic network. The metabolomic network in the stationary growth phase is less susceptible to changes over time, and is also characterized by a lower dispersion of samples from one aging group.

The distribution of the main metabolites, depending on the stage of development are shown on the heat maps (**Figure 17**). At the initial stages of *D. tricolor* growth, amino acids and organic acids of the TCA cycle accumulated in the undifferentiated mycelium. Subsequently, at the stage of differentiated growth (7th - 21st days of growth), the concentrations of these compounds decreased and slightly increased on the 35th day of growth. On the 14th day of growth, the amount of glucose and some unidentified monosaccharides, as well as trisaccharides increased. On the 21st day of growth, mainly fructose, arabinose, sugar acids and some unidentified monosaccharides with retention time (Rt) 21–22 minutes accumulated. On the 35th day, the total amount of carbohydrates significantly decreased, but the accumulation of phosphate increased.

The dynamics of changes in the metabolomic profile of *L. vetlinianus* was very different from *D. tricolor*. While in *D. tricolor* the maximum concentrations of most metabolites were observed at the beginning of the exponential growth phase, in *L. vetlinianus* the concentration changes of small molecules varied greatly at all growth stages. The initial stage of *L. vetlinianus* growth was characterized by active accumulation of monosaccharides (glucose, fructose and mannose) and a number of unidentified sugars (Rt 20–22 min). Amino acids

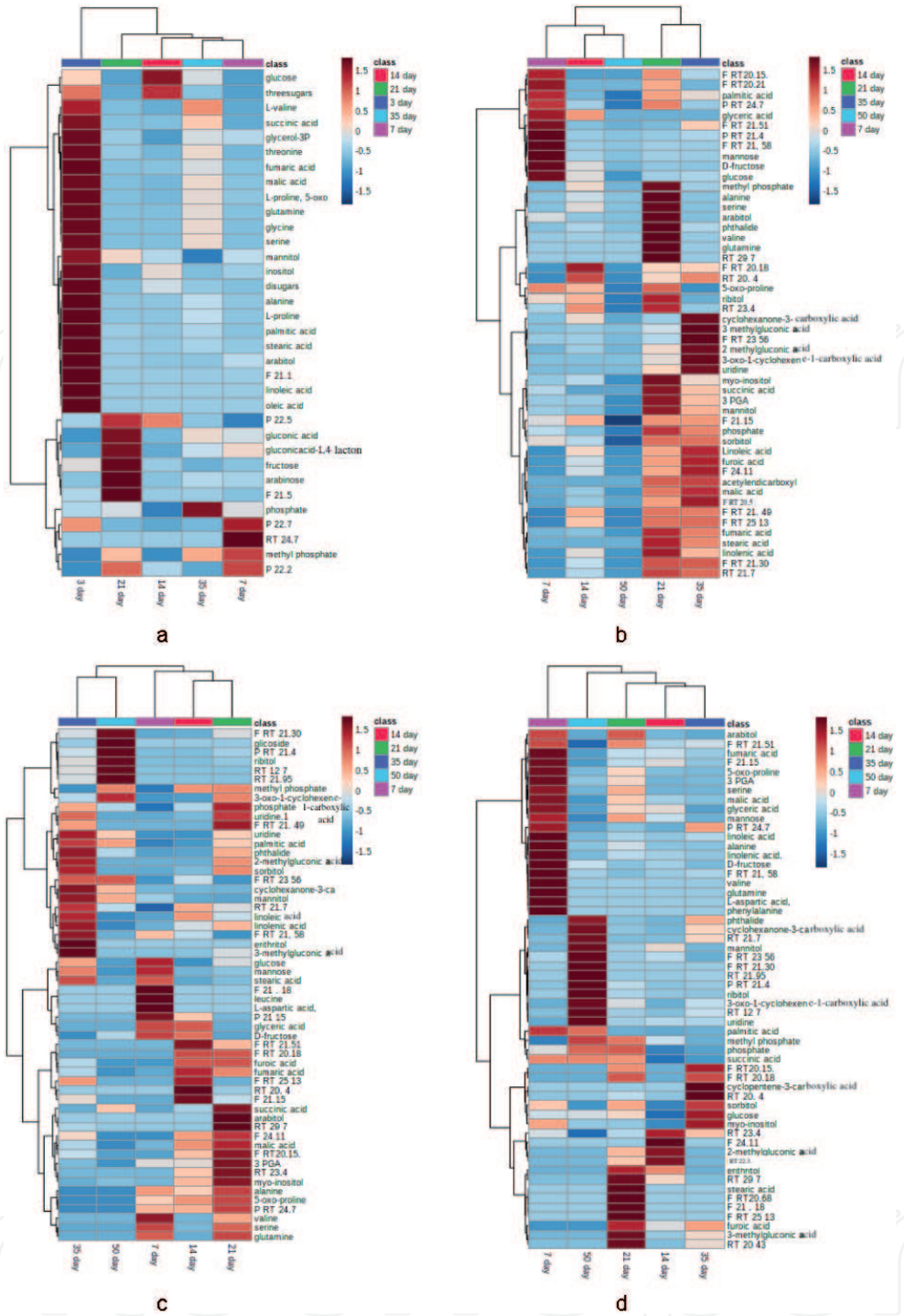


Figure 17.
Heat map of metabolites changes in the ontogenesis of fungi: a- D. tricolor *LE-BIN 2266; b -* L. vetlinianus *LE-BIN 2339; c -* L. vetlinianus *LE-BIN 2335;* d - L. vetlinianus *LE-BIN 3253 (for unidentified metabolites: Rt – Retention time, P- pyranose, F – Furanose).*

accumulated in young cultures of *L. vetlinianus* LE-BIN 2339 and LE-BIN 3253. In *L. vetlinianus* LE-BIN 2335, the maximum amino acid content was found on the 21st day of growth. In general, the strain LE-BIN 2335 with the least intensive growth was characterized by a very poor metabolomic profile on the 50th day of growth, while the faster-growing strains accumulated glycosides, sugars, and cyclic compounds on the 50th day of growth.

Cyclic compounds are mainly dominated in stationary phase of growth. But in strain *L. vetlinianus* LE-BIN 2335, the maximum content of phthalide was recorded in the mycelium on the 21st day of growth, and then it decreased. Perhaps this is due to

the excretion of phthalide into the medium or to the uneven distribution of phthalide clusters on the mycelium. In *L. vetlinianus* LE-BIN 3253, which forms the smallest number of aggregates, the maximum of phthalide concentration in the mycelium was recorded on the 50th day of growth. The isolation of the 50th day into a single cluster is probably associated with a significant accumulation of phthalide and other cyclic compounds from the mycelium of this strain. Fatty acids dominated mainly at the end of the exponential and the beginning of the stationary growth phase. Di- and three-saccharides dominated in the young (7 days-old) and in aging (50 days-old) cultures.

The strain differences of *L. vetlinianus* in the composition of the metabolomic profile, described above, are illustrated on the 7th and 35th days of growth, that is, in the exponential and stationary growth phases in **Figure 18**.

In the methanol extracts of *S. crispa* amino acids (alanine, valine, isoleucine, serine, threonine, proline), sugar alcohols (glycerol, ribitol), and sugars (fructose, glucose, melibiose, sucrose), organic acids and phosphate predominated at the beginning of the log phase (day 7) of growth. On the 14th day of growth, as in *D. tricolor*, the amount of monosaccharides (Rt 18–22 min) increased. At the same time, the qualitative composition of other metabolites in the *S. crispa* mycelium practically did not differ on the 7th and 14th days of growth. With the onset of the stationary growth phase (21 days), the total amount of sugars decreased.

On the 28th day of growth, the composition of the metabolomic profile of *S. craspa* did not differ much from the three-week-old cultures, but the total quantitative content of amino acids was lower than in younger cultures by 30–35%.

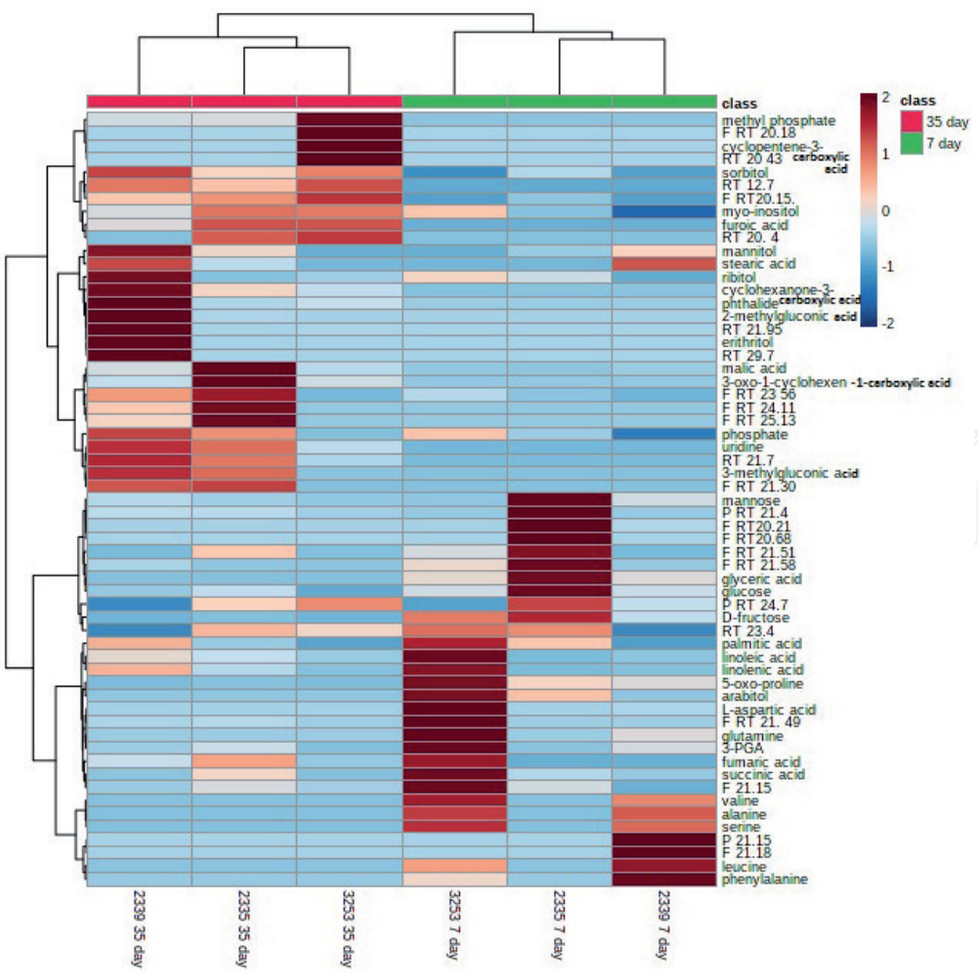


Figure 18.
Heat map of metabolites of L. vetlinianus *strains at different stages of ontogenesis (for unidentified metabolites: Rt – Retention time, P- pyranose, F – Furanose).*

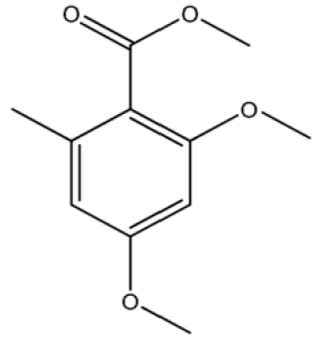
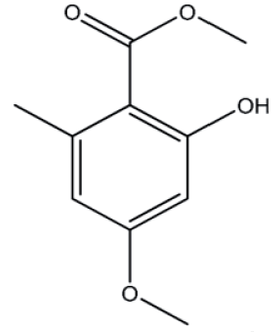
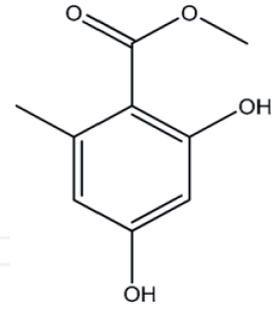
S. crispa **strain number**	**Cultivation time (days)**	**Compounds (μg/g mycelium)**		
		Methyl ester of sparassol	**Sparassol**	**Methyl ester of orsellinic acid**
				
LE-BIN 043	21	—	875.0 ± 97.3	4375.5 ± 802.8
	35	509.2 ± 55.4	2598.5 ± 505.1	124.1 ± 67.5
LE-BIN 2902	21	—	905.0 ± 108.4	4013.5 ± 604.1
	35	7.6 ± 2.2	4915.7 ± 678.2	2706.3 ± 303.3
LE-BIN 2967	21	—	181.9 ± 20.9	197.6 ± 19.1
	35	17.6 ± 4.4	1638.1 ± 80.8	424.9 ± 54.3

Table 1.
Composition of phenolic compounds in the mycelium of S. crispa *strains at different cultivation periods.*

The composition of phenolic compounds synthesized by *S. crispa* and their change with the age of culture is presented in the **Table 1**. In strains 2902 and 043, methyl-orsellinate dominated in the mycelium on the 21st day of growth; its amount was 4.5–6 times higher than the amount of sparassol. In *S. crispa* LE-BIN 2967, the concentrations of these compounds were the same. On the 35th day of growth in strain 2902, the amount of sparassol increased by 4 times, the amount of methyl orsellinate decreased by 35%. In strain LE-BIN 2967, the content of sparassol increased 9.5 times, and methyl orsellinate increased 2 times. In *S. crispa* LE-BIN 043, the amount of sparassol increased by 3 times, while the amount of methyl-orsellinate, on the contrary, decreased by more than 10 times. Also, all cultures with age in the medium accumulated methyl ester of sparassol and compounds with molecular weights of 298 and 356.

Phenolic compounds found in the culture of *L. vetlinianus* and *S. crispa* are derivatives of orsellinic acid and are widespread in fungi of various taxonomic groups. In recent studies, much attention is paid to the biosynthesis, as well as the biological activity of these compounds [20, 21]. Orsellinates are believed to be involved in allelopathic interactions, exhibiting a rather weak antifungal effect [16, 22, 23]. Orsellinic acid itself is formed in the acetate-malonate pathway and is an intermediate for many phenolic compounds [24]. Many natural phthalides display a variety of biological activities. It was stated that phthalides are responsible for numerous bioactivities, however their exact mechanism of action is not studied yet [25]. Phthalides are known to be responsible for antimicrobial, antifungal, cytotoxic, enzyme inhibiting, antitumor and plant hormone like activities [26, 27]. Also there are some results suggested that phthalides acted as inhibitors of oxidative and inflammatory stress.

4. Conclusion

According to the results, the metabolomic analysis by GC–MS with subsequent processing of the metabolomic matrix by multivariate statistics methods makes it possible to reveal the species and strain specificity of biochemical changes in fungal mycelium during ontogenesis. The exponential phase of mycelium growth is characterized by pronounced differences during of growth, which manifests itself both in the analysis of specific compounds and in the modeling of the statistical model of the metabolic network. The metabolomic network in the stationary growth phase is less susceptible to changes over time, and is also characterized by a lower dispersion of samples from one aging group. High concentrations of phthalide accumulated in *L. vetlinianus* and phenolic compounds synthesized by *S. crispa* revealed in some LE-BIN strains under certain cultivation conditions allow considering these strains as promising objects for biotechnology and the study of phenolic compounds metabolism in fungi.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

BSTFA	bis—3-methyl-silyl-3-F-acetamide
BWA	beer-wort agar
CE-MS	capillary electrophoresis-mass spectrometry
F	furanose
GC–MS	gas chromatography–mass spectrometry
LC–MS	liquid chromatography-mass spectrometry
MEA	malt extract agar
NMR	nuclear magnetic resonance
P	pyranose
PC	principal components
PCA	principal component analysis
PGA	phosphoglyceric acid.
Rt	retention time
TCA	tricarboxylic acid

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