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Growth of *Paecilomyces variotii* fungus on salt-agar medium and on natural and chemically preserved wood

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Abstract: *Growth of Paecilomyces variotii fungus on salt-agar medium and on natural and chemically preserved wood*. *Paecilomyces variotii* Bainer fungus (*Pv*) is one of filamentous fungi classified as belonging to Ascomycetes, Deuteromycetes groups, which are able to grow on many of softwood and hardwood species. These fungi (molds) commonly found around the world are responsible for the decrease of functional aesthetics of lignocellulosic materials and even soft rot of wooden materials. The growth of the fungi may be the reason for the drop of the material value, technological difficulties and, eventually, financial loses. It was indicated that *Pv* may cause yellow discoloration of oak, particularly during kiln drying of wood. The fungus is able to grow on softwood species as well. The aim of this research was to identify the growth rate of *Pv* fungus on salt-agar medium in comparison to other filamentous fungi and to compare its growth on Scots pine wood both natural and chemically preserved with model wood preservative. It was observed that the growth rate of *Pv* fungus during double-species tests on salt-agar medium was different depending on the species of the second fungus. *Trichoderma viride* and *Chaetomium globosum* fungi limited to some extent the growth of *Pv* fungus; however, no apparent antagonism was observed. The model wood preservative at 200g/m^2 retention effectively controlled the growth of *Pv*, which in the case of natural Scots pine wood caused complete coverage of the wood surface.

Keywords: filamentous fungi, growth, wood, model wood preservative, efficacy

INTRODUCTION

Paecilomyces variotii fungus as one of filamentous fungi is classified as belonging to Ascomycetes, Deuteromycetes groups, which very often cause mould of lignocellulosic materials, particularly timber. These groups are considered to be cosmopolitan organisms, known and commonly found around the world. Because of very little nutritious requirements, moulds are able to grow on the surfaces of many organic and even inorganic materials contaminated by the traces of organic substances. Many of softwood and hardwood species are susceptible to mould attack [Clausen and Yang 2003, Fojutowski et al. 2007, 2009, 2011, 2013, 2014]. Filamentous fungi during the short period of growth mainly deteriorate functional aesthetics of lignocellulosic materials causing their discoloration and disfigurement, but if the mould effect on wood is prolonged some of the fungi are able to cause even soft rot of wooden materials by producing cellulitic enzymes (cellulases and hemicellulases). In any case, the growth of the fungi may be reason for the drop of the material value, technological problems with the processing of wood infested with molds and, eventually, financial loses. It was indicated that *Paecilomyces variotii* Bain. fungus (*Pv*) may cause yellow discoloration of oak, particularly during kiln drying of wood [Bauch et al. 1991, Cofta 2006, Gustafsson and Lundquist- Gustafsson 2008, Wegener and Fengel 1988]. It was found in a laboratory tests that *Paecilomyces variotii* fungus easily caused a clear, yellow discoloration of the wood of *Quercus robur* L., *Quercus petraea* (Matt). Liebl., and *Castanea sativa* Mill., but did not cause discoloration of *Quercus rubra* L. wood. It is assumed that this is due to the specific causative reactions between the compounds in the heartwood of *Quercus robur* L., *Quercus petraea* (Matt.) Liebl. or *Castanea sativa* Mill. and the metabolites of the fungus. The fungus is able to grow on softwood species as well.

The aim of this research was to identify the growth rate of *Paecilomyces variotii* fungus on salt-agar medium in comparison to other filamentous fungi and to compare its growth on Scots pine wood both natural and chemically preserved with model wood preservative.

MATERIALS AND METHODS

The individual fungi growth rates and their interactions were tested on salt-agar medium. Pure cultures of the following, individual fungi often used in testing resistance of building materials [Instrukcja ITB….1998] were used: *Aspergillus niger* (DSM 12634), *Chaetomium globosum* (DSM 1962), *Ophiostoma piliferum* (Fries:Fries) H.&p. Sydow (DSM 4920), *Penicillium funiculosum* (DSM 2213), *Paecilomyces variotii* (DSM 1961) and *Trichoderma viride* (DSM 63065). A suspension of mould spores of the density of 1×10^6 conidia/cm^3 of the individual fungi was prepared. Filter paper discs of the diameter of 5 mm, immersed for 2 seconds in the suspension were placed as inocula (infestation) on salt-agar medium in Petri dishes of the diameter of 90 mm. The inoculum was placed close to one edge of a Petri dish in the case of a one-species test or by two edges of a Petri dish, opposite to each other in the case of double-species test (distance ~70 mm). Incubation conditions were: the temperature of 27 ± 1^oC and relative humidity of 90%. The growth of fungi was measured in mm (also calculated in %, taking maximum of the distance as 100%) after 4, 5, 6, 7, 8,14, 18, 19 and 20 days or to the moment when one fungus species meets the other. The interactions of fungi were observed and recorded at the moment when the fungi met, according to the following conventional scale: 0 – no further increase in fungus growth, + – further increase in fungus growth, and i – zone of inhibition (mm).

A method adapted from building procedures [Instrukcja ITB....1998] was used for mycological testing of *Paecilomyces variotii* (*Pv*) growth on natural and preserved wood. The materials used in the tests was Scots pine (*Pinus sylvestris* L.) sapwood of the quality meeting the requirements of [EN 113] standard and mean density of approx. 550 kg/m^3. Before the exposure to fungi samples were sterilized with steam in an autoclave (20 min., 121^{o}C). A set of 6 wooden discs of the nominal diameter of 25 mm (tangential/radial surface) and 4 mm thick cut out from the sapwood was used for each variant of tested wood samples, i.e. natural wood (control wood) and samples treated with model wood preservative. A preservative containing ammonium salts of phosphoric and sulphuric acids and QAC (quaternary ammonium compound, benzyl-$C_{12\text{-}16}$-alkyldimethyl chloride), IPBC (3-iodo-2-propynylbutcarbamate) and disodium octaborate was used. The samples of wood were preserved by brushing method using 200 or 100 or 50 g of the model wood preservative/m^2.
A suspension of mould spores of *Pv* fungus of the density of 1×10^6 conidia/cm^3 was sprayed on the surface of tested wood samples placed individually on the surface of salt-agar medium in Petri dishes with 90 mm diameter and outside height of 15 mm, and incubated at the temperature of 27 ± 1^oC and relative humidity of 90%. The activity of the fungi spores used in the test was evaluated by checking their growth on salt-agar medium and on control Scots pine wood samples. After 4 weeks the growth of mycelium on the surface of test samples was evaluated using the following scale:
0 – no growth of fungi on a sample visible under microscope,
1 – trace growth of fungi on a sample, hardly visible to the naked eye but well visible under microscope or growth limited to the edges of a sample, visible to the naked eye,
2 – growth of fungi on a sample, visible to the naked eye, but less than 15% of the surface is covered with fungus,
3 – over 15% of the surface is covered with fungus visible to the naked eye.
A standard evaluation was completed with the estimation of the percentage of the sample surface overgrown by mycelium and the grade of the intensity of the mycelium growth (1 – small; 2 – medium, 3 – strong).

Additionally, the content of ergosterol, as a method for fungi growth assessment, was determined by spectrophotometric method [Seitz 1977, Gutarowska 1999, Gutarowska and Żakowska 2002] after extraction from mycelium. The absorbance of methanol solutions obtained by extraction was measured at λ=282.6 nm using a Beckman DU 640

spectrophotometer. The ergosterol content was determined from the calibration curve in µg and expressed as quantity on the surface of the sample (5.3 cm^2). The change (reduction or increase) of the ergosterol content in tested wood in comparison to the content in control wood was calculated as well (Eq. 1).

$$R=100\% - (B*100\%/K) \quad (1)$$

R – reduction [%]
B – concentration of ergosterol in samples treated with fungicide [µg/5.3cm^2]
K – concentration of ergosterol in control samples (not treated with fungicide) [µg/5.3cm^2]

RESULTS

Trichoderma viride (*Tv*) fungus demonstrated the fastest growth rate between tested fungi in one-species test; while *Ophiostoma piliferum* demonstrated the slowest (Table 1). The growth rate of *Paecilomyces variotii* (*Pv*) fungus was also fast; however, two-fold slower than that of *Tv*. Moreover, it was observed that only in the test with *Pv* after 7 days of incubation a colony of *Pv* emanating from new spores spread by the fungus appeared on all surfaces of medium.

Table 1. The individual growth of tested filamentous fungi from one inoculum on salt-agar medium in Petri dishes

Fungus[1)]	Fungus growth time [day]								
	4	5	6	7	8	14	18	19	20
	Mean growth of fungus [%][2)]								
Tv	72	92							
Pv	**25**	**35**	**47**	**50**					
An	22	32	39	46	55				
Cg	25	36	47	54	63	95			
Pf	9	12	15	18	20	38	49		
Op	13	18	23	28	35	72	87	91	95

[1)] *Tv – Trichoderma viride, Pv – Paecilomyces variotii, An – Asperillus niger, Cg – Chaetomium globosum, Pf – Penicillium funiculosum, Op –Ophiostoma piliferum,*
[2)] The percent of maximum distance possible for growth.

The growth rate of *Pv* fungus in double-species tests on salt-agar medium was different depending on the species of the second fungus (Table 2). *Tv* and *Cg* fungi limited to some extent the growth of *Pv* fungus; however, no apparent antagonism was observed. Only in test *Pv-Tv* the weak growth of *Pv* was noticed after the mycelium of both fungi met, but in other double-species tests both fungi species stopped their growth the moment they met.

Paecilomyces variotii fungus grew fast and completely covered the surface of nutrient medium in Petri dishes within 3-5 days after infection. The test with wood showed, as a consequence of high activity of the fungus observed on nutrient medium, that the fungus was able to cover completely the surface of control wood not only at the end of the test period (28 days), but already during the five days after incubation (Table 3). At least 75% coverage of control wood surface by fungus/fungi is required for test validity. Therefore, the activity of *Paecilomyces variotii* spores met the requirements of the method. The fungus covered the surface of control wood in the highest grade of 3.0, meaning more than 15% of the wood surface, but in fact it was 100%, with grade 2 of the growth intensity. The results of *Pv* fungus growth on control wood also confirm, that the fungus is able to overgrow the wood surface very fast.

The results of susceptibility tests of Scots pine sapwood treated against mould fungi with different retentions of model wood preservative (Table 3) show that *Pv* fungus was able to

attack the surface of all tested wood samples; however, to different extents depending on the preservative retention and with weak intensity – grade 1.

Table 2. The growth of tested filamentous fungi in double-species test in relation to *Paecilomyces variotii* in Petri dishes (to different inocula on salt-agar medium opposite each other)

Main fungus: Paecilomyces variotii/Test fungus[1)]	Fugi growth time [days]					State after both fungi met	Growth rate [mm/day]
	4	5	6	7	8		
	Distance overgrown by fungi [mm]						
Pv	18	27	35	36		0	5.14
An	17	24	31	33		0	4.71
Pv	15	24	31	31	31	0	3.88
Cg	20	26	34	37	39	0	4.88
***Pv** (individual)*	**20**	**28**	**37**	**40**			5.71
Pv	16	24	35	40	41	0	5.12
Op	9	13	18	21	21	0	2.62
Pv	18	27	36	39	50	0	6.25
Pf	6	9	12	13	14	0	1.75
Pv	15	17				+	3.40
Tv	53	53				0	10.60

[1)] legend as to Table 1;

The growth of *Pv* fungus on wood with 50 and 100g/m^2 of preservative was so large (grade 3 and 2; 100% and 40%, respectively) that the wood was classified as not resistant to mould fungi. The growth of *Pv* fungus in standard grade (=1) may be considered acceptable in terms of building requirements only at 200g/m^2 retention of wood preservative. However, the growth of the fungus, in terms of wood surface percentage coverage, was 10%. The ergosterol reduction was observed for wood with 200 g/m^2 retention of wood preservative; however, for wood with 100 and 50 g/m^2 retention of wood preservative an increase in ergosterol content was observed. This was probably due to the fact that wood preservative retention, which was distinctly smaller from toxic limits of wood preservative, had a stimulating effect on fungi growth. Such results were also obtained in the test with total colour change measurement [Fojutowski at al. 2012, 2013]. Generally, the results confirm different susceptibility of wood to *Pv* attack depending on wood preservative retention.

Table 3. The state of attack by *Paecilomyces variotii* fungus on Scots pine sapwood samples treated with model wood preservative after 4 weeks from infestation, determined by visual grading of mould fungi growth on wood surface and instrumental measuring of the ergosterol content in wood

Fungus	Model wood preservative retention [g/m^2]	Visual evaluation of fungal growth on the samples surface			Mean ergosterol content	
		Grade	[%]	Intensity	[µg/5.3cm^2]	change[1)][%]
Paecilomyces variotii	200	1.0	10	1	45.6	–65.0
	100	2.0	40	1	139.3	+7.1
	50	3.0	100	1	170.8	+31.2
	0	3.0[2)]	100[2)]	2	130.1	-

[1)] – reduction, + increase; [2)]

CONCLUSIONS

1. The assessments of fungal growth on the surface of Scots pine wood samples by visual evaluation, total wood colour change measurement and the ergosterol content determination differ significantly.

2. Tested fungi, i.e. *Aspergillus niger, Chaetomium globosum, Ophiostoma piliferum, Penicillium funiculosum* and *Trichoderma viride* did not show antagonism to *Paecilomyces variotii* fungus in double-species tests on salt-agar medium; however *Trichoderma viride* and *Chaetomium globosum* limited, to some extent, the growth of *Paecilomyces variotii* fungus.
3. *Paecilomyces variotii* fungus grows very fast on natural Scots pine wood causing complete coverage of the wood surface during 5 days in laboratory conditions.
3. The Scots pine sapwood treated with model wood preservative at 200g/m^2 retention demonstrates enhanced resistance to *Paecilomyces variotii* fungus in relation to natural wood, but by 50 and 100g/m^2 retention some stimulation of the fungus growth was observed in the ergosterol content.
4. The ergosterol content determination, as an instrumental method, brought more detailed results in terms of fungi growth evaluation than descriptive, visual examinations. The instrumental method is supplemented by a descriptive assessment.

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Streszczenie: *Wzrost grzyba Paecilomyces variotii na pożywce solno-agarowej oraz na drewnie naturalnym i zabezpieczonym chemicznie*. Grzyb *Paecilomyces variotii* Bainer (*Pv*) to jeden z grzybów strzępkowych należących do grupy Ascomycetes, Deuteromycetes, zdolnych do rozwoju na wielu iglastych i liściastych gatunkach drewna. Grzyby te (pleśnie) powszechnie występujące w całym świecie powodują pogorszenie funkcjonalnych i estetycznych właściwości materiałów lignocelulozowych, a nawet szary rozkład materiałów drzewnych. Wzrost tych grzybów może być powodem obniżenia wartości materiałów, trudności technologicznych w przerobie drewna i, ewentualnie, strat ekonomicznych. Wykazano, że *Pv* może powodować żółknięcie drewna dębu, zwłaszcza podczas sztucznego suszenia tego drewna. Grzyb ten jest również w stanie rozwijać się na drewnie iglastym. Celem badań było rozpoznanie szybkości wzrostu grzyba *Pv* na pożywce solno – agarowej w porównaniu z innymi grzybami strzępkowymi oraz porównanie jego wzrostu na naturalnym drewnie sosny zwyczajnej i zabezpieczonym chemicznie modelowym środkiem ochrony drewna. Zaobserwowano, że szybkość wzrostu grzyba *Pv* w dwugatunkowym badaniu na pożywce solno - agarowej była różna w zależności od gatunku drugiego z współrosnących grzybów. Grzyby *Trichoderma viride* i *Chaetomium globosum* ograniczały do pewnego stopnia wzrost grzyba *Pv*; jednakże nie zaobserwowano występowania antagonizmu. Modelowy środek ochrony drewna w retencji 200g/m^2 skutecznie ograniczał wzrost grzyba *Pv*, który w przypadku naturalnego drewna sosny zwyczajnej powodował całkowite pokrycie powierzchni drewna.

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