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In vitro assessment of ligninolytic and cellulolytic activities for 14 *Agaricomycetes* species, new to Bryansk Oblast (European Russia)

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Summary. Xylotrophic basidiomycete fungi take a unique place in the functional structure of forest ecosystems because, on the one hand, they possess an extensive complex of enzymes involved in lignin modification and degradation, and, on the other hand, they synthesize enzymes capable of cellulose decomposition. Two groups of wood-destroying fungi are widely known in this respect – brown-rot fungi producing cellulolytic enzymes and white-rot fungi possessing not only cellulases but also ligninolytic oxidative enzymes. Currently, the physiology, biochemistry and genetics of basidial fungi are being actively researched. Thanks to the intensive development of bioinformatics resources, the transcriptomes, proteomes and secretomes of higher fungi are being analysed. At all three levels, both the biochemical mechanisms of degradation of different wood types by basidiomycetes are being studied and the spectrum of enzymes of the lignocellulolytic complex involved in these processes is being revealed. However, despite the identified general regularities, the specific mechanism of wood degradation is determined by individual peculiarities of fungal enzyme systems engaged in this process. The demand for lignocellulolytic complex enzymes for biotechnology purposes continues to grow steadily, since in addition to their ability to modify complex organic polymers, these enzymes break down a wide range of substrates of both natural and anthropogenic origin. New biotechnologically promising producers of ligninases and cellulases with high biodegradation potential are constantly searched for.

In this work, we present data on 14 species of xylotrophic basidiomycete fungi new to Bryansk Oblast, including little-known species *Conferticium ravum*, *Phlebia tremelloidea*, *Physisporinus crocatus*, with information on woody substrates and habitats occupied within the territory of the Bryanskiy Les State Nature Reserve, as well as data on general distribution and finds of these species in adjacent regions. Among hosts for revealed species of wood-inhabiting fungi, the main forest-forming trees such as *Betula pendula*, *Picea abies*, *Pinus sylvestris*, *Populus tremula*, and *Quercus robur* are noted. The majority of fungal cultures are obtained from basidiospores and basidiomata grown on coniferous wood. Herbarium specimens of the identified species are catalogued and stored in the Mycological Herbarium of the Komarov Botanical Institute RAS (LE), and fungal strains are deposited in the Komarov Botanical Institute Basidiomycetes Culture Collection (LE-BIN, St. Petersburg, Russia).

All collected specimens and pure cultures of studied aphyllophoroid fungi are determined and verified based on both microscopic features and molecular genetic data. Physiological and biochemical characterization, including assessment of their growth rate and detection of enzymatic activity by rapid screening, was given for 16 strains of wood-dwelling fungi. The linear growth rate was measured by culturing pure cultures on standard MEA medium. The activities of ligninolytic and cellulolytic enzyme complexes were registered using the application method that is widely applied for the primary biochemical screening of strains in many culture collections worldwide.

The agarized medium containing ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) was used for detection of oxidative enzyme activity while the cellulase activity was studied on the agarized medium with CMC (carboxymethyl cellulose). The strain LE-BIN 4006 of *Phlebia tremelloidea* showed high ligninolytic and cellulolytic potential and rapid colony growth rate. This strain can be recommended for further biotechnological applications. The strains LE-BIN 4422 of *Emmia latemarginata* and LE-BIN 3999 of *Phanerochaete livescens* demonstrated high cellulolytic complex enzyme activity despite the detected medium colony growth rate and medium oxidative enzyme activity. Thus, based on the screening results, three strains of fungi belonging to the order *Polyporales* have been identified as being of practical interest for use in biotechnological delignification and remediation processes. The importance of screening studies on active enzymatic producers among not only widespread taxa, but also by including rare and little-collected species of fungi are demonstrated.

The article contains 2 figures, 1 table, 48 references.

Keywords: Basidiomycota; biodiversity; cellulases; DNA barcodes; ligninases; pure culture; xylotrophic fungi

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Научная статья
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Оценка *in vitro* лигнинолитической и целлюлолитической активности 14 видов *Agaricomycetes*, новых для Брянской области (Европейская Россия)

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Аннотация. Ксилотрофные базидиальные грибы занимают уникальное место в функциональной структуре лесных экосистем, поскольку, с одной стороны, обладают обширным комплексом ферментов, участвующих в модификации и деградации лигнина, а с другой стороны, синтезируют ферменты, способные разлагать целлюлозу. В этом отношении широко известны две группы

дереворазрушающих грибов – грибы бурой гнили, продуцирующие целлюлолитические ферменты, и грибы белой гнили, обладающие не только целлюлазами, но и лигнинолитическими окислительными ферментами. В настоящее время активно изучаются физиология, биохимия и генетика базидиальных грибов. Благодаря интенсивному развитию биоинформационных ресурсов анализируются транскриптомы, протеомы и секретомы высших грибов. На всех трех уровнях изучаются как биохимические механизмы деградации базидиомицетами различных древесных пород, так и выявляется спектр ферментов лигноцеллюлолитического комплекса, участвующих в этих процессах. Однако, несмотря на выявленные общие закономерности, конкретный механизм разрушения древесины определяется индивидуальными особенностями ферментных систем грибов, участвующих в этом процессе. Спрос на ферменты лигноцеллюлозного комплекса для целей биотехнологии продолжает неуклонно расти, поскольку помимо способности модифицировать сложные органические полимеры эти ферменты разлагают широкий спектр субстратов как природного, так и антропогенного происхождения. В настоящее время продолжается поиск новых биотехнологически перспективных продуцентов лигниназ и целлюлаз, обладающих высоким биодеградационным потенциалом.

В данной работе представлены сведения о 14 новых для Брянской области видах ксилотрофных базидиомицетов, в том числе малоизвестных видах *Conferticium ravum*, *Phlebia tremelloidea*, *Physisporinus crocatus* и информация о занимаемых древесных субстратах и местообитаниях на территории государственного природного заповедника «Брянский лес», а также данные об общем распространении и находках этих видов в соседних регионах. Среди древесных субстратов для выявленных видов деревообитающих грибов отмечены основные лесообразующие породы – *Betula pendula*, *Picea abies*, *Pinus sylvestris*, *Populus tremula*, *Quercus robur*. Большинство культур грибов получено из базидиоспор и базидиом, собранных на древесине хвойных пород. Гербарные образцы идентифицированных видов каталогизированы и хранятся в Микологическом гербарии Ботанического института им. В.Л. Комарова РАН (LE), а штаммы грибов депонированы в Коллекции культур базидиомицетов Ботанического института им. В.Л. Комарова (LE-BIN, Санкт-Петербург, Россия).

Все собранные образцы и чистые культуры изученных афиллофороидных грибов определены и верифицированы на основе как микроскопических признаков, так и молекулярно-генетических данных. Для 16 штаммов дереворазрушающих грибов дана физиолого-биохимическая характеристика, включающая оценку скорости их роста и выявление ферментативной активности методом экспресс-скрининга. Линейную скорость роста определяли путем культивирования чистых культур на стандартной среде MEA. Активность лигнинолитических и целлюлолитических ферментных комплексов регистрировали с помощью метода, широко применяемого для первичного биохимического скрининга штаммов во многих мировых коллекциях культур. Для выявления окислительной активности ферментов использовали агаризованную среду, содержащую ABTS (2,2'-азино-бис 3-этилбензотиазолин-6-сульфокислота), а целлюлазную активность изучали на агаризованной среде с добавлением карбоксиметилцеллюлозы. Штамм LE-BIN 4006 *Phlebia tremelloidea* показал высокий лигнинолитический и целлюлолитический потенциал и быструю скорость роста колоний. Этот штамм может быть рекомендован для дальнейшего биотехнологического использования. Штаммы LE-BIN 4422 *Emmia latemarginata* и LE-BIN 3999 *Phanerochaete livescens* продемонстрировали высокую активность ферментов целлюлолитического комплекса, несмотря на среднюю скорость роста колоний и среднюю активность окислительных ферментов. Таким образом, на основе результатов скрининга выявлены три штамма грибов из порядка *Polyporales*, представляющие практический интерес для использования в биотехнологических процессах делигнификации и

ремедиации. Показана значимость скрининговых исследований активных продуцентов ферментов не только среди широко распространенных таксонов, но и за счет включения редких и малоизученных видов грибов.

Ключевые слова: Basidiomycota, биоразнообразие, целлюлазы, ДНК-штрих-коды, лигниназы, чистая культура, ксилотрофные грибы

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Introduction

Basidial fungi are an important part of forest communities due to their involvement in the biodegradation of plant residues and the production of a unique set of biologically active substances. The lignocellulosic biomass of woody plants is known to consist of three main components: cellulose (40-60%), hemicellulose (20-40%) and lignin (10-25%) [1]. Wood decomposition is largely driven by xylotrophic *Agaricomycetes*, an essential group of wood-inhabiting fungi with a peculiar range of ligninolytic and cellulolytic enzyme complexes.

The extracellular enzyme systems of basidial fungi include a spectrum of multiple forms of oxidoreductases that interact with lignocellulose (heme-containing, flavin-containing and copper-containing enzymes). The exogenous enzymes of the ligninolytic complex represented by laccases (EC 1.10.3.2), lignin peroxidases (EC 1.11.1.14), versatile peroxidases (EC 1.11.1.16), and manganese peroxidases MnP (EC 1.11.1.13) are key agents in the process of lignin modification, the most resistant to chemical and microbiological destruction of wood biopolymer [2, 3]. White-rot basidiomycete fungi perform decomposition of lignin *via* formation of radicals by oxygen (laccase) or hydrogen peroxide (some peroxidases) [4].

Cellulose degradation in the plant cell wall is carried out by endocellulases (EC 3.2.1.4), exocellulases (cellobiohydrolases, EC 3.2.1.91; glucanohydrolases, EC 3.2.1.74), and beta-glucosidases (EC 3.2.1.21). The functional role of cellulolytic enzymes secreted by lignicolous fungi is the hydrolytic degradation of the main wood polysaccharides (cellulose, hemicellulose) to oligo-, di- and monosaccharides [5]. Cellulose and hemicellulose are decomposed

predominantly by brown-rot fungi, but also by wood-destroying white-rot fungi and soft-rot ones [6].

Different species of xylotrophic fungi produce both single enzymes and multienzyme compositions. Indeed, white rot basidiomycetes are capable of producing multiple laccase isoenzymes (i.e., products of different non-allelic genes) that can perform different functions. Currently, studies are carried out not only to characterise laccases isolated from different fungal species, but also comparative investigations of laccase isoenzymes obtained from the same fungus to establish the range of biological functions of this group of enzymes [7]. In recent years, the study of the regulation mechanisms of laccase and peroxidase gene expression has gained interest due to the need to understand the physiological role of various isoforms produced by fungi. At present, the general mechanisms of transcriptional regulation of oxidative enzymes are not completely clear. Some authors suggest that the existence of numerous genes producing isoforms is a consequence of the diversity of physiological functions performed by laccases and peroxidases throughout the fungal life cycle (delignification, fruiting body development, pathogenesis, pigment formation during the period of asexual reproduction, competitive interactions, etc.) [8-10].

Isolation and study of laccase isoforms with new physicochemical properties can help us not only in understanding physiological regulatory mechanisms of biosynthesis of these enzymes, but also in their application for biotechnological purposes. Therefore, the role of individual enzymes and multienzyme complexes in the processes of biotransformation and biodegradation of lignocellulosic substrate remains a subject of study for the last several decades.

Since the enzyme assemblages of the lignocellulolytic complex probably reflect the adaptation of *Agaricomycetes* to unique ecological niches, this group of fungi has enormous potential for applications in various industries [11-15, et al.]. The search for wood-inhabiting fungi with high lignin- and cellulolytic potential for practical purposes is conducted in two interrelated directions: revealing new fungal producers with high enzymatic activity and the subsequent selection of highly effective inducers and promoters of these enzymes [16].

This article aims to investigate the enzymatic potential of xylotrophic basidiomycete fungi registered for the first time for Bryansk Oblast, including rare species, and focuses on the selection of the most promising strains for use in biotechnological processes.

Materials and methods

Data sampling. Basidiocarps of lignicolous aphyllophoroid fungi were collected at different types of forests on the territory of the Bryanskiy Les State Nature Reserve (Bryansk Oblast, European part of Russia) during route surveys in 2015–2021. For each sample, the category of the colonized substrate, tree species, forest community type, date of collection and geographical coordinates were noted. The geographical coordinates of studied localities were measured by the Garmin 64st GPS navigator.

Morphological identification of fruit bodies. Microscopic identification of dried fungal specimens were performed using a AxioScope A1 microscope (Carl Zeiss, Germany), a LOMO Mikmed-6 microscope (Russia) with a standard set of chemicals (5% KOH, Melzer's reagent, 0.1% Cotton Blue) based on key monographs on European poroid and corticioid fungi [17-19] as well as some modern taxonomy articles. The names of the fungal species are given according to the Index Fungorum database [20].

Isolation and verification of fungal pure cultures. The isolation of *Agaricomycetes* in pure culture by solid-phase cultivation, as well as the subsequent microscopic characterisation of the isolates, was carried out according to the previously described techniques [21, 22].

Fungal genomic DNA was amplified directly from 14-day-old pure cultures, which were grown on standard MEA medium (1.5% w/v malt extract "Conda" (Madrid, Spain), pH 5.8, and 2% w/v agar "Difco" (Kansas City, MO, USA)) in the dark at 25°C, with the Phire Plant Direct PCR Master Mix Kit (Thermo Fisher Scientific, Lithuania). A complete ITS1–5.8S–ITS2 region of nrDNA was amplified by the primer pair ITS1F/ITS4B [23, 24]. PCR products were purified using the CleanMag DNA (Evrogen, Moscow, Russia) purification kit, and then sequenced with the BrilliantDye Terminator v3.1 Cycle Sequencing Kit (NimaGen, Nijmegen, the Netherlands). Sequencing products were purified with the Nimagen D-Pure Dye-Terminator Cleanup kit before being analyzed on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Raw data were edited and assembled in MEGA 6 [25].

The newly obtained sequences were compared with the available sequences using a megablast search in the NCBI database, and then newly generated sequences were deposited into NCBI GenBank [26].

Growth measurement. To obtain data on linear growth rate, the strains were grown in Petri dishes with a diameter of 90 mm on standard MEA medium in the dark at 25°C. Inoculation of strains was carried out with mycelial discs (7 mm diam.) placed on the nutrient medium in the centre of a Petri dish with the mycelial layer down. The growth rate was studied for 5 weeks by measuring the colony diameter in two mutually perpendicular directions every two days starting from the third day until a Petri dish was completely overgrown. The growth of strains was characterised by the colony radius by 7, 14, 21 and 28 days, assessing the growth rate by the rate of cup overgrowth: fast growth (F) — 1 week, medium (M) — 2–3 weeks, and slow (S) — 4 weeks or more.

Detection of enzymatic activity. There known several qualitative tests for rapid detection of ligninases (syringaldazine well test [27], bromophenol blue plate assay [28], guaiacol agar plate assay [29]) and cellulases (filter paper degradation), dye diffusion from a cellulose-dye complex (cellulose azure agar), cellulose agar clearance (cellulose agar), esculin plus iron agar (esculin agar) [30]. In addition, researchers use a semi-quantitative fluorometric method to determine the activity of hydrolases and oxidases in the total homogenate, based on the interaction of enzymes with specific substrates bound to fluorochromes – methylumbelliferone or aminomethyl coumarin [31]. At the same time, within this

study the activities of the ligninolytic and cellulolytic enzyme complexes in the investigated strains were registered using the application method [22, 32]. This method is widely used in many collections worldwide during primary biochemical screening of culture strains. Moreover, this technique is included in the list of standard operating procedures of the Komarov Botanical Institute Basidiomycetes Culture Collection (LE-BIN).

The inoculum was obtained by growing the strains on MEA at 25°C for 2 weeks. Then, mycelial discs (7 mm in diam.) from the marginal zone of an actively growing colony were placed with the mycelial layer upwards in three units in a 90 mm Petri dish with medium containing: a) 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (0.1% w/v ABTS "Sigma" (St. Louis, MO, USA) and 2.0% w/v agar "Difco") for detection of oxidative enzyme activity (OA), or b) carboxymethyl cellulose (1.0% w/v CMC "Chemapol" (Praha, Czech Republic) and 1.0% w/v agar "Difco") for detection of cellulase activity (CA).

The oxidoreductase activity was measured in 48 hours after inoculation, based on the ability of the strains to oxidize the substrate containing ABTS with the appearance of emerald-green staining. The qualitative activity of cellulolytic enzymes in fungal cultures was recorded in 48 hours after inoculation on CMC agar by the presence of a lightened zone around the inoculums. The clear zone was detected using a solution of I in KI (0.5% I in 2% KI). To determine the intensity of the medium colour changes or lightening reactions of the medium, the following criteria were used: the diameter of the coloured/lightening zone is 10 – <15 mm – slightly positive reaction (low activity); the diameter of zone is ≥15 – 25 mm – positive reaction (medium activity); the diameter of zone is more than 25 mm – strongly positive reaction (high activity).

The cultivation in all experiments was performed in at least three repetitions. Microsoft Excel and OriginPro 7.5 software were used for statistical data processing.

Results and discussion

A total of 14 species of aphyllophoroid fungi from five orders of the class *Agaricomycetes* (*Basidiomycota*) have been revealed for the first time in Bryansk Oblast as a result of the mycological studies carried out within the Bryanskiy Les State Nature Reserve. Among them 10 fungal species are shared with Oryol Oblast, three species – with Kaluga Oblast, two species – with Kursk Oblast, and one species – with Smolensk Oblast (Fig. 1.). At the same time, four species (*Fibroporia gossypium*, *Leptoporus mollis*, *Phlebia tremelloidea*, *Physisporinus crocatus*) have not been registered to date in neighbouring Russian regions.

Details of the recorded species locations are given below, with data on substrata and habitats, as well as numbers of herbarium specimens stored in the Mycological Herbarium of the Komarov Botanical Institute RAS (LE), and strains kept in the Komarov Botanical Institute Basidiomycetes Culture Collection (LE-BIN, St. Petersburg, Russia). For each species, a brief ecological summary, information on the general distribution and finds in adjacent regions, and the

physiological and biochemical characteristics of strains isolated in pure culture are provided.

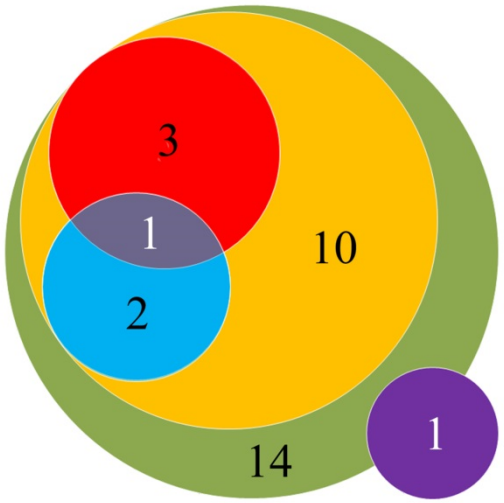

Fig. 1. The numbers of fungal species new to Bryansk Oblast (green circle), common to adjacent Russian regions: Kaluga (3 species, red circle), Kursk (2 species, blue circle), Oryol (10 species, yellow circle), Smolensk (1 species, violet circle) Oblasts

Class *Agaricomycetes*
Order *Auriculariales*

Aporpium canescens (P. Karst.) Bondartsev & Singer (Fig. 2, *A*) — on a fallen trunk of *Populus tremula* in herb-rich aspen forest mixed with ash and maple, 07.08.2015, LE-BIN 3585 (*ex basidiospores*). This deciduous-dwelling fungus has been delimited from an American taxon *A. caryae* (Schwein.) Teixeira & D.P. Rogers based on the differences in pore and spore sizes, as well as in ITS nrDNA sequences [33]. *A. canescens* is quite common in the European Russia, and in adjacent Russian regions it is known from Kaluga and Oryol Oblasts [34]. The strain LE-BIN 3585 of *A. canescens* showed oxidative enzymes with high activity (24.8 ± 2.4 mm) and cellulolytic enzymes with low activity (10.4 ± 0.4 mm) in combination with medium growth rate.

Order *Boletales*

Serpula himantioides (Fr.) P. Karst. — on a fallen trunk of *Pinus sylvestris* in herb-rich spruce forest mixed with oak and pine, 07.10.2021, LE F-342503, LE-BIN 4757 (*ex basidiocarp*). The fungus causes an intense brown rot, mainly developing on various coniferous woods in humid boreal forests. *S. himantioides* prefers old-growth and relatively undisturbed forests, but also there are occasional records in wood construction material [35]. Widespread species in Russia, and in neighbouring regions it was revealed in Oryol Oblast [36]. The strain LE-BIN 4757 of *S. himantioides* was characterised by the absence of oxidoreductase

production, which is characteristic of brown rot fungi, medium cellulase activity (22.0 ± 0 mm) and medium rate of overgrowth in Petri dishes.

Order *Hymenochaetales*

Xylodon flaviporus (Berk. & M.A. Curtis ex Cooke) Riebesehl & Langer (Fig. 2, *B*) — on a fallen trunk of *Betula pendula* in herb-rich aspen forest mixed with birch and maple, 22.08.2017, LE F-342508, LE-BIN 3975 (*ex basidiospores*). It is a widely distributed species, more common in Central and Southern Europe [18], as well as in the European part of Russia, including Kaluga [37] and Oryol [38] Oblasts. This white-rot fungus grows on fallen trunks and large branches of varied deciduous trees, preferably on *Betula* spp. The strain LE-BIN 3975 of *X. flaviporus* exhibited medium levels of both ligninolytic (16.8 ± 1.4 mm) and cellulolytic (18.1 ± 0.9 mm) enzyme activities, and a slow growth rate.

Order *Polyporales*

Antrodiella serpula (P. Karst.) Spirin & Niemelä — on a fallen trunk of *Salix caprea* in herb-fern spruce mixed forest with aspen, 24.08.2017, LE F-342496, LE-BIN 3998 (*ex basidiocarp*). This fungal species inhabits on a wide range of deciduous trees, and it exhibits successional relationships with *Mensularia* species [18]. In adjacent Russian regions *A. serpula* is known for Kaluga [39], Kursk [40], and Oryol [38] Oblasts. The strain LE-BIN 3998 of *A. serpula* showed a maximum oxidative enzyme activity (32.2 ± 1.2 mm) with a complete absence of cellulolytic enzyme activity and a slow growth rate.

Ceriporia bresadolae (Bourdot & Galzin) Donk (Fig. 2, *C*) — on a fallen branch of *Pinus sylvestris* in blueberry-mosses pine forest, 24.08.2017, LE F-342497, LE-BIN 3994 (*ex basidiospores*); on a fallen branch of *Pinus sylvestris* in herb-mosses pine forest with spruce, 08.10.2021, LE F-342504, LE-BIN 4766 (*ex basidiospores*). The species is widely distributed in temperate and boreal zones of the northern hemisphere, but for a long time its records have been misidentified as a coniferous-dwelling representatives of *C. purpurea* s. lato [41]. Decorticated hard twigs of *Pinus* ssp. are a favourable substrate of *C. bresadolae* along with fallen, tough branches and logs of other coniferous trees. The fungus has a scattered distribution in Russia, including Oryol Oblast among nearby regions [36]. Both *C. bresadolae* isolates (LE-BIN 3994 and LE-BIN 4766) were characterised by medium ligninolytic (22.5 ± 1.0 and 17.7±0.3 mm respectively) and cellulolytic complex enzyme activities (18.2 ± 1.4 and 18.5 ± 0.7 mm accordingly) and medium colonization rate of Petri dishes.

Crustoderma dryinum (Berk. & M.A. Curtis) Parmasto — on a fallen trunk of *Picea abies* in herb-mosses pine forest with spruce, 08.10.2021, LE F-342505, LE-BIN 4770 (*ex basidiospores*); on a fallen trunk of *Picea abies* in herb-sphagnum spruce forest with pine, 09.10.2021, LE F-342507, LE-BIN 4765 (*ex basidiospores*). The fungus grows as a brown-rot saprotroph mostly on coniferous wood at the middle stages of decay. *C. dryinum* is considered as a specialist species of old-growth spruce forests in North-Western European Russia [42]. The species is distributed among boreal zone of Russia, and in adjacent regions it was

registered for Oryol Oblast [43]. For both strains of *C. dryinum* (LE-BIN 4765 and LE-BIN 4770), the absence of oxidative enzyme activity and high cellulolytic activity (28.0 ± 0 and 29.1 ± 1.4 mm, correspondingly) and slow growth rate were observed.

Emmia latemarginata (Durieu & Mont.) Zmitr., Spirin & Malysheva — on a fallen trunk of *Betula pendula* in blueberry pine forest, 19.08.2020, LE F-342502, LE-BIN 4422 (*ex basidiospores*). This fungus grows mainly on dead wood of deciduous trees, including buried at the ground or burned woody remnants. Widespread species in Russia, and in neighbouring regions there known records from Kursk [44] and Oryol [38] Oblasts. The strain LE-BIN 4422 of *E. latemarginata* showed medium ligninase activity (19.0 ± 0.5 mm), high cellulase activity (27.0 ± 1.4 mm) and medium growth rate.

Fibroporia gossypium (Speg.) Parmasto — on a fallen trunk of *Picea abies* in herb-mosses pine-dominated forest with spruce, 08.10.2021, LE F-342506, LE-BIN 4771 (*ex basidiocarp*). This wood-decaying fungus causes an aggressive brown rot in both nature conditions and humid unventilated indoors. In forests the species develops on coniferous trunks and stumps, sometimes with basidiocarps covering a litter around the base of dead trees [44]. *F. gossypium* is a species with a sporadic distribution but more frequent in forests with moist and swamp soils [18]. Hitherto, the species has not been recorded in adjacent Russian regions, but it was found in different regions of the European part of Russia, the Urals, Siberia, and the Russian Far East [44]. The strain LE-BIN 4771 of *F. gossypium* expressed no oxidoreductase activity and a medium level of cellulolytic activity (21.8 ± 0.2 mm) with a medium growth rate.

Leptoporus mollis (Pers.) Quél. (Fig. 2, *D*) — on a fallen trunk of *Pinus sylvestris* in blueberry pine forest, 19.08.2020, LE F-342501, LE-BIN 4429 (*ex basidiospores*). The species is widely distributed in Russia [36], but it was not found in the regions adjacent to Bryansk Oblast. This lignicolous fungus grows on large-scale lying trunks and stumps of various coniferous trees (*Abies*, *Larix*, *Picea*, *Pinus*), and especially often on *Pinus* spp. [18]. The strain LE-BIN 4429 of *L. mollis* revealed medium levels of ligninase (19.5 ± 0.6 mm) and cellulase (19.4 ± 0.7 mm) activities together with a slow rate of overgrowth in Petri dishes.

Phanerochaete livescens (P. Karst.) Volobuev & Spirin (Fig. 2, *E*) — at base of a dry standing tree of *Quercus robur* in herb-rich aspen forest with oak, 24.08.2017, LE F-342498, LE-BIN 3999 (*ex basidiospores*). This corticioid fungus is widespread in nemoral and hemiboreal zones of Eurasia, inhabiting various angiosperm hosts, such as *Acer*, *Alnus*, *Carpinus*, *Corylus*, *Fagus*, *Padus*, *Populus*, *Quercus*, *Ulmus* [45]. The species is registered for 13 regions of Russia, and among neighbouring regions it was recorded in Oryol Oblast [36]. The strain LE-BIN 3999 of *P. livescens* demonstrated medium level of oxidoreductase activity (21.3 ± 1.2 mm) and the highest level of cellulase activity out of all strains studied (42.7 ± 1.3 mm). The rate of overgrowth of Petri dishes by colonies of this strain was middle.

Phlebia tremelloidea (Bres.) Parmasto (Fig. 2, *F*) — on a fallen trunk of *Quercus robur* in oak forest with maple and hazel, 26.08.2017, LE F-342499, LE-BIN 4006 (*ex basidiospores*). This phlebioid fungus is known as a rare slowly-growing

boreonemoral species, which is adapted to colonization of homogeneous substrata, like decorticated wood or uncracked bark [46]. *P. tremelloidea* is collected from only six regions in the European part of Russia, including Arkhangelsk, Kirov, Leningrad, Nizhny Novgorod, Tver Oblasts, and the Republic of Mordovia [37]. The strain LE-BIN 4006 of *P. tremelloidea* combined a high production of lignocellulolytic enzymes (29.3 ± 1.1 and 25.4 ± 1.0 mm, respectively) with a fast growth rate.

Physisporinus crocatus (Pat.) F. Wu, Jia J. Chen & Y.C. Dai — on a fallen trunk of *Picea abies* in herb-rich black alder forest with maple, oak and spruce, 18.08.2020, LE F-342500, LE-BIN 4426 (*ex basidiospores*). The fungus grows on stumps, dead lying trees and coarse woody debris of conifers, more rarely on broad-leaved trees, in moist forests and alluvial environments [18]. *P. crocatus* is considered as an indicator species of forests with a minimal anthropogenic impact in North-Western European Russia [42]. The species is not known to be found in neighbouring regions of Russia. The strain LE-BIN 4426 of *P. crocatus* was highly active by the ABTS oxidoreductase test (25.6 ± 0.8 mm) and medium active by the CMC cellulase test (18.3 ± 0.6 mm) and had a slow growth rate.

Order *Russulales*

Conferticium ravum (Burt) Ginns & G.W. Freeman (Fig. 2, *G*) — on a fallen trunk of *Populus tremula* in herb-rich aspen forest mixed with ash and maple, 07.08.2015, LE-BIN 3587 (*ex basidiospores*). The growth on dead wood of *Populus* spp. is one of distinguishing ecological features for this fungus, which causes a white rot. The species is rare in Europe [17], with a sporadic distribution in Russia [43]. In adjacent Russian regions, *C. ravum* was registered in Oryol Oblast [38]. Despite the fast growth rate, the strain LE-BIN 3587 of *C. ravum* was the lowest in oxidative activity (10.4±0.6 mm) and detected no cellulolytic activity.

Laxitextum bicolor (Pers.) Lentz (Fig. 2, *H*) — on a fallen trunk of *Populus tremula* in herb-fern aspen forest with birch, 22.08.2017, LE F-342495, LE-BIN 3971 (*ex basidiospores*). Common and widespread species in Europe [17], inhabiting humid herb-rich deciduous and mixed forests. The preferable substrata are dead wood of *Alnus*, *Betula*, *Corylus*, *Fraxinus*, *Populus*, *Quercus*, and *Sorbus* [47]. *L. bicolor* was recorded in Oryol [38] and Smolensk [48] Oblasts among neighbouring Russian regions. The strain LE-BIN 3971 of *L. bicolor* demonstrated medium activity of ligninolytic complex enzymes (20.1 ± 0 mm) and no production of cellulolytic complex enzymes at medium growth rate.

The production of ligninases during solid-phase cultivation was found to be noted in all studied strains related to white rot fungi belonging to the orders *Auriculariales*, *Polyporales* and *Russulales*. The strains of brown rot fungi, *Serpula himantioides* LE-BIN 4757 and *Fibroporia gossypium* LE-BIN 4771, showed medium growth rate coupled with medium cellulolytic activity, and the slow-growing strains *Crustoderma dryinum* LE-BIN 4765 and LE-BIN 4770 exhibited high cellulase activity. Similarly, high cellulase activity was observed in fungi of the order *Polyporales*, in particular, in the medium-growing *Emmia latemarginata* LE-BIN 4422 and *Phanerochaete livescens* LE-BIN 3999 as well as the fast-growing strain *Phlebia tremelloidea* LE-BIN 4006 (Table).

Fig. 2. Basidiomata of some fungal species studied: *A* - *Aporpium canescens*, *B* - *Xylodon flaviporus*, *C* - *Ceriporia bresadolae*, *D* - *Leptoporus mollis*, *E* - *Phanerochaete livescens*, *F* - *Phlebia tremelloidea*, *G* - *Conferticium ravum*, *H* - *Laxitextum bicolor*. Scale bar - 1 cm

Results of the express assays of oxidative and cellulolytic enzymes of *Agaricomycetes* pure cultures studied

Strains	Species	GenBank accession number	Rot type	Growth rate	Enzymatic activity (diameter, mm)		Substrate	Voucher specimen
					ABTS	CMC		
AURICULARIALES								
LE-BIN 3585	*Aporpium canescens*	OP700293	WR	M	24.8±2.4	10.4±0.4	*Populus tremula*	—
BOLETALES								
LE-BIN 4757	*Serpula himantioides*	OL764365	BR	M	n/d	22.0±0	*Pinus syl-vestris*	LE F-342503
HYMENOCHAETALES								
LE-BIN 3975	*Xylodon flaviporus*	OP700296	WR	S	16.8±1.4	18.1±0.9	*Betula pendula*	LE F-342508
POLYPORALES								
LE-BIN 3998	*Antrodiella serpula*	OP700297	WR	S	32.2±1.2	n/d	*Salix caprea*	LE F-342496
LE-BIN 3994	*Ceriporia bresadolae*	—	WR	M	22.5±1.0	18.2±1.4	*Pinus syl-vestris*	LE F-342497
LE-BIN 4766		OP700304	WR	M	17.7±0.3	18.5±0.7	*Pinus syl-vestris*	LE F-342504
LE-BIN 4765	*Crustoderma dryinum*	OP700303	BR	S	n/d	28.0±0	*Picea abies*	LE F-342507
LE-BIN 4770		OP700305	BR	S	n/d	29.1±1.8	*Picea abies*	LE F-342505
LE-BIN 4422	*Emmia late-marginata*	OP700300	WR	M	19.0±0.5	27.0±1.4	*Betula pendula*	LE F-342502
LE-BIN 4771	*Fibroporia gossypium*	OL840818	BR	M	n/d	21.8±0.2	*Picea abies*	LE F-342506
LE-BIN 4429	*Leptoporus mollis*	OP700302	WR	S	19.5±0.6	19.4±0.7	*Pinus syl-vestris*	LE F-342501
LE-BIN 3999	*Phanero-chaete livescens*	OP700298	WR	M	21.3±1.2	42.7±1.3	*Quercus robur*	LE F-342498
LE-BIN 4006	*Phlebia tremelloidea*	OP700299	WR	F	29.3±1.1	25.4±1.0	*Quercus robur*	LE F-342499
LE-BIN 4426	*Physispori-nus crocatus*	OP700301	WR	S	25.6±0.8	18.3±0.6	*Picea abies*	LE F-342500
RUSSULALES								
LE-BIN 3587	*Conferticium ravum*	OP700294	WR	F	10.4±0.6	n/d	*Populus tremula*	—
LE-BIN 3971	*Laxitextum bicolor*	OP700295	WR	M	20.1±0	n/d	*Populus tremula*	LE F-342495

Note. Rot type: BR – brown rot, WR – white rot; Growth rate: F – fast, M – medium, S – slow; ABTS – 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid; CMC – carboxymethyl cellulose; n/d – not detected.

The ABTS agar test showed typically high oxidative enzyme activity in basidiomycete fungi belonging to the order *Polyporales* [32], namely the slow-growing strains of *Antrodiella serpula* LE-BIN 3998 and LE-BIN 4426 *Physisporinus crocatus*, and the fast-growing *Phlebia tremelloidea* LE-BIN 4006. The strain of *Aporpium canescens* LE-BIN 3585 (order *Auriculariales*) also revealed rather high oxidoreductase activity with a medium growth rate. The lowest level of production of ligninolytic complex enzymes was detected in a representative of the order *Russulales*, the fast-growing strain of *Conferticium ravum* LE-BIN 3587. At the same time, no cellulolytic enzyme activity was detected in the latter isolate.

As a result of the screening performed, a representative of the order *Polyporales*, *Phlebia tremelloidea* LE-BIN 4006, combining high ligninolytic and cellulolytic potential (diameter of the color-changing zone of agarized medium – more than 25 mm) with a rapid growth rate, was selected. This strain can be recommended for use in bioconversion and bioremediation technologies. Strains of fungi from the order *Polyporales*, *Emmia latemarginata* LE-BIN 4422 and *Phanerochaete livescens* LE-BIN 3999, were characterized by medium growth rate, high cellulase production, and medium ligninase activity, that also allows us to refer these strains to biotechnological potential. The strain of *Physisporinus crocatus* LE-BIN 4426 with high oxidative and medium cellulolytic enzyme activities could not be used for biotechnology purposes, since this strain has a slow growth rate.

Conclusions

The data obtained on 16 isolates of 14 xylotrophic basidiomycete species registered in Bryansk Oblast for the first time made it possible to determine strains-producers promising for applied biotechnology based on delignification processes. Besides rather common species of aphyllophoroid fungi, rare and poorly known species demonstrated either high values of individual enzymatic systems activity (e.g. cellulase in *Crustoderma dryinum*) or significant levels of all lignocellulolytic complex enzymes (*Phanerochaete livescens*, *Phlebia tremelloidea*, *Physisporinus crocatus*). Therefore, screening studies aimed at identifying fungal strains with high enzymatic activity should be carried out not only among widespread taxa, but also by including rare, narrow-ranging and/or stenobiont species in the analysis. In addition, *ex situ* isolation of rare xylotrophic basidiomycete fungi ensures the conservation and genetic stability of these fungal species.

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