Investigations on the growth of mycelium cauliflower fungus on composites filled with rapeseed straw with a polymer matrix Mater-Bi® NF01U

Michał Starzycki
Dominik Paukszta*
Elżbieta Starzycka

Plant Breeding and Acclimatization Institute - National Research Institute (IHAR-PIB)
Strzeszynska 36 st., 60-479 Poznan, Poland
*Poznan University of Technology, Institute of Chemical Technology and Engineering,
M. Sklodowskiej-Curie 2 Sq., 60-965 Poznan, Poland
e-mail: michals@nico.ihar.poznan.pl or michal_star@wp.pl and dominik.paukszta@put.poznan.pl

ABSTRACT

The objective of the investigations was to demonstrate growth potentials of cauliflower mushroom **Sparassis crispa** - (Wulf. Ex. Fr) Fr. in in vitro conditions. Experiments were carried out employing biodegradable composites which were used as substrates for cauliflower fungus development. The substrates contained unmodified rapeseed straw and a Mater-Bi® NF01U polymer matrix of the Italian Novamont Company. The first step of the process aimed to obtain this material involves cutting up rape stems into small pieces (1 – 2 mm) and removing parenchyma. Composite materials containing 30 % rapeseed straw were obtained using a single-screw extruder (D = 25 mm, L/D = 25). The process was conducted at 30-35 worm rotations per minute. The obtained composite in the form of granules was dried and later used in investigations. Experiments on mycelium growth were carried out using a strain of **Sparasis crispa** (Wulf. Ex. Fr) Fr. isolated in 2003 from natural conditions which was inoculated onto PDA substrate. The species of the used in the experiments was unequivocally identified on the basis of the performed DNA ribosomal sequencing (ITS-2) which was followed by the comparison of its DNA with the DNA published in the Gene Bank (GenBanc 2009). The obtained isolate was characterised by considerable capabilities for aggressive control of the substrate in 'in vitro' conditions. In addition, in the course of initial experiments, the composites with the biodegradable matrix containing native lingocellulosic materials containing the **S. crispa** exhibited totally biodegradable properties.

INTRODUCTION

The investigations aimed at obtaining a pure culture of the **Sparassis crispa** (Wulf. Ex. Fr) Fr. mushroom and were designed to check mycelium growth possibilities of the cauliflower mushroom as well as its capabilities to form carpophores in conditions *in vitro*. The biodegradable composites used in the study constituting a substrate for the development of mushroom with a Mater-Bi® NF01U polymer matrix provide a potential good protective material against other fungi which are not always beneficial. The cauliflower mushroom is a legally protected species in Poland and, therefore, our concern regarding its maintenance in natural site conditions is fully justified (list of Polish mushroom species under legal protection; Directive of the Minister of Environment of July, 9th 2004).

MATERIAL AND METHODS

Biodegradable composites used as a substrate for the development of mushrooms with a Mater-Bi® NF01U polymer matrix (Italian company Novamont) filled with a comminuted, non-modified rapeseed straw were employed in the described experiments. Rapeseed straw of Californium variety was harvested in 2007 at Swadzim Agricultural Experimental Station (Poznań University of Life Sciences). Following initial break down with the assistance of a flail-type crusher, lignified parts of straw were separated from the parenchymal tissue using an air separator. A vibrating screen was used to obtain a fraction with particle size of 1-2 mm. Composite materials containing 30% rapeseed straw were obtained by extrusion method employing a single-screw extruder (D = 25 mm, L/D = 25). The process was conducted at the velocity of 30-35 rotations of the screw per minute. The obtained composite material in the form of granulate was subjected to drying and was then used for further investigations. The following plate combinations were prepared from the obtained composites:
K – control, pure PDA substrate,
I and II – plates with addition of pine sawdust,
III and IV - plates with addition of pine sawdust and rapeseed straw,
30% - plates with the addition of 30% rapeseed straw.
The above combinations of plates were sterilised at the temperature of 100°C (Photo 2).

Mycelium growth was investigated using for this purpose a strain of **Sparassis crispa** (Wulf. Ex. Fr) Fr. mushroom isolated from natural conditions in 2003 which was inoculated onto PDA medium and subjected to sequencing in order to examine its species purity (Photo 1), (GenBanc 2008).

Photo 1. Cauliflower mushroom **Sparassis crispa** (Wulf. Ex. Fr) Fr. in its natural environment.

Observations of mycelium development on experimental substrates containing composites were conducted for 21 days simultaneously taking measurements (mm) of radially growing hyphae of **S. crispa** (Photos 4A and 4B). In addition, possibilities of *in vitro* development of carpophores were checked on PDA substrates (Photo 3).

Photo 2. Sterilised composite plates before placing them on PDA substrates.

Statistical calculations were performed with the assistance of the "Statistica 9-64 bit" package.

RESULTS

The obtained results are presented in the form of photographic documentation and a statistical diagram.

On the basis of the performed sequencing of ribosomal DNA (ITS-2), the species membership of the applied **Sparassis crispa** (Wulf. Ex. Fr) Fr. mushroom was confirmed unequivocally by comparing its DNA to the DNA published in the Gene Bank (GenBanc 2008). The examined **S. crispa** isolate was characterised by considerable capabilities of aggressive occupation of the substrate as well as production of carpophores in conditions *in vitro* (Photo 3).

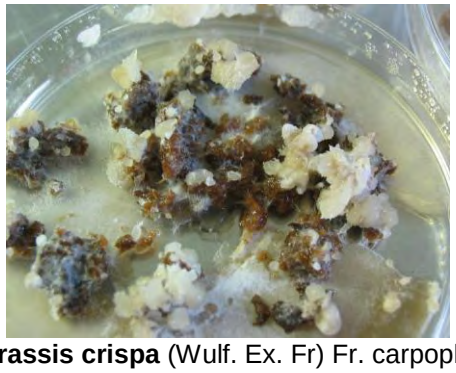
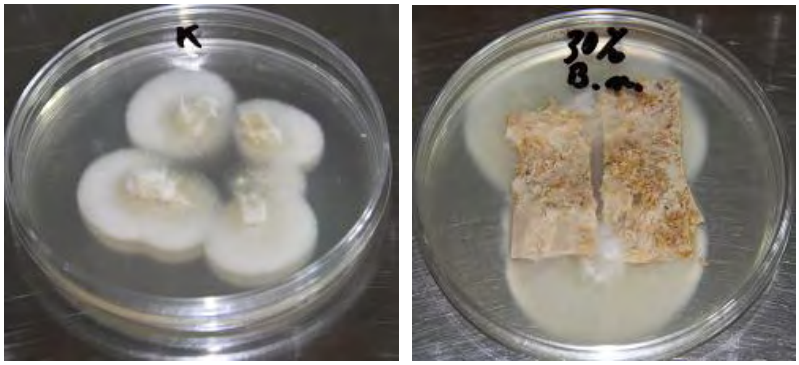
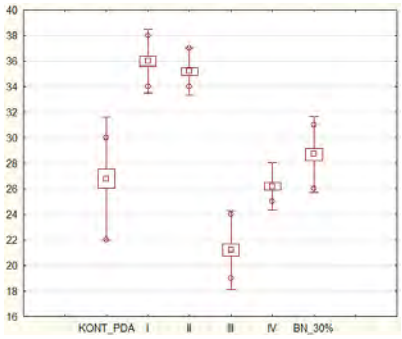


Photo 3. Developing **Sparassis crispa** (Wulf. Ex. Fr) Fr. carpophores in *in vitro* condition.



A. B.

Photo 4A and 4B. Comparison of hyphae development of the **S. crispa** mushroom on the PDA substrate (A) and with composite containing rapeseed straw (B).



□ - Mean
⊡ - Standard error of the mean
T - Standard deviation

Figure 1. Statistical diagram showing growth of the **Sparassis crispa** (Wulf. Ex. Fr) Fr. mycelium following 21-day culturing in conditions *in vitro*.

DISCUSSION AND CONCLUSION

It turned out that the best combinations for the mycelium growth of the cauliflower mushroom were two composites: I and II – polymer plates with the addition of pine sawdust. The next best conditions for mycelium growth were created by plates with the addition of 30% rapeseed straw. The remaining combinations (III and IV) were, practically speaking, worse with respect to the examined trait in comparison with the control PDA substrate. Statistical correlations are presented in Figure 1.

In addition, in the course of initial investigations, composites with the biodegradable matrix containing native lignocellulosic materials (Garbarczyk J. *et al.,* 2008; Mal-Nam Kim *et al.,* 2000) using mushroom **Sparassis crispa** (Wulf. Ex. Fr) Fr. demonstrated total biodegradability.

The results of these experiments can be implemented in forest cultivations especially bearing in mind considerable losses observed in stands due to extreme climatic conditions.

Windfalls as well as trees dying off as a result of accidental infestation provide a good substrate for fungi frequently harmful for forest economy. In order to prevent uncontrolled pathogen attacks (Łakomy P., 2004, Raziq F., Fox R. T. V., 2004, Thorpe K., Webber J., 2004) on damaged stumps and stubs, a protective solution was put forward consisting in the inoculation of windfalls by mycelium of cauliflower fungus. Initiated in vitro experiments on biodegradable composite materials containing additions of rapeseed straw may be used in future as protection capsules with the aim to protect mycelium of **S. crispa** (or other species) against natural biotic or abiotic stresses which accompany growth of these mushrooms.

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