Available online: www.notulaebotanicae.ro
Print ISSN 0255-965X; Electronic 1842-4309
Not Bot Horti Agrobo, 2018, 46(1):156-160. DOI:10.15835/nbha46110959
Original Article

The Effect of Selected Cultivation Factors on the Growth of Mycelium of *Pleurotus cystidiosus* Miller

Luiza DAWIDOWICZ*, Agnieszka JASIŃSKA, Marek SIWULSKI

*Poznań University of Life Sciences, Faculty of Horticulture and Landscape Architecture, Department of Vegetable Crops, Dąbrowskiego Street 159, 60-594 Poznań, Poland; loocy7@op.pl (*corresponding author); jasinska.a@gmail.com; marek.siwulski@up.poznan.pl*

Abstract

Pleurotus cystidiosus occurs in natural areas in North America. Its carpophores are characterized by a slightly sweet and mild taste. Fruiting bodies of *P. cystidiosus* are valued as source of nutrients and biologically active substances. The aim of the study was the selection of the optimal incubation temperature and the best substrate for the fastest mycelium growth. Two cultivars of *P. cystidiosus* – B1 and B122 was taken. Incubation of mycelium was performed in the temperature range from 15 to 30 °C. The influence of a substrate made of wheat straw, hemp shives and energetic grasses – *Panicum virgatum* and *Miscanthus* × *giganteus*. The experiment compared the growth of mycelium on a substrate with wheat straw (control sample) and with wheat straw and the addition of hemp shives and energetic grass in increasing amounts: 10, 20 and 30%. It has been shown that the growth of mycelium of tested strains on different substrates varied. The best growth of mycelium occurred on the substrate with wheat straw and the addition of hemp shives, *P. virgatum* and *M.* × *giganteus* in the amounts of 20% and 30%. The temperature of incubation also had a significant impact on the growth of the mycelium of *P. cystidiosus*. The optimal temperature was 25 °C. An investigation of the mutual dependence between morphological and qualitative characteristics of the type of agar medium allows optimization of the production of *P. cystidiosus*. The development of a low-cost and simple method for the production of *P. cystidiosus* can contribute to the introduction of this species into intensive cultivation. Use of waste materials in mushroom production will significantly increase the profitability of crops and reduce their costs.

Keywords: cultivation of edible mushrooms, energetic grass, hemp shives, incubation temperature, mycelium, organic substrate, oyster mushroom, wheat straw

Introduction

Pleurotus cystidiosus belongs to the kingdom of *Fungi*, phylum *Basidiomycota*, class *Agaricomycetes*, order *Agaricales*, family *Pleurotaceae*, genus *Pleurotus*. This species was first identified in North America by O. K. Miller in 1969. In literature, the name Miller's Oyster Mushroom, The Abalone Mushroom and the Maple Oyster Mushroom can be found as well as a Japanese name *Ohiratake* and Chinese name *bao yu gu* (Hanelt, 2001; Stamets, 2011).

Cap of *P. cystidiosus* convex to hemispherix, eventually plane, measuring 2-5 cm wide and cream to off-white in colour. Cap edge often irregular. Gills broad, sometimes widely spaced, strongly deccurent with irregular edges. Stem thick, centrally or eccentrically attached and relatively short (Fig. 1). The mycelium of *P. cystidiosus* resembles an oyster strain – white, racing linearly, soon fluffy white and aerial. But *P. cystidiosus* produces darkly pigmented arthroconidia forming a black pigment on the mycelium or basidiomata. As it grows outwards, black droplets form, radiating outwards from the centre as the mycelium matures. These are coremia – stalk-like cells whose tops are fitted with liquid droplets of black spores (Fig. 2) (Petersen *et al*., 1997; Zervakis, 1998; Croan, 2004; Bao *et al*., 2004; Lechner *et al*., 2004; Selvakumar *et al*., 2008; Abdullah *et al*., 2012; Usami *et al*., 2014; Stamets, 2011).

In natural conditions, *P. cystidiosus* occurred on all continents except Antarctica (Vilgalys *et al*., 1996; Zervakis and Balis, 1996; Lechner *et al*., 2004). It has a widespread distribution on angiosperm wood. In a natural environment it is encountered on dead tree stumps especially deciduous trees. *P. cystidiosus* occurs often in the form of groups consisting of several larger and smaller specimens that arise from a common base or imbricately are arranged one above the other (Moncalvo, 1995; Zervakis *et al*., 2004).

In cultivation, *P. cystidiosus* can be grown on substrates prepared on a base of straw and various types of agricultural,

Received: 15 May 2017. Received in revised form: 05 Sep 2017. Accepted: 08 Sep 2017. Published online: 19 Sep 2017.

horticultural, forestry and textile industry waste (Cohen *et al*., 2002; Croan, 2004; Lau *et al*., 2013; Usami *et al*., 2014). It is cultivated mainly in Asia, particularly in China, Thailand and Taiwan (Hanelt, 2001; Stamets, 2011; Usami *et al*., 2014).

The caps and stems of *P. cystidiosus* are edible. In addition to the culinary qualities of fruiting bodies, *P. cystidiosus* has a high nutritional value (proteins, fibre, minerals and vitamins) and the content of biologically active substances with proven health-promoting properties. The biological activity of these mushrooms has been confirmed in several laboratory tests and clinical trials, which showed, among others, their antitumor, anti-inflammatory, antiallergic, antiatherosclerotic, antibacterial, antiviral, antifungal, immunomodulatory and hepatoprotective properties as well as lowering blood sugar levels and the effect of blood cholesterol This mushroom is low in calories due to the limited content of lipids (Manzi and Pizzoferrato, 2000; Wasser, 2002; Croan, 2004; Thekkuttuparambil and Kainoor, 2007; Karaman *et al*., 2010; Abdullah *et al*., 2012; Patel *et al*., 2012; Lau *et al*., 2013; Siwulski *et al*., 2014; Usami *et al*., 2014).

Earlier documentation states that oyster mushrooms can be grown on a variety of waste materials, such as: various types of straw and sawdust, cotton waste, chopped and corn stover, waste from the production of palm oil, tea leaves, chopped cocoa pods (Vilgalys *et al*., 1996; Croan, 2004; Lau *et al*., 2013; Usami *et al*., 2014). During the last few years, there has been a very dynamic development in the production of edible and medicinal mushrooms. This development was mainly caused by the ready availability of low-cost, waste materials from the agricultural, textile and wood processing industries, which may suggest a potential base for their crops including the cultivation of *P. cystidiosus*. Due to the increasing scarcity of cereal straw, a lot of research is undertaken to find an alternative substrate. It may be the straw of energetic grasses that is grown with an emphasis on maximum biomass. Energetic grasses can be used as an easily available substrate. In a similar way, the problem with hemp shives, the waste from the textile industry, can also solved. Hemp shives, in addition to the substrate, reduces the percentage of pathogenic infections. Use of these waste materials in oyster mushroom production will significantly increase the profitability of the crop and reduce its costs. There will also be a new way to solve the waste problem. Development of a low-cost and simple method for the production of *P. cystidiosus* may contribute to the introduction of this species into commercial cultivation. Determination of the morphological and qualitative characteristics of this type of substrate and growing conditions allow optimizing production in order to obtain the best yield of fruiting bodies with the best characteristics, for consumption and as a raw material for obtaining biologically active substances (Vilgalys *et al*., 1996; Siwulski and Sobieralski, 2004; Sobieralski *et al*., 2011; Usami *et al*., 2014; Dawidowicz and Siwulski, 2017).

Research of this project was intended to clarify the mechanisms of growth of mycelium of *P. cystidiosus* depending on various factors, especially the temperature of incubation and the type of substrate. The aim of the experiment was the selection of the optimal incubation temperature and the type of substrate with the addition of hemp shives and energetic grass straw on which mycelium growth was the fastest.

Fig. 1. Fruiting bodies of *P. cystidiosus*

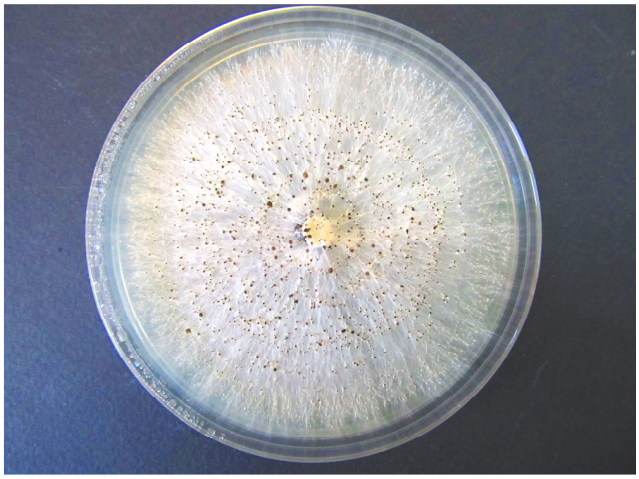

Fig. 2. Mycelium of *P. cystidiosus*

Materials and Methods

Experimental setup

The experiment was carried out from January to April 2017 at the Biological Laboratory of the Department of Vegetable Crops, in the Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences. All laboratory experiments were established in a fully randomized design, 3 replications in 2 series.

Biological material

The subject of research in all experiments was two strains of *P. cystidiosus* – B1 and B122 – from the Collection of Edible and Medicinal Mushrooms Department of Vegetable Crops, Poznań University of Life Sciences.

Temperature of incubation

Wheat agar medium was used in the experiment in Petri dishes 90 mm: wheat – extract from 200 g of wheat grains,

22 g of agar, 3 g of glucose per 1 litre of medium. The agar medium was inoculated with mycelium of *P. cystidiosus.*

Incubation of the mycelium was performed in a thermostat in the temperature range from 15 to 30 °C at intervals of every 5 °C, relative humidity 80-90%. The rate of mycelial growth was studied by measuring the diameter of the surface of the medium overgrown by mycelium. The measurements were to be performed on the 5th, 7th, 10th and 14th day of incubation.

The type of substrate

The control substrate was prepared from a wheat straw. In the experiment, the substrate with wheat straw and the addition of hemp shives and energetic grasses – *Panicum virgatum* L. and *Miscanthus* × *giganteus* – in increasing amounts: 10, 20 and 30% were used. The substrate was prepared from a wheat straw and supplements cut into chaff 4-5 cm long. The substrate for the experiment was moistened to a moisture content of about 65% using deionized water. The substrates were placed in the bacteriological test tubes and subjected to sterilization. After cooling to room temperature, the substrates were inoculated with a 1-cm layer of granular mycelium. Tubes with inoculated substrates were placed in a thermostat, which were maintained at a temperature of 25 °C and relative humidity 80-90%. The incubation was carried out in darkness for 21 days. The measure of mycelium growth was the length of the substrate in a biological tube overgrown by hyphae after 7th, 10th, 14th and 21th days of incubation.

Statistical analysis

The results of research were statistically analysed. When comparing the experimental results, the analysis of variance for factorial experiments was applied (STAT, level of significance $\alpha = 0.05$).

Results and Discussion

Temperature of incubation

A comparison of the growth of mycelium of *P. cystidiosus* in various temperatures after 5th, 7th, 10th and 14th days of incubation is presented on Fig. 1. It has been shown that growth of mycelium on different temperature was various. The best growth of mycelium of *P. cystidiosus* occurred at the temperature of 25 °C. At the temperatures 30 °C and 20 °C, mycelial growth was already significantly lower. At the temperature of 15 °C, mycelial growth was the lowest. This may be due to the fact that this mushroom in its natural environment grows in a sub-tropical climate and 30 °C is too high a temperature, while 15-20 °C too low for the proper growth of mycelium. Too low temperatures, i.e. below 22 °C, and too high, i.e. above 28 °C, inhibit the growth of mycelium. On the other hand, the longer-lasting temperature of 34-35 °C causes mycelium to decay (Ziombra 1996). Temperature 25 °C for growth of mycelium *P. cystidiosus* reccomends Stamets (2011). For the growth of mycelium, most species of oyster mushrooms are at a recommended temperature of 25 °C (Ziombra, 1998; Siwulski and Sobieralski, 2004; Gapiński *et al.*, 2007; Siwulski *et al.*, 2007; Stamets, 2011). Chang and Hayes (1978) and Kalberer (1992) determine the optimal temperature depending on the oyster mushroom species in the range of 22-28 °C.

The type of substrate

A comparison of the growth of mycelium of *P. cystidiosus* on a substrate with *Panicum virgatum* in addition 10, 20 and 30% is presented in Fig. 2. It has been shown that the growth of mycelium of *P. cystidiosus* on different substrates varied. The fastest growth of mycelium was on a substrate with a 20% and 30% addition of *P. virgatum.*

A comparison of the growth of mycelium of *P. cystidiosus* on a substrate with *Miscanthus* × *giganteus* in addition 10, 20 and 30% is presented in Fig. 3.

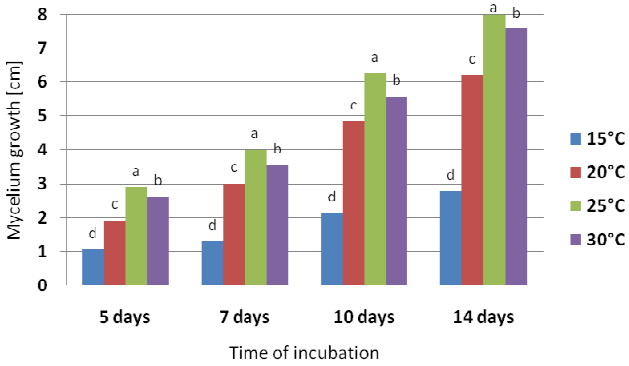

Fig. 1. A comparison of the growth of mycelium of *P. cystidiosus* in various temperatures

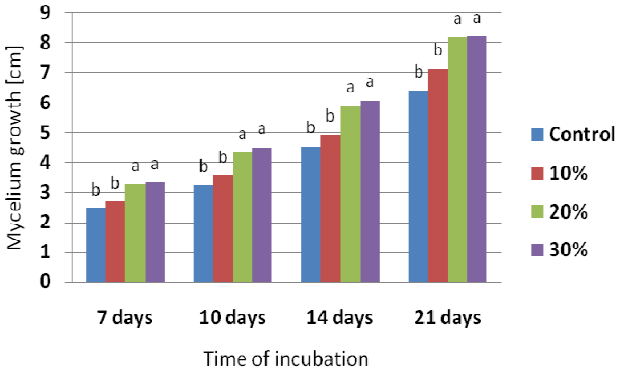

Fig. 2. A comparison of the growth of mycelium of *P. cystidiosus* in a substrate with the varied addition of *P. virgatum*

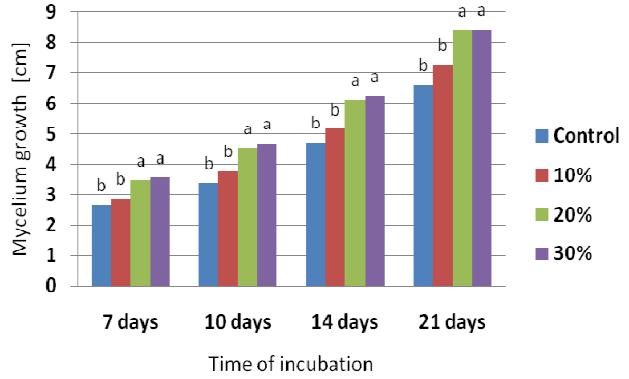

Fig. 3. A comparison of the growth of mycelium of *P. cystidiosus* in a substrate with the varied addiction of *M.* × *giganteus*

It has been shown that the growth of mycelium of *P. cystidiosus* on different substrates varied. As in the case above, the fastest growth of mycelium was on a substrate with a 20% and 30% addition of *M.* × *giganteus*.

A comparison of the growth of mycelium of *P. cystidiosus* on a substrate with hemp shives in addition 10, 20 and 30% is presented in Fig. 4.

It has been shown that the growth of mycelium of *P. cystidiosus* on different substrates varied. The fastest growth of mycelium was on a substrate with 20% and 30% addition of hemp shives.

The mycelium growth of oyster mushrooms depends on many factors, among others: an agar medium, the incubation temperature, the type of substrate, additives, which also confirms the research of Ziombra (1998) and Gapiński *et al*. (2001). Previous self study has confirmed the use of hemp shives and energetic grass straw as a component of the substrate for many species of cultivated mushrooms. It has been shown that the growth of mycelium of this species and cultivars was dependent on the diversity of the growing substrate. It has been shown that the use of substrates from mixtures of various substrates and organic additives is preferable to homogeneous substrates because of the richer mycelial growth medium, greater nitrogen and carbon diversity, and improved water and air conditions resulting from a more complex substrate structure. Moreover, it was shown particularly useful *Miscanthus* sp. straw (the mycelium overgrew at a rapid rate, fruiting bodies were of good quality) due to the high content of easily available and assimilable compounds compared to other media (eg cellulose and simple sugars). In addition, it was confirmed that the usefulness of hemp shives and their antiseptic properties have been proven to inhibit the growth of competing organisms in relation to the mycelium cultivated mushrooms, providing a quick overgrowth of mycelium through the substrate while guaranteeing yield. Moreover, hemp shives have a structure of one-year wood and the concentration of lignin and cellulose used by cultivated mushrooms is much higher than in the commonly used in mushroom cultivation cereal straw. The specific spongy structure of hemp shives promotes good water absorption and retention, which prevents the substrate from drying out and stimulates mycelium growth and yield (Jasińska *et al*., 2010; Siwulski *et al*., 2010a; Siwulski *et al*., 2010b; Sobieralski *et al*., 2011; Jasińska *et al*., 2012; Siwulski *et al*., 2013; Jasińska *et al*., 2014a; Jasińska *et al*., 2014b). Use of waste materials from the textile industry and the straw of energetic grasses in mushroom cultivation is also described by Hadar *et al*. (1993), Pani *et al*. (1997), Kirbag and Akyuz (2008), Akyuz and Yildiz (2008).

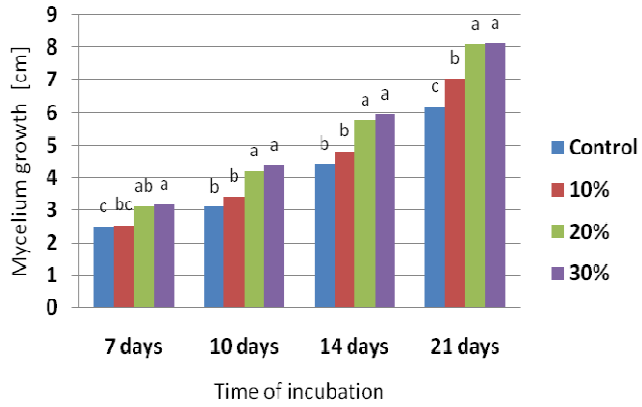

Fig. 4. A comparison of the growth of mycelium of *P. cystidiosus* in a substrate with a varied addition of hemp shives

Conclusions

The optimal incubation temperature for the growth of mycelium of *P. cystidiosus* was 25 °C. The best substrate for the growth of mycelium of *P. cystidiosus* was wheat straw with the addition of hemp shives, *P. virgatum* and *M.* ×*giganteus* in amounts of 20 and 30%.

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