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Fungi Surviving on Treated Wood and Some of Their Physiological Properties

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Wood is still widely used as a construction material but in spite of great assortment of antifungal chemicals, incidents of treated-wood damage still happen. The objective of the present investigation was to identify the fungal species that survived after wood treatment and make a primary screening of some physiological properties important for fungi in wood colonization. Fungal strains from *Alternaria, Cladosporium, Fusarium, Penicillium, Phoma, Trichoderma* and *Ulocladium* genera were isolated and identified. The primary endoglucanase and phenoloxidase activity discoloration assays showed that wood colonization ability of fungi isolated from treated wood differed among the strains and some of them had not only cellulolytic but even ligninolytic activity as well. The spectrophothometric analysis of fungal enzymatic activity on wood showed that *Alternaria alternata* 8/15-2 was most active tyrosinase producer and *Cladosporium herbarum* 8/15-1 – laccase producer whereas peroxidase activity level and variation tendency of all strains was alike. Among studied strains only *Penicillium* genus representatives had medium acidification ability. *Alternaria alternata* 8/15-2 was the hardiest to wood preservatives fungal strain. The study of fungal physiological properties could help in selecting wood preservatives, in elucidation of reasons of their inefficiency and creating of new ones.

Keywords: fungi, phenoloxidases, endoglucanase, organic acids, wood preservatives.

1. INTRODUCTION

Wood is still widely used as a construction material in building but in spite of great assortment of antifungal chemicals, incidents of treated-wood damage still happen. The mycological damage of treated wooden constructions and finishing details caused by fungi (wood rot, moulds and "blue staining") may result in great economical losses, lower product quality and even endanger human health indoors.

Soft rot is considered to be the first decay stage found in a general sequence of wood colonization. It caused by fungi from the *Ascomycota* and *Deuteromycota*. Cylindrical cavities within secondary cell walls or cell wall erosion are formed after lignocellulose matrix in wood attack by fungal enzymes [1]. Though it's a shallow wood damage and it cause insignificant mass loss (7 % – 8 % in 30 month in Scots pine) compared with brown- and white-rot but even these few percents can drastically decrease the impact bending strength [2].

The ability of soft rot fungi to adapt to various environmental conditions and their physiological properties causes difficulties in searching means against wood damage. Soft rot fungi can be found even in dry environments and are mostly known to occur where brown- and white-rot are inhibited by factors such as high moisture content, low aeration and presence of preservatives or high temperatures [1, 3 – 5].

Traditional wood protection methods involve treating with synthetic chemicals composed with aromatic compounds or heavy metals. Some of soft rot fungi are distinguished by their extreme tolerance to heavy metals [6 – 8]. There are reports that oxalic acid produced by fungi presumably precipitates copper into insoluble form of the oxalate, rendering the copper metabolite inert [9]. Other ways of fungal self-protection involves extracellular mucilaginous material that acts as a protective layer surrounding the organism, fungal ability to absorb different metal ion amounts into cell wall and other intercellular structures [6, 10]. Due to the unspecificity of ligninolytic extracellular enzymes (phenoloxidases) some of fungi can degrade not only lignin but some aromatic compounds that are used in wood protection (*e.g.* pentachlorophenol) as well [11, 12]. There are reports about soft rot fungi isolated on creosote-, pentachlorophenol- and CCA-treated wood [13, 14].

Soft rot fungi growing on wood-based materials indoors not only lower esthetic value of wood but constitute menace for human health. They produce volatile compounds and/or mycotoxins as well as spores themselves that could raise significant human health danger (allergic, respiratory diseases) resulting from their inhalation [15, 16].

Taking into account all above mentioned soft rot fungal significance, the studies of their properties are of extreme importance. It is necessary to develop a better database on the fungal diversity on treated wood as well as investigate their physiological-biological properties.

The present investigation was prompt by a case of mould damage of six buildings under construction. Wooden roof joists were treated with wood preservative but after some time a fungal development was noticed on these wooden constructions (wood discoloration and dark mycelium were seen). The objective of the present investigation was to identify the fungal species that survived after wood treatment and make a primary screening of some physiological properties important for fungi in treated wood colonization. For that purpose fungal isolates from treated wood were identified and their

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ligninolytic and cellulolytic activities, medium acidification ability and sensitivity for antifungal chemicals were estimated.

2. MATERIALS AND METHODS

2.1. Fungal isolation

Wood samples were taken from 15 points (in 6 buildings) of the joists where discoloration or mycelium growth on treated wood was visually noticed. The samples were washed under tap water and then with sterile water, blotted and briefly surface-sterilized by flame. Small pieces of wood (approximately (5×5×10) mm in size) in threes per Petri dish were placed half-submerged in the medium. Three Petri dishes for one examination point were prepared. Malt extract agar (Liofilchem S. r. l.) medium with the addition of 100 µg/ml chloramphenicol (for bacterium growth inhibition) was used for fungi and malt extract agar with 4 µg/ml benomyl and 100 µg/ml chloramphenicol for basidiomycetous fungus isolation. The dishes were incubated for 10 days at 24 °C ±2 °C [13]. Fungi were identified according to cultural and morphological features [17 – 21]. Fungal occurrence frequency was calculated as percentage of a number of the same fungal species isolates in 6 examined buildings. Fungal isolates were deposited in the Culture collection of the Laboratory of Biodeterioration Research in Nature Research Centre.

2.2. Fungal cellulolytic ability measurement

Fungal cellulolytic ability was evaluated by primary estimation of their endoglucanase (EC 3.2.1.4, CM-cellulase) activity. This enzyme is important in cellulose decomposition as it hydrolyzes glucosidal linkages, gradually splitting away glucosidal residuals and resulting in reduced sugar formation.

Fungal isolates were cultivated on modified Czapek medium: $NaNO_3$ – 2 g; KH_2PO_4 – 1 g; $MgSO_4 \cdot 7H_2O$ – 0.5 g; KCl – 0.5 g; $FeSO_4$ – 0.01 g, carboximetylcellulose – 10 g; agar – 20 g; H_2O – 1000 ml, at 24 °C ±2 °C temperature for 5 days. The endoglucanase activity was detected by flooding of the 0.1 % aqueous solution of Congo Red and it revealed by a pale orange zone around the colony. The activity was evaluated by the width (mm) of this zone [22].

2.3. Ligninolytic activity measurements

Primary evaluation of fungal strain phenoloxidases (peroxidase, laccase and tyrosinase) that take part in lignin degradation, activity was estimated according to Bavendamm method. The fungal colonies were grown in Petri dish on Czapek agar medium with an addition of 0.2 % of gallic acid. The phenoloxidase activity was evaluated by the width (mm) of brown colored zone around the colony after 5 and 7 cultivation days at 24 °C ±2 °C temperature [23].

The more precise studies of peroxidase, laccase and tyrosinase activity were fulfilled by cultivating fungi in liquid modified Czapek medium with sawdust (1 g/50 ml) as a sole carbon source after 4, 7 and 10 days. The assay method for peroxidase (EC 1.11.1.7) activity was based on the colorimetric evaluation of the oxidation product of o-dianisidine reagent in the presence of H_2O_2 (using a green filter) [23]. The activity was calculated according to the coefficient of micromolar extinction which is 0.0128 and was expressed as units per mol^{-1} (U mol^{-1}).

Tyrozinase (EC 1.14.18.1) activity was measured spectrophotometrically at 420 nm every 20 s for 2 min. using a method based on the estimation of optical density of reaction products formed during oxidation of pyrocatechin [24]. Enzymatic activity was expressed as units per mol^{-1} (U mol^{-1}).

Laccase (EC 1.10.3.2) activity was measured according to Ravin H.A. and Harvard M.D. using p-phenylene-diaminechloride spectrophotometrically at 530 nm and was expressed as units per mol^{-1} (U mol^{-1}) [25].

2.4. Measurement of organic acid production (medium acidification) coefficient

Fungal ability to produce organic acids (to acidify the medium) was tested on modified Czapek agar medium with 5 % glucose and 0.005 % bromphenol blue indicator. The medium (5 ml) was poured into test-tubes. Fungi cultures were sawn with a needle and the tubes incubated at 24 °C ±2 °C temperature 7 days. The essay was performed with three replicate test-tubes per every fungal strain. The yellow coloration of the medium beneath the growing fungi showed the presence of organic acids. The acidification coefficient was expressed as a ratio of yellow colored medium column height and whole medium column height [26].

2.5. Fungal sensitivity to antifungal chemicals estimation

Six different wood preservatives on offer were used for the evaluation of fungal sensitivity to antifungal chemicals: Borolitas (Lithuania), WT Sodium Hypochlorite (Finland); “Anti-mould liquid” (Germany); “Boramon” (Poland); “Arlitas” (Lithuania) and Complete Wood Treatment (UK). The assay was carried out by agar-diffusion method in Petri dishes (90 mm diameter) on malt agar medium. Filter paper discs (6 mm diameter), soaked with antifungal chemicals, were placed in the centre of the dish on the sowed out fungal lawn. The assay was performed in three replicates. The zone width (mm) of suppressed fungal growth around the disc revealed the fungicidal action of the preservatives tested and expressed fungal sensitivity to the chemicals [27].

2.6. Statistical analysis

All experiments were performed in triplicates. The obtained results of fungal primary endoglucanase and phenoloxidase activity, as well as, peroxidase, laccase, tyrosinase activity and medium acidification ability measurements were processed using Microsoft Excel 2003, Statistica 5.

3. RESULTS AND DISCUSSION

3.1. Fungal species isolated from preservative-treated wooden constructions

Fungal strains isolated from treated wooden joists belonged to 7 different genera (Table 1). *Cladosporium* and *Fusarium* genera were dominating among isolated

species. 31.3 % of all species identified belonged to *Cladosporium* genus and among them *Cladosporium cladosporioides* had the highest occurrence frequency (83.3 %). *Fusarium* genus species make up 31.3 % as well and the highest occurrence frequency (50.0 %) among them was that of *Fusarium sp.* Other detected fungal genera were presented by less species diversity but some of them had high occurrence frequency: *Penicillium sp.* and *Ulocladium chartarum* – 50.0 %. *Trichoderma harzianum* and *Phoma sp.* had the lowest occurrence frequency (16.7 %) in our study. Basidiomycetous fungi that cause wood decay were not detected.

Table 1. Fungi isolated from treated wooden joists in the buildings studied

Fungi	Occurrence frequency, %
Alternaria alternata (Fr.) Keissler	13.3
Cladosporium cladosporioides (Fresen.) de Vries	33.3
Cladosporium herbarum (Pers.) Link ex S. F. Gray	26.7
Cladosporium sphaerospermum Penz.	20.0
Cladosporium spongiosum Berk. et Curt.	6.7
Cladosporium variabile (Cooke) de Vries	13.3
Fusarium acuminatum Ell. et Ev.	6.7
Fusarium avenaceum (Fr.) Sacc.	6.7
Fusarium culmorum (W. G. Smith)	6.7
Fusarium equiseti (Corda) Sacc.	13.3
Fusarium solani (Mart.) Appel et Wollenw. emend Snyd. et Hans.	6.7
Fusarium sp.	20.0
Penicillium brevicompactum Dierckx	13.3
Penicillium sp.	20.0
Phoma sp.	6.7
Trichoderma harzianum Rifai	6.7
Ulocladium chartarum (Preuss) Simmons	20.0

The fungi isolated from treated wood during our investigation are wide spread, naturally found in soil, saprotrophs and most of them (except *Penicillium* and *Trichoderma*) are plant pathogens. *Alternaria, Cladosporium, Phoma* as well as *Ulocladium* genus fungi have black pigment (melanin) that caused distinctly seen dark grey discoloration on wood joints studied [21]. *Trichoderma* as well as *Penicillium* usually form green colonies because of their spores that could be seen on wood surface nevertheless they rarely have been reported to cause soft rot [14]. Most of fungi isolated from treated wood during our investigation (*Alternaria alternata, Cladosporium cladosporioides, C. herbarum, Fusarium solani, Ulocladium chartarum*) are recognized as soft-rot fungi and they have been found even on painted or preservative-treated wood [2, 4, 13, 14]. There are reports of *Trichoderma harzianum* and *Phoma sp.* detection on treated wood as well [13, 14]. Kim *et al.* reported that even 45 % of the isolates from CCA-treated wood radiata pine board stored at the yard in Korea have been *Phoma* species though in our study it was isolated only once. *Cladosporium* isolates were widely spread in our investigation and its frequent occurrence is mentioned in many other reports [13, 14, 17, 21].

Such species as *Alternaria alternata, Cladosporium* species*, Penicillium brevicompactum* and *Trichoderma harzianum* have been mentioned among species on wooden building construction in previous our investigations and isolated from wood blocks treated with preservatives Arlitas, Asepas-1, Dispersan in soil contact [28, 29].

Cladosporium spp. and *Penicillium spp.* are toxinogenic fungi and their conidia are easily distributed through the atmosphere therefore they could cause a threat of human health indoors – may be the reason of allergy and respiratory diseases indoors. Mycotoxins of *Alternaria alternata* causing toxic leucopenia in man have been also reported and it's considered to be an important source of allergens as well [21, 30 – 32]. Some of *Fusarium* species could be also human pathogens causing mycoses [33]. *Ulocladium chartarum* wasn't mentioned among human pathogens thought it often found in damp buildings on wood-based and other materials [34]. Consequently the accumulation of above mentioned fungi is really undesirable in dwelling rooms.

3.2. Fungal cellulolytic activity

Fungi could use wood as a substratum with the help of their vital functioning and in this way damage it. The primary analysis showed that endoglucanase – one of the three enzymes that takes part in cellulose decomposition in wood was characteristic of all tested fungal strains that have been isolated from treated wood (Fig. 1).

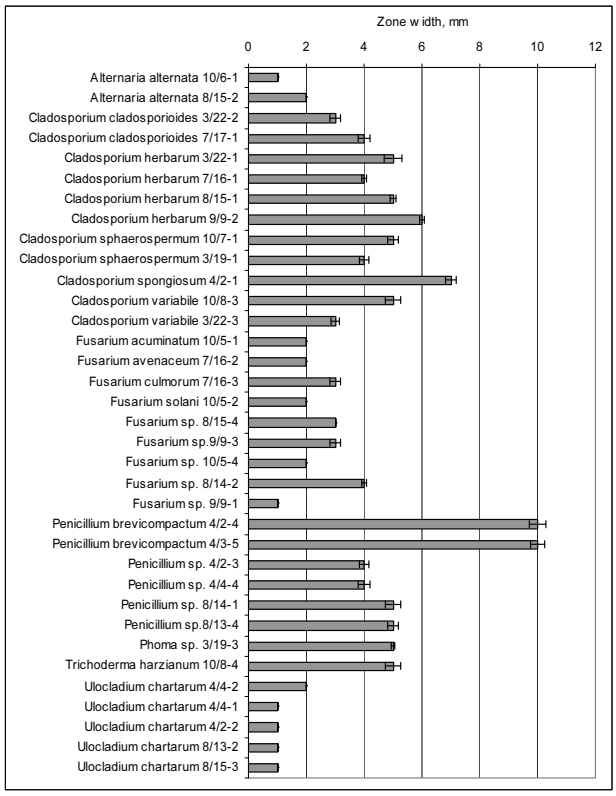

Fig. 1. Primary screening of endoglucanase activity of the fungi isolated from treated wooden joists

Penicillium brevicompactum 4/2-4 and 4/3-5 strains revealed the highest endoglucanase activity among all tested fungi isolated from treated wood – the discolored zone reached even 10 mm. The lowest endoglucanase

activity was demonstrated by *Ulocladium* and *Alternaria* genus strains – it wasn't more than 2 mm.

The endoglucanase activity of fungi from treated wood is not very high compared with those from plant wooden remnants. In our previous studies the discolored zone of the fungal strains from plant wooden remnants usually reached more than 10 mm [35]. These results demonstrated that fungal endoglucanase activity depended on isolation substratum. The same conclusion was reported by Pedersen M. et al. with *Ulocladium* species fungi from different isolation sources [36].

3.3. Fungal ligninolytic activity

Fungi that develop on wood sometimes are able to degrade lignin as well. The phenoloxidases (peroxidase, tyrosinase and laccase) that take part in lignin decomposition are not characteristic of every fungus.

The primary screening of fungal strains from treated wood showed that some of them were able to produce phenoloxidases (Fig. 2). Furthermore, it was noticed that this ability varied in time. For example, *Alternaria alternata* 8/15-2 showed phenoloxidase activity after 5 cultivation days but after 7 days brown zone around the colony revealing fungal activity wasn't noticed.

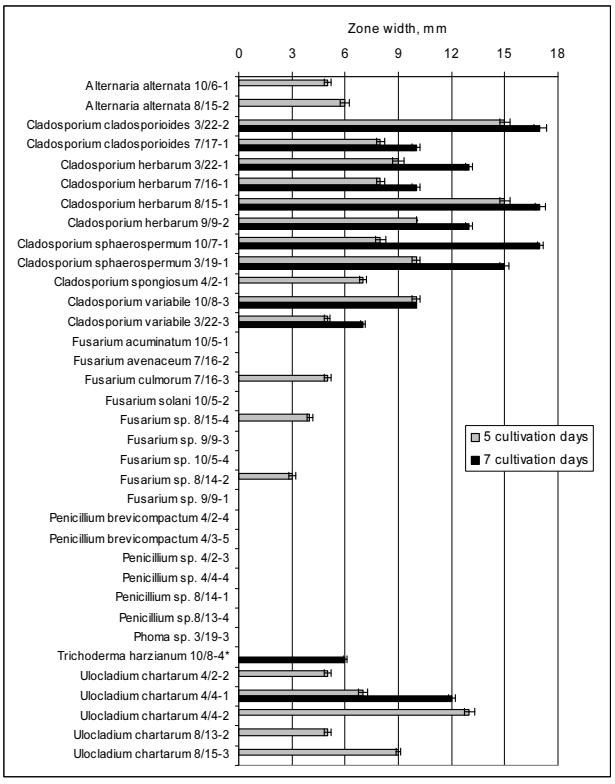

Fig. 2. Primary screening of phenoloxidase activity of the fungi isolated from treated wooden joists (*– brown color zone, indicating phenoloxidase activity, emerged under colony)

Cladosporium genus fungi showed the highest and most durable phenoloxidase activity. In some cases brown colored zone reached even 17 mm after 7 days (*C. cladosporioides* 3/22-2, *C. herbarum* 8/15-1, *C. sphaerospermum* 10/7-1). *Fusarium* genus strains were weaker producers of phenoloxodases compared with other studied fungi. Only some of them (*Fusarium culmorum* 7/16-3, *Fusarium. sp.* 8/15-4 and *Fusarium sp.* 8/14-2) showed slight activity (respectively, zone width 5, 4 and 3 mm) after 5 cultivation days but after 7 days no phenoloxidase activity was noticed though there are data about high ligninolytic activity of *Fusarium* strains. Their dependence on isolation substrata and cultivation time was reported as well [37]. The phenoloxidase acivity of *Penicillium* genus strains isolated from treated wood wasn't detected but they had quit high (among tested strains) endoglucanase activity.

The primary endoglucanase and phenoloxidase activity discoloration assays showed that wood colonization ability of fungi isolated from treated wood differed among the strains and some of them had not only cellulolytic but ligninolytic activity as well, i. e. they are able to decompose cellulose and lignin. In this way they can cause more damage.

The liquid-faze fermentation with sawdust as natural carbon souse was used for the more precise analysis of phenoloxidases – peroxidase, tyrosine and laccase production during 10 cultivation days. The most active phenoloxidase producers from different genera were selected for this research.

The studied strains showed peroxidase activity after 4 cultivation days under liquid-phase fermentation on sawdust as carbon source (Fig. 3). The peroxidase activity of all studied strain varied in the same way in the course of the cultivation: it was the lowest after 4 cultivation days and the highest after 7 days. After 4 cultivation days among all studied strains *Alternaria alternata* 8/15-2 showed the highest peroxidase activity (20.31 U mol^{-1}) but after 7 and 10 days *Cladosporium herbarum* 8/15-1 was the most active peroxidase producer.

The results showed that the level of peroxidase activity depended on fungal strain but the variation tendency was the same of them all. It was found that fungal peroxidase activity on wood compared to that on graminaceous substrata, the peroxidase activity of certain strains was higher on the latter. E. g., *Myrothecium verrucaria* showed the 70 U mol^{-1} peroxidase activity after 8 days of cultivation on rye straw under liquid-faze conditions whereas the peroxidase activity on wood of fungal strains studied was the highest after 7 days and fluctuated between 23 U mol^{-1} and 27 U mol^{-1}, i. e. it was 2,7 times lower [38].

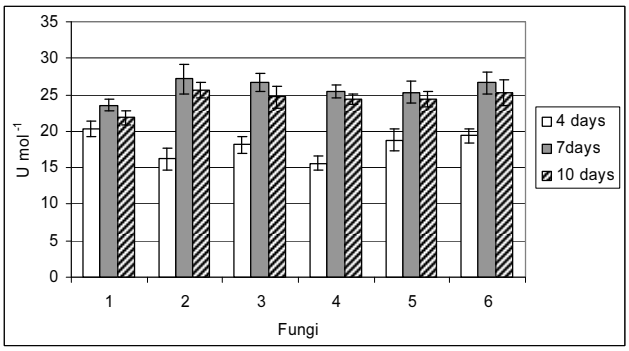

Fig. 3. Peroxidase activity (U mol^{-1}) of fungi: 1 – *Alternaria alternata* 8/15-2; 2 – *Cladosporium herbarum* 8/15-1; 3 – *C. cladosporioides* 3/22-2; 4 – *Fusarium culmorum* 7/16-3; 5 – *Fusarium sp.* 8/15-4 and 6 – *Ulocladium chartarum* 4/4-2, from treated wood during 10-day cultivation in modified Czapek medium with sawdust as a sole carbon source

The highest tyrosinase activity was detected after *Alternaria alternata* 8/15-2 4-day cultivation under liquid-phase fermentation conditions on wood (1.088 U mol^{-1}) (Fig. 4). The activities of other strains studied were markedly lower. The tyrosinase activity varied in the course of cultivation very individually, e. g. the activity of *Alternaria alternata* 8/15-2 and *Cladosporium herbarum* 8/15-1 and *Fusarium culmorum* 7/16-3 gradually decreased while that of *Fusarium sp.* 8/15-4 and *Ulocladium chartarum* 4/4-2 had the highest after 7-day cultivation (0.216 U mol^{-1} and 0.108 U mol^{-1} respectively).

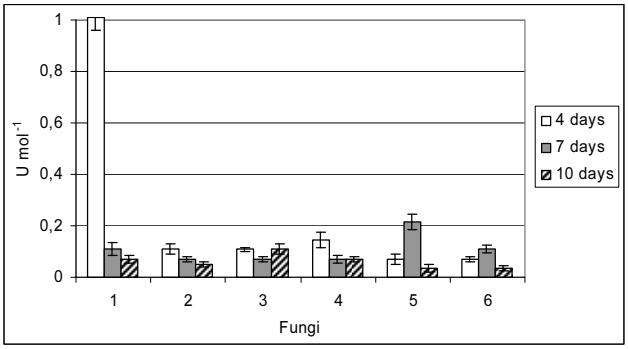

Fig. 4. Tyrosinase activity (U mol^{-1}) of fungi: 1 – *Alternaria alternata* 8/15-2; 2 – *Cladosporium herbarum* 8/15-1; 3 – *C. cladosporioides* 3/22-2; 4 – *Fusarium culmorum* 7/16-3; 5 – *Fusarium sp.* 8/15-4 and 6 – *Ulocladium chartarum* 4/4-2, from treated wood during 10-day cultivation in modified Czapek medium with sawdust as a sole carbon source

The tyrosinase activity of the strains studied during cultivation on wood wasn't high (fluctuated about 0.1 U mol^{-1} with the exceptions of *Alternaria alternata* 8/15-2 and *Fusarium sp.* 8/15-4) compared with the activity of some strains on straw. E. g., *Myrothecium verrucaria* showed 1 U mol^{-1} tyrosinase activity when lignin degradation reached 10 % after 8 cultivation days [38].

The investigation showed that laccase activity of all fungal strains studied on wood was low and the highest activity that showed *Cladosporium herbarum* 8/15-1 was 0.147 U mol^{-1} after 10-day cultivation under liquid-phase fermentation conditions, respectively (Fig. 5). The laccase activity of other strains studied fluctuated between 0.019 and 0.010 after 4-day, between 0.027 and 0.013 after 7-days and between 0.058 U mol^{-1} and 0.012 U mol^{-1} after 10-day cultivation. The activity of some fungi (*Galactomyces geotrichum, Myrothecium verrucaria, Mortierella verticillata*) on straw fluctuated in similar limits after 8-day cultivation [38].

The activity of phenoloxidases varied in time. The laccase activity of some strains (*Alternaria alternata* 8/15-2, *Cladosporium cladosporioides* 3/22-2 and *Fusarium sp.* 8/15-4) gradually increased in the cause of 10 days, the others (*Fusarium culmorum* 7/16-3 4, *Ulocladium chartarum* 4/4-2) had the highest activity on 7-day cultivation which decreased later. The oscillatory nature of fungal enzyme production was noted by other investigators [39] and the data of peroxidase and laccase activities of *Fusarium* and *Cladosporium* genus strains as well as their ability to degrade polycyclic aromatic hydrocarbons was reported [40, 41].

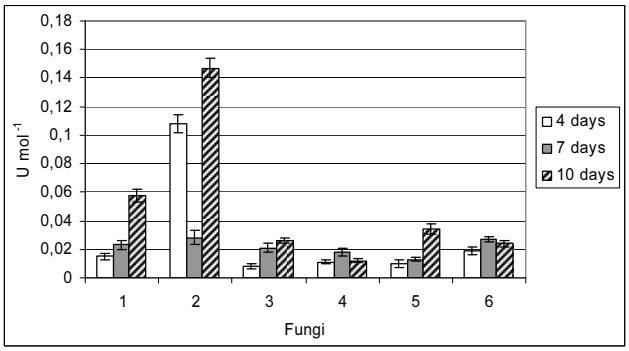

Fig. 5. Laccase activity (U mol^{-1}) of fungi: 1 – *Alternaria alternata* 8/15-2; 2 – *Cladosporium herbarum* 8/15-1; 3 – *C. cladosporioides* 3/22-2; 4 – *Fusarium culmorum* 7/16-3; 5 – *Fusarium sp.* 8/15-4 and 6 – *Ulocladium chartarum* 4/4-2, from treated wood during 10-day cultivation in modified Czapek medium with sawdust as a sole carbon source

The spectrophotometric analysis of fungal enzymatic activity on wood showed that *Alternaria alternata* 8/15-2 was markedly most active tyrosinase producer and *Cladosporium herbarum* 8/15-1 – laccase producer whereas peroxidase activity level and variation tendency of all strains was alike.

3.4. Organic acid production

Organic acids are supposed to be linked with fungal metal tolerance and in this way are important for survival on metal-based treated wood. The examination of the fungi isolated from treated wood showed that only strains of *Penicillium* genus were able to produce organic acids and acidify the medium (Fig. 6) though their acidification coefficient differed. The *Penicillium sp.* 8/13-4 strain had the highest acidification coefficient and reached 0.62 and the lowest acidification coefficient that of *Penicillium sp.* 4/2-3 make up only 0.11.

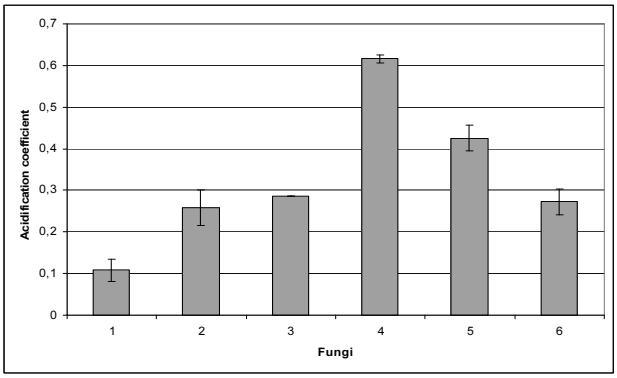

Fig. 6. Medium acidifiation coefficient of fungi from treated wood: 1 – *Penicillium sp.* 4/2-3; 2 – *Penicillium brevicompactum* 4/2-4; 3 – *Penicillium brevicompactum* 4/3-5; 4 – *Penicillium sp.* 8/13-4; 5 – *Penicillium sp.* 8/13-5 and 6 – *Penicillium sp.* 8/14-1

3.5. Fungal sensitivity to different wood preservatives

The assay with different wood preservatives revealed more distinctly the tolerance to various chemicals and adaptation abilities of fungal strains isolated from treated

wood. Most antifungal chemicals suppressed their development though the suppression degree differed notwithstanding fungal strains have been isolated from the same ecological niche – treated wood. *Cladosporium cladosporioides* 3/22-2, *Fusarium culmorum* 7/16-3, *Penicillium sp.* 8/13-4 and *Penicillium brevicompactum* 4/3-5 were sensitive to 5 from 6 studied preservatives. *Fusarium* genus strains were found to be less sensitive to wood preservatives (their growth suppression zones fluctuated between 1.67 mm and 5.75 mm) compared to studied *Cladosporium, Penicillium* and *Ulocladium* genus fungi the suppression zones of that fluctuated between 2 – 16.5, 1.75 – 13.5 and 2.25 – 13.5 mm respectively (Fig. 7).

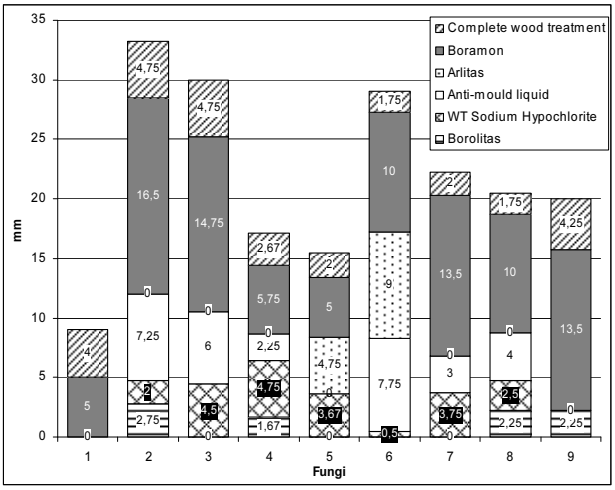

Fig. 7. Fungal sensitivity to different wood preservatives (growth suppression zones, mm – numbers on the columns): 1 – *Alternaria alternata* 8/15-2; 2 – *Cladosporium cladosporioides* 3/22-2; 3 – *C. herbarum* 8/15-1; 4 – *Fusarium culmorum* 7/16-3; 5 – *Fusarium sp.* 8/15-4; 6 – *Penicillium sp.* 8/13-4; 7 – *P. brevicompactum* 4/2-4; 8 – *P. brevicompactum* 4/3-5 and 9 – *Ulocladium chartarum* 4/4-2

Alternaria alternata 8/15-2 was the hardiest to wood preservatives fungal strain that, according to our results, showed the highest among the strains studied tyrosinase activity on wood. Only two preservatives Complete Wood Treatment and Boramon were efficient enough to suppress the growth of this strain. Furthermore the biodegradable abilities of *Alternaria alternata* in creosote treated wood were reported by other investigators [42]. *Cladosporium herbarum* 8/15-1 that distinguish itself by high laccase activity developing on wood haven't the advantage among strains studied regarding resistance to antifungal chemicals.

In our case the investigation results haven't confirmed the idea of organic acids role in fungal survival and preservative inactivation [9]. *Penicillium* genus strains that had ability to acidify the medium were nonresistant to majority of studied wood preservatives though in some cases growth suppression zones were narrower compared with other strains that didn't possess this ability. E. g., the growth suppression zones of *Penicillium* strains studied were narrower compared with *Cladosporium* strains when Complete Wood Treatment was used (1.75 mm – 2 mm and 4.75 mm respectively).

The fungal resistance to certain metal based preservatives could be explained by their tolerance to metal ions but it's demonstrated, as well, that micro concentrations of certain metal ions could even increase fungal enzymatic activity and in this way they may become more aggressive colonizers of treated wood [6, 43].

It's not easy to predict all factors that determine fungal development on wood, and prevent it from biodeterioration. In our studies wooden constructions studied were well protected from basidiomycetous fungi but the growth of fungi causing wood discoloration and soft rot was observed. The main reason of this phenomenon is great adaptability of soft rot fungi that enables them to survive under unfavorable conditions. Wood colonization could be supported by a wide range of conditions that must be taken into consideration in order to avoid or lessen the fear of damage. During our investigation airborne fungi (*Cladosporium, Penicillium* genus species) were isolated from wooden joints as well as those often found in soil (*Fusarium, Alternaria* genus species). Therefore even timbering storage avoiding soil contact is important in further wood protection from fungal infection. Furthermore certain environmental conditions are needed for fungal spore germination and further development. In our case the buildings were unfinished up, they were unheated and humid. These conditions definitely favored the growth and development of fungi though the cases of soft-rot appearance in relative dry sites could happen as well [1]. On wood and in other microenvironments it is typical for many fungi to live and grow in close proximity to each other. It was noticed that mixed fungal cultures could lead to a higher enzyme production (through synergistic interaction) [44]. Extracellular phenoloxidase enzymes are implicated in the offensive/defensive strategies employed by fungi during interaction and one fungus could induce phenoloxidase activity of another [45, 46]. According to our results of primary enzymatic studies with solitary strains fungi that haven't ability to produce phenoloxidases (*Fusarium* and *Penicillium* strains) were isolated together with that producing these enzymes (*Cladosporium, Alternaria* or *Ulocladium* genus strains). Therefore our obtained results on fungal enzymatic activity may differ during fungal interaction and more thoroughly analysis is needed. Furthermore the results of the enzymatic activity depend on substratum: measurements on sawdust revealed the laccase activity of *Fusarium* genus strains that showed no phenoloxidase activity on Czapek medium.

These fungal physiological properties as well as their great adaptability to chemicals and tolerance to heavy metals raise high and complex requirements for creation of new wood preservatives. On the other hand adapted to polluted environment fungal strains may be used in bioremediation of treated wood after service. As enzymatic activity depends on many factors the investigation results demonstrated in the present work don't provide sufficient information about fungal survival reasons on treated wood and more detailed investigations under closer to natural conditions (fungal consortium development on treated wood) are needed. The study of undesirable mycological damage of treated wood and the elucidation of reasons and laws of fungal survival could help in creation more efficient preservatives.

4. CONCLUSIONS

1. The investigation showed that treated wooden joists were colonized by fungi of 7 different genera (*Alternaria, Cladosporium, Fusarium. Penicillium, Phoma, Trichoderm* and *Ulocladium*) and among them *Cladosporium cladosporioides* had the highest detection frequency (33.3 %).

2. Fungal screening for cellulolytic activity (activity that is important in wood assimilation and makes possible the fungal development on wood) showed that all studied fungal strains isolated from treated wood have endogluconase activity but the degree of this activity differed.

3. The phenoloxidases (peroxidase, tyrosinase and laccase) that take part in lignin decomposition (and supposed to degrade preservatives of aromatic compound origin) were not characteristic of every fungi studied.

4. Fungi that have survived on treated wood showed different sensitivity to other antifungal chemicals. *Alternaria alternata* 8/15-2 – the most active tyrosinase producer (1.088 U mol^{-1}after 4 days cultivation) was most tolerant among studied strains to wood preservatives of different chemical composition but the majority of studied wood preservatives suppressed the development of *Penicillium* genus strains that had ability to acidify the medium.

5. The assays on sensitivity of fungal strains isolated from treated wood to different wood preservatives could help in selection of effective wood protection means.

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