SHORT COMMUNICATIONS

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IN VITRO GROWTH OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) MYCELIUM ON COMPOSITES FILLED WITH RAPESEED STRAW

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Key words: biodegradable composites, lignocellulosic materials, *Pleurotus ostreatus in vitro*

The preliminary *in vitro* experiments on growth of *Pleurotus ostreatus* on biodegradable composites with lignocellulosic materials were carried out. The composites with Mater-Bi® NF01U matrix filled by 20% and 30% native rapeseed straw were tested as substratum for the fungus growth.

The authors initiated *in vitro* experiments on medium on biodegradable composites (Mal-Nam et al. 2000, Starzycki et al. 2011) which could be used for protective purposes (as plates) of oyster mushroom mycelium against biotic and abiotic stress in amateur or commercial cultivation, for example, on tree stumps left after forest clearcutting.

When biological protection of stumps with *P. ostreatus* against wood decaying fungi is considered (Łakomy 2004) it may be important to protect *P. ostreatus* mycelium during the initial development on stump surface, in order to prevent random infection of tree stumps by other, frequently pathogenic, fungal species. Protective properties of composites which provide a shield for the inoculated mycelium also constitute a barrier against its excessive drying. In amateur cultivation, polyethylene foil (plastic bags) which does not undergo biodegradation is usually used (Sher et al. 2011).

Under laboratory conditions, substrates containing polysaccharides with appropriately thermally treated cereal kernels or potato dextrose agar (PDA) are employed for majority of mushroom species, including *P. ostreatus*.

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In the course of preliminary *in vitro* experiments with *P. ostreatus*, it was demonstrated that the fungus was capable of strong growth on composites with rapeseed straw and Mater-Bi® matrix containing native lignocellulosic materials.

Biodegradable composites which are used as substrates for mushroom cultivation, containing a polymer Mater-Bi® NF01U of Italian company Novamont filled with ground, unmodified rapeseed straw were used in the presented experiments.

Rapeseed straw cv. 'Californium' was harvested in 2007 at Swadzim Agricultural Experimental Station (Poznań University of Life Sciences). Following preliminary fragmentation, lignified straw particles were separated from the parenchymal tissue using an air separator. An "Analysette 3" Spartan vibrating screen of Fritsch Company was employed to obtain 1-2 mm particle fraction.

Composite materials containing 20% and 30% rapeseed straw were obtained by extrusion method using a single screw extruder (D = 25 mm, L/D = 25). Temperature conditions of the composite extrusion process are presented in Table 1. The process was carried out at screw velocity of 30–35 rotations per minute. The obtained composite in the form of granules was dried and then used for further investigations.

An isolate obtained from the fruiting body of *P. ostreatus* growing under natural conditions in 2003 (Phot. 1), was used in the study. The isolate had a considerable capability for rapid growth and produced on the applied substrate profuse quantities of carpophores *in vitro* (Phot. 2).

Ribosomal DNA sequencing (ITS 1) proved that the isolate represented *P. ostreatus*, with no foreign biotic DNA contaminations (GenBank® 2008). Apart from phenotypic observations of mushroom development, a novel method of DNA

Table 1

Temperature on extruder zones during extrusion of composites (°C)

Zone I	Zone II	Zone III	Head
115	145	165	150

Phot. 1. *Pleurotus ostreatus* used for mycelium isolation

Phot. 2. Subsequent stages of *Pleurotus ostreatus* fruit bodies growth *in vitro*

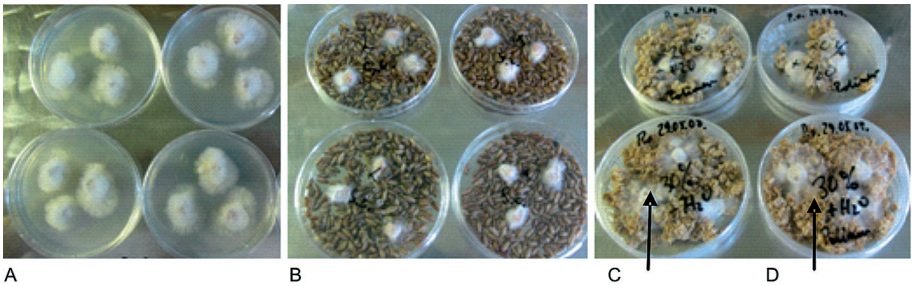
Table 2

DNA sequences (ITS 1) of *Pleurotus ostreatus* which was compared with DNA oyster mushroom used in research (GenBank® 2008)

The number of nucleotides sequenced	Sequences of nucleotides					
1	tttccgtagg	tgaacctgcg	gaaggatcat	taatgaattc	gctatggagt	tgttgctggc
61	ctctaggggt	atgtgcacgc	ttcactagtc	tttcgaccac	ctgtgaactt	ttggtagatc
121	gcgaagtcgt	tctctcaagt	cgtcagactt	ggattgctgg	gatttaaaca	tctcggtgtg
181	actacgcagt	ctatttactt	atacacccca	aatgtatgtc	tacgaatgtc	atttaatggg
241	ccttgtgcct	ataaaccata	atacaacttt	caacaacgga	ctcttggctt	ctcgcatcga
301	tgaagaacgc	agcgaaatgc	gataagtaat	gtgaattgca	gaattcagtg	aatcatcgaa
361	tctttgaacg	caccttgcgc	cccttggtat	tccgaggggc	atgcctgttt	gagtgtcatt
421	aaattctcaa	actcactctg	gttttttcca	attgtgatgt	ttggattgtt	gggggctgct
481	ggccttgaca	ggtcggctcc	tcttaaatgc	attagcagga	cttctcattg	cctctgcgca
541	tgatgtgata	attatcactc	atcaatagca	cgcatgaata	gagtctagct	ctctaatcgt
601	ccgcaaggac	aatttgacaa	tttgacctca	aatcaggtag	gactacccgc	tgaacttaa

sequencing essential for unambiguous species determination was employed in this study. The result shown in Table 2 is significant because it indicates the pure isolate of *P. ostreatus* (no heterogeneity).

Pleurotus ostreatus inoculum in the form of three agar cubes with mycelium was placed on Petri dishes with appropriately prepared composites containing 20% and 30% rapeseed straw (control – PDA substrate, Sigma). The substrate was enriched with *Secale cereale* kernels prepared in the autoclave (triple sterilization of 20 min each during 6 days at 120°C). Fragments of mycelium were placed in three places of the substrate and its growth was measured in millimetres (Phot. 3) after 7 days. The inoculated Petri dishes were kept in of the phytotron with programmed thermo- (12 h 15°C / 12 h 10°C) and photoperiod (12 h light / 12 h darkness), stimulating the growth of *P. ostreatus*.



Phot. 3. Fungus growth on medium: A – PDA medium 3% (Sigma), standard, B – autoclaved *Secale cereale* kernels, C – polymer composites with 20% native rapeseed straw, D – polymer composites with 30% native rapeseed straw

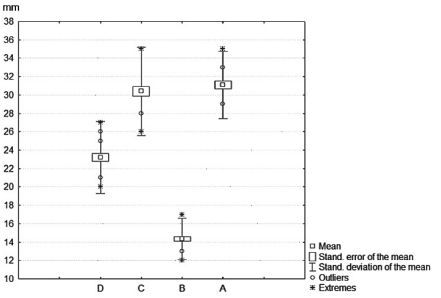


Fig. 1. Statistical relationship in experimental combinations with *Pleurotus ostreatus* growth: A – PDA medium 3% (Sigma), standard, B – autoclaved *Secale cereale* kernels, C – polymer composites with 20% native rapeseed straw, D – polymer composites with 30% native rapeseed straw

The differences in mycelium growth were assessed with the Student's t-test (two-track, paired, Microsoft Excel 2007) and with Statistica-9 software (Statistica... 2010) (Fig. 1).

Similarly to the majority of forest mushrooms, oyster mushroom (*P. ostreatus*) mycelium produces specific enzymes, for example, cellulases as well as lignifying enzymes capable of polysaccharide and lignin degradation. It was reported earlier that complete decomposition by *P. ostreatus* of, for example, poplar stumps required the period of 4 years (Gapiński and Ziombra 1984).

In this paper, the authors initiated investigations in *in vitro* conditions on the development of oyster mushroom mycelium on biodegradable composites with a view of their possible application for protection of mycelium in natural forest environment.

Only in combinations A and C no statistically significant differences occurred (Table 3). There was no negative influence of the applied composites on the development of the oyster mushroom mycelium.

It seems that plates made from composites by a press moulding method can serve as shields of mycelium cultures on stumps of broad-leaved trees after their cutting (for instance: energetic willow or other trees). In addition, composite granules could possibly be utilised as a carrier (substrate) for longer storage of commercial mushroom cultures which will be the object of subsequent experiments.

This study is being continued with species other than *P. ostreatus*.

Table 3

Mean value and Student's t-test of mycelium growth (*Pleurotus ostreatus*) on PDA medium, on *Secale cereale* grain and on biodegradable composites containing 20% and 30% of rapeseed straw

Combination – the base	Mean – $\bar{x}$	Comparison of the combination	Student's t-test
Polymer composites with 20% native rapeseed straw			
D	22.5	A to D	4.61E-12**
Polymer composites with 30% native rapeseed straw			
C	29.3	A to C	0.079667
Secale cereale kernels prepared in autoclave			
B	14.8	A to B	1.08E-20**
PDA medium			
A	30.6		

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