Asian Journal of Mycology 4(1): 274–278 (2021) ISSN 2651-1339

www.asianjournalofmycology.org **Article**
Doi 10.5943/ajom/4/1/10

Low temperature induces *Polyporus umbellatus* sclerotia formation on nutrient media

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Pasailiuk MV 2021 – Low temperature induces *Polyporus umbellatus* sclerotia formation on nutrient media. Asian Journal of Mycology 4(1), 274–278, Doi 10.5943/ajom/4/1/10

Abstract

This experiment demonstrates that *Polyporus umbellatus* produces sclerotia on Wort Agar (WA) and Malt Extract Agar (MEA) at low temperature. After six months of cultivation on WA at 4±1ºC, *P. umbellatus* 2510 formed white irregular-formed sclerotia grouped at the edges of the petri dish while *P. umbellatus* 2511 formed white spherical sclerotia in the center of the petri dish. After four months of cultivation on MEA at 4±1ºC, *P. umbellatus* 2511 formed white spherical sclerotia near the walls of the petri dish.

Key words – cultivation – malt extract agar – stress – wort agar

Introduction

Polyporus umbellatus (Pers.) Fr., a rare fungus found in Ukraine (Didukh 2009), is listed in IUCN Red lists in some countries (Bohlin et al. 2006). Young fruit bodies of *P. umbellatus* are edible, and their sclerotia have been used in traditional Chinese medicine for centuries (Bandara et al. 2015, Zeng et al. 2011). Medical use of sclerotia has become a determining factor in the need to cultivate this species, since natural resources of the fungus are depleted (Guo et al. 2002). Therefore, in the literature, there are many studies on the cultivation of sclerotia of *P. umbellatus* in artificial conditions.

The possibility of sclerotia formation has been studied by modifying the nutrient media by adding vitamins, macroelements, microelements and changing the sources of carbon, nitrogen, other organic and inorganic compounds in the medium. It was found that only fructose can stimulate the sclerotial formation of *P. umbellatus* as a carbon component and the source of nitrogen is peptone. Vitamins, macronutrients and trace elements have no relationship with the sclerotial formation (Liu & Guo 2009). Xing et al. (2011) reported fructose and maltose as the suitable carbon sources for the induction of sclerotia of *P. umbellatus* directly from hyphae in artificial environments. In addition, pH is an important factor for the induction of sclerotial growth. Lee et al. (2013) investigated the favorable conditions for mycelial growth of *P. umbellatus* and its symbiont *Armillaria mellea*. When *P. umbellatus* and *A. mellea* were dually cultured, sclerotia were induced on basal media supplemented with glucose, fructose and maltose as carbon sources at pH 4~6, while nitrogen sources that induced sclerotia were basal media supplemented with peptone and yeast extract for 60 days under dark conditions.

Xing et al. (2015) proved that the addition of exogenous oxalic acid during the formation of sclerotia causes a delay in their differentiation (up to 9 days or more) and inhibits sclerotial biomass while significantly reducing lipid peroxidation depending on the concentration. Early studies of

Submitted 2 Febuary 2021, Accepted 26 May 2021, Published 18 June 2021
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Xing et al. (2013a) have shown that *P. umbellatus* sclerotial development is closely associated with a high oxidative state, and H_2O_2 accumulation was observed in cell walls or around the organelle membranes of mycelial cells using transmission electron microscopy. Thus, antioxidants in the medium will inhibit the formation of sclerotia of *P. umbellatus* by reducing the content of reactive oxygen species (ROS).

Therefore, it is important to maintain the antioxidant-prooxidant balance of the medium. For example, Xing et al. (2013b) reported the concentration-dependent effects of vitamin C (5–15 mg mL^{-1}) that reduced ROS generation and inhibited sclerotial formation; However, a low concentration of vitamin C (1 mg mL^{-1}) successfully induced sclerotial differentiation and increased ROS production. Thus, conditions that increase the level of ROS stimulate the formation of sclerotia. *Polyporus umbellatus* cultivated under such conditions when exposed to low temperatures induced sclerotial morphogenesis on sawdust-based media (Xing et. 2013a, Xing et. 2013b).

The aim of this study is to determine the effect of cultivation at low temperatures on the formation of sclerotia *P. umbellatus* on widely used nutrient media MEA and WA.

Materials & methods

Mushroom cultures

The fungi studied, *P. umbellatus* 2510 and *P. umbellatus* 2511, were obtained from the IBK mushroom culture collection at the M.G. Kholodny Institute of Botany, National Academy of Sciences (NAS) of Ukraine, Kyiv, Ukraine (Bisko et al. 2016, World Data Centre for Microorganisms 2021).

Nutrient media and culturing fungi

Fungal isolates were cultivated at 22±1°C on WA (8° by Baling, pH 6.0) and MEA (pH 6.0) media. Complete growth of fungal mycelium on petri dishes was determined and then the cultures were stored in a refrigerator at 4±1ºC for nine months or 22±1°C for four months. Macromorphological features of the mycelium were recorded according to the standard method proposed by Stalpers (1978).

Data analysis

The results were processed using Statistica 8.0 (StatSoft Inc., Tulsa, Oklahoma, USA). All experiments were conducted using four biological replicates, x±y represents mean ± standard deviation in all cases.

Results

The terms of complete overgrowth on Petri dishes by *P. umbellatus* mycelium were similar for strains on the same medium. On the WA, complete overgrowth on Petri dishes by mycelium of both strains took longer than that on the MEA (Table 1). The growth rate of colonies of *P. umbellatus* was similar for strains (Table 1).

Table 1 Duration (days) until complete overgrowth on nutrient media in petri dishes by *Polyporus umbellatus*

Name	strain	MEA	WA
Polyporus umbellatus	2510	15.25±1.23	18.5±0.58
	2511	14.75±0.5	18.25±0.5

Sclerotia were not induced when continuously cultivated at 22°C. Mycelium became yellow, more mature, and drops of exudate appear. But sclerotia of *P. umbellatus* were induced by continuous cultivation at 4±1°C (Fig 1).

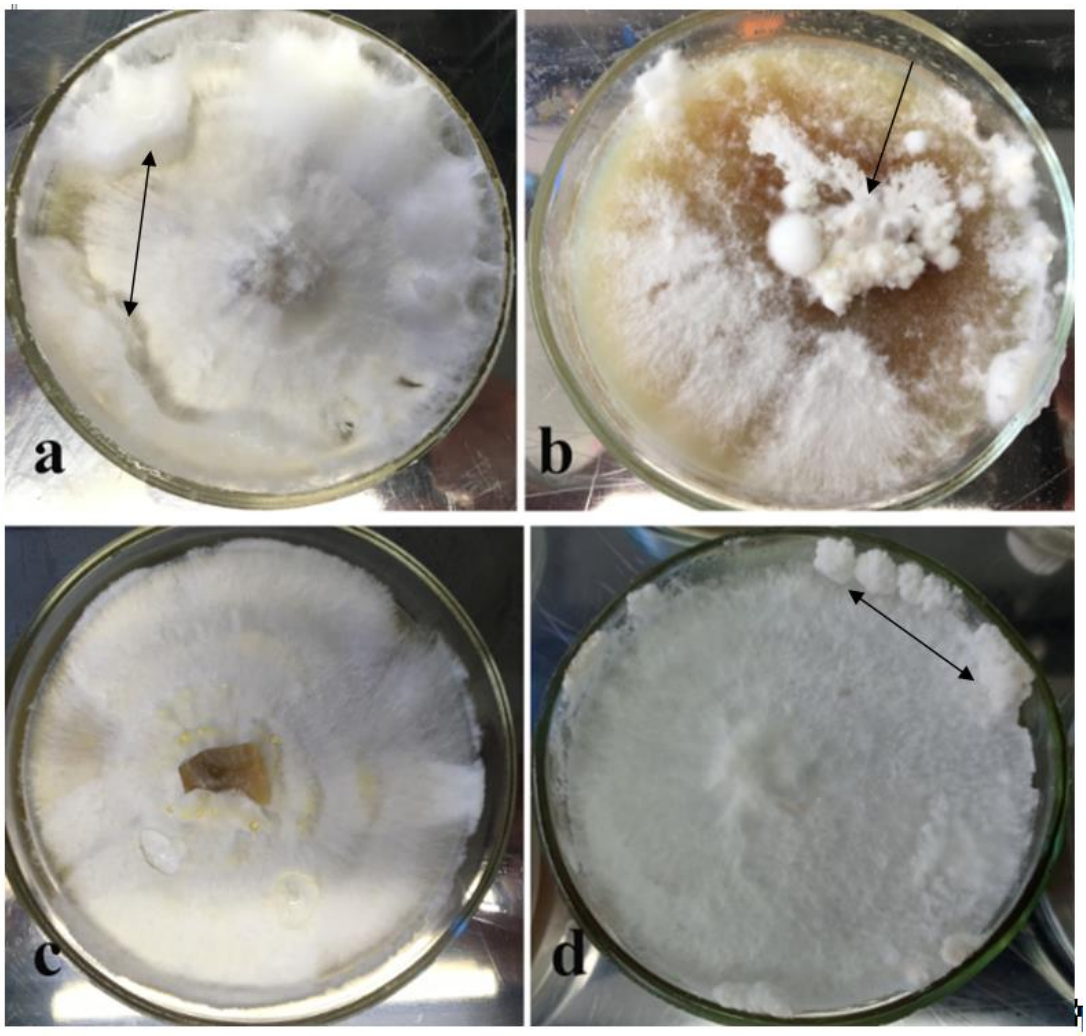

Fig. 1 – Sclerotia of *Poyporus umbellatus* 2510 (a), *Poyporus umbellatus* 2511 (b, d) and mycelium of *Poyporus umbellatus* 2510 (c) on WA (a, b) and MEA (c, d) at 4±1°C. Arrow heads indicate the sclerotia.

The timing of sclerotia formation varies, depending on the type of medium selected for cultivation. On WA, *P. umbellatus* 2510 and 2511 formed sclerotia after 6 months of cultivation at 4±1°C (Fig 1a, b). On MEA, only *P. umbellatus* 2511 formed sclerotia after 4 months of cultivation at 4±1°C (Fig 1d). *Polyporus umbellatus* 2510 did not form sclerotia even after 7 months of cultivation at 4±1°C (Fig 1c).

The morphology of sclerotia are different according to strains, and also for same strain on different media. *Polyporus umbellatus* 2510 formed white irregular sclerotia grouped at the edges of the petri dishes on WA (Fig. 1a). *Polyporus umbellatus* 2511 formed white spherical sclerotia in the center of the petri dish (Fig. 1b) and white spherical sclerotia near the walls of the petri dish (Fig. 1d).

Discussion

Polyporus umbellatus forms sclerotia underground. In nature, sclerotia help fungi to survive challenging conditions such as freezing temperatures, desiccation, microbial attack, and so on (Smith et al. 2014). The sclerotia of *P. umbellatus* contain polysaccharides that promote anti-tumor and immuno-modulating activities (Yang et al. 2004, Zeng et al. 2011). They have been used to treat edema and promote diuretic processes (Ying et al. 1987). *P umbellatus* has been used as a natural medicine in China for more than 2500 years. It has also been used as a medicinal antidote (Xing et al. 2015). The rarity of *P. umbellatus* in the environment has prompted scientists to induce

sclerotial formation in artificial conditions. Much interest has been focused on *P. umbellatus* sclerotial production directly from hyphae instead of old sclerotia in laboratory conditions (Xing et al. 2013b).

Temperature changes provoke *P. umbellatus* sclerotia formation, but the timing depends on the cultivation conditions. Overgrowth of *P. umbellatus* mycelium on petri dishes containing WA is longer, and sclerotia formed two months later than on the MEA. This fact is important for understanding the biology of this species.

Therefore, the type of nutrient medium is an important factor for the formation of *P. umbellatus* sclerotia, but the low temperature is a decisive factor. Despite the fact that sclerotia had a clearly visible relief surface, rudiments of fruiting bodies were not formed. This can be explained by rather small reserves of nutrient medium in the Petri dish with a capacity of 15 ml, while the fruiting bodies of the fungus in natural conditions reach 2.5 to 3 kg (Pasailiuk 2020a).

However, the formation of sclerotia in cultivation conditions at low temperature does not require the presence of *A. mellea*, whereas in other works the presence of this fungus is necessary for the formation of sclerotia (Lee et al. 2013).

Our results are consistent with the results of Xing et al. (2013b), where the formation of sclerotia also occurred at low temperatures, but on a medium prepared from corn, sawdust and wheat bran. In the same study, it was demonstrated that the cause that provokes the formation of sclerotia is ROS, the level of which increases due to decreasing temperature. As ROS levels increase, the hyperoxidant state becomes more severe. As a result, ROS in *P. umbellatus* mycelial cells accumulated to such an extent that the fungus formed sclerotia to adapt to the new conditions by means of reducing intracellular oxygen concentrations and keeping away from the oxidative stress state (Hansberg & Aguirre 1990).

Thus, stressful conditions provoke sclerotia formation during the cultivation of *P. umbellatus* mycelium on a suitable medium. A similar effect of stress on the acceleration of fruiting for *Hericium coralloides* cultivated by the direct confrontation method with other cultures was observed (Pasailiuk 2020b).

Therefore, there is an assumption that stressful conditions in general not just lowers the temperature during the cultivation of the species but will stimulate the formation of *P. umbellatus* sclerotia. Verification of this assumption will be the basis for further work to determine ways to stimulate the formation of *P. umbellatus* sclerotia.

These results indicated that *P. umbellatus* produce sclerotia on WA and MEA at low temperature on the artificial media. From these experimental results, we are now searching for new nutrient media and other stress factors to improve the efficiency of sclerotial production in the laboratory conditions.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The author thanks Nina Fontana, PhD candidate for editorial assistance with the manuscript.

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