



Growing of *Polyporus umbellatus*

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Abstract

Polyporus umbellatus is a valuable edible and medicinal fungus. Growth of two *P. umbellatus* strains 2510 and 2511 was studied on various plant substrates and their compositions. Fruit bodies of *P. umbellatus* were produced after successful mycelial colonization of the substrates with added freshly fallen oak leaves, wheat and sunflower seed shells. Using this growing method, the small fruit bodies (2.5 × 3.5 cm, 60–70 g) of *P. umbellatus*, strain 2511, developed on day 78, including 25 days of cultivation on MEA, 35 days of cultivation on substrate containing 50 g of wheat grain and sunflower husk with ratio 80:20, and 18 days cultivation on substrate containing 600 g freshly fallen oak leaves.

Key words – fruiting body – mycelial growth – plant substrate

Introduction

Polyporus umbellatus (Pers.) Fr. is a rare fungus and has been introduced to the Red Data Book of Ukraine (Didukh 2009). In the Red Lists of Estonia its conservation status is critically endangered, endangered in Croatia, vulnerable in Czech Republic, Hungary, Latvia, Norway, near threatened in Denmark, Finland, Great Britain, Sweden, and the Netherlands, according to the IUCN Red List Categories. This fungus is widely distributed in East Asia, Europe and North America (Bohlin et al. 2006, Kunca 2011, Bisko et al. 2018).

This mushroom is commonly known as “Zhu Ling” in China and widely used in traditional Chinese medicine for centuries. Its young fruiting bodies are regarded as edible. The fruiting body of *P. umbellatus* is composed of numerous caps (Bandara et al. 2015). The multiple circular pilei arising from a common stem make its very distinct species (Ryvarden & Melo 2014). The fruit bodies are whitish, becoming brown with age, their characteristics pores are circular to angular (Ryvarden & Gilbertson 1994, Stamets 2002). This fungus usually grows at the base of trunks, roots and stumps of oak, birch in the deciduous and mixed forests from June to October (Kotlaba 1984), forming underground sclerotia (Zhao & Zhang 1992, Guo et al. 2019). The sclerotia have been used to treat edema and promote diuretic processes (Ying et al. 1987). They contain the polysaccharides that promote the antitumor and immunomodulating activities (Yang et al. 2004, Zeng et al. 2011). This fungus has a diuretic effect on pathogenic dampness and it is being used in traditional medicine combinations to treat oliguria, diarrhea, acute nephritis, thirst, urination disturbance, sunstroke, jaundice, cirrhosis, and ascites (Ying et al. 1987, Wu 2005, Liu & Liu 2009, Zhao et al. 2009). The sclerotia of *P. umbellatus* were successfully cultured in China with *Armillaria mellea* (Vahl) P. Kumm, both on the artificial media and under natural conditions using burying and root inoculation methods (Choi et al. 2003, Lee et al. 2013).

While the sales commercial products of sclerotia and fruit bodies of *P. umbellatus* trend to increase, the resources of *P. umbellatus* in the wild will soon exhaust (Guo et al. 2002). Therefore it is necessary to cultivate the *P. umbellatus* under artificial or semi artificial methods (Huang & Liu 2007, Xing et al. 2013). In this study, we aim to test the new cultivation methods for *P. umbellatus* productions by using the composition of various plant substrates without *A. melea*.

Materials & Methods

Mycelial culture

The pure cultures of *P. umbellatus* strains 2510 and 2511 were obtained from the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany (IBK) of the National Academy of Sciences of Ukraine (Bisko et al. 2016, 2018, World Data Center for Microorganism 2019).

Culture growth on nutrient agar medium

Polyporus umbellatus culture was isolated onto malt extract agar (MEA), pH 6, and incubated at 26°C for 25 days (Bisko et al. 2016). The radial growth rate of the mycelium (mm/day) was calculated according to Lomberg & Solomko (2012).

Spawn preparation on plant substrates

Polyporus umbellatus spawn was prepared in two consecutive stages, including the different substrates and their combinations (Table 1).

Table 1 Substrate compositions.

Components of the substrates (%)	Substrates				
	WgSs	WgBsWsPs	wheat	leaf	WsOl
Wheat grain (Wg)	80	50	100	–	–
Sunflower seed shells (Ss)	20	–	–	–	–
Beech shavings (Bs)	–	25	–	–	–
Wheat straw (Ws)	–	12.5	–	–	50
Peanut shells (Ps)	–	12.5	–	–	–
Oak leaves (Ol)	–	–	–	100	50

Notes – WgSs: wheat grain/sunflower seed shells, WgBsWsPs: wheat grain/beech shavings/wheat straw/peanut shells, wheat: wheat grain, leaf: oak leaves, WsOl: wheat straw/oak leaves

Substrates components were cooked as described by Pasailiuk et al. (2019). Wheat grain (10 kg) was cooked for 25–30 min in 10 liters of water. After drying, one kilograms of wheat grain was mixed with 12 g of gypsum (CaSO₄·2H₂O) and 3 g of chalk (CaCO₃). Sunflower seed shells and peanut shells were pre-dried. Beech shavings (*Fagus sylvaticus*) was obtained during the shredding of healthy beech wood and the size of the wooden particles was 10×10–40×1 mm. Wheat straw was dried and chopped to 2.5–5 cm particles size. Forest litters were collected under the oak (*Quercus robur*) and dried. All substrates were moisturized to 60% humidity, and filled to 0.5 liters glass jars and flasks (WgSs, WgBsWsPs, wheat substrates) or put into 4 liters polyethylene bags (WsOl and leaf substrates). Fifty grams of each WgSs, WgBsWsPs and wheat substrate were put into the jars and flasks, autoclaved at 121°C for 90 min. Each jar or flask was inoculated with 1/4 of the mycelial colony on a 90 mm petri dish and incubated at 26°C for 35 days.

Six hundred grams of each leaf substrate and WsOl (pH 4.6 and 5.5) were autoclaved twice at 121°C for 1 hour. After cooling down, 1/3 part of the 35 days old culture of *P. umbellatus* were transferred to each substrate and incubated at 22°C, under the light maximum 200 lux/darkness for 12:12 hours, 75% humidity content, and ventilated at 1–3 room volumes air exchange per hour. The mycelium colonization rate of each substrate was monitored daily and recorded by measuring of the height of the mycelium which covered the substrate from four mutually perpendicular sides. The

average values were calculated by adding four values of heights and dividing by four and the % of colonization the substrate by mycelium was determined as:

$$\text{Percentage of colonization (\%)} = (h_r+h_l+h_a+h_f)/h_s \times 100\%$$

Where:

h_r , h_l , h_a , h_f – height of mycelium covering the right, left, back and front side of jar/flask/bag, correspondently, h_s – height of substrate in the jar/flask/bag.

Macro-morphological features of the mycelium were described according to the standard methods proposed by Stalpers (1978). The morphological characteristics at day 15 on MEA media, day 19 on the wheat substrate, day 28 on the WgSs, WgBsWsPs, and wheat substrates, and day 23 on leaf and WsOl substrates were recorded.

Primordia and fruiting bodies of *Polyporus umbellatus* on plant substrates

The primordia and young fruit bodies of *P. umbellatus* 2511 on the WsOl and leaf substrates were recorded. Their morphology, color, size and location in the substrates were described.

Statistical analysis

Data analysis was carried out using Statistica 8.0 software (StatSoft Inc., USA). Significant differences between values are indicated at $p \leq 0.05$.

Results

Mycelial growth rate and morphology of *Polyporus umbellatus*

The growth rate of *P. umbellatus* was 2.97–3.01 mm/day on nutrient media, thus this fungus belongs to slow-growing cultures (2–4 mm/day). The mycelial morphology of *P. umbellatus* colonies of the strains 2510 and 2511 on MEA was dense, opaque, white wooly, with concentric circles formed with cottony aerial hyphae (Fig. 1a, b). The colony margin is raised above the substrate, and became yellow after incubation for 35 days.

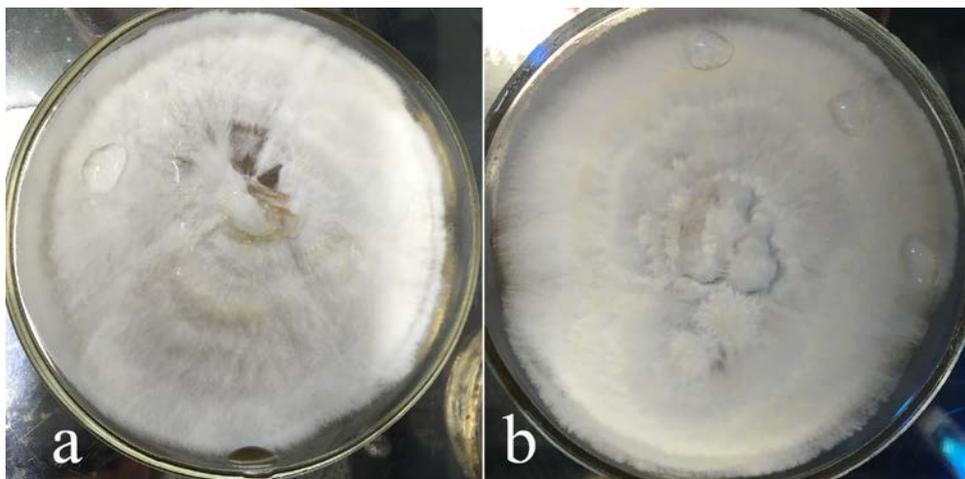


Fig. 1 – *Polyporus umbellatus* mycelial colonies incubated at 26°C for 15 days. a strain 2510. b strain 2511.

Morphology and growth rate of *Polyporus umbellatus* on WgSs, WgBsWsPs and wheat substrates

After incubation for 21 days, strain 2510 was growing better on WgSs substrate (Fig. 2), while strain 2511 was able growth well on wheat media (Fig. 3).

The WgSs and WgBsWsPs substrates were equally suitable for the mycelial growth of *P. umbellatus*. Also, these two substrates were significantly less susceptible to contamination compared to wheat (5% vs 25%). No significant differences were observed in the mycelial morphology of strains grown on different substrates. The mycelial colonies had distinctive cotton white and fluffy surface (Figs 4, 5).

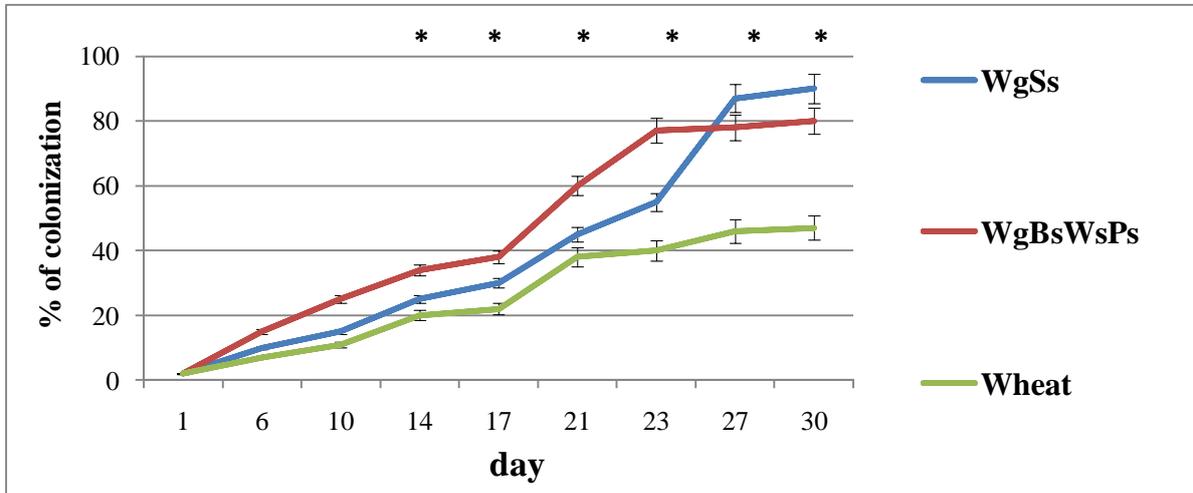


Fig. 2 – Colonization rate of *Polyporus umbellatus* strain 2510 on various substrates.

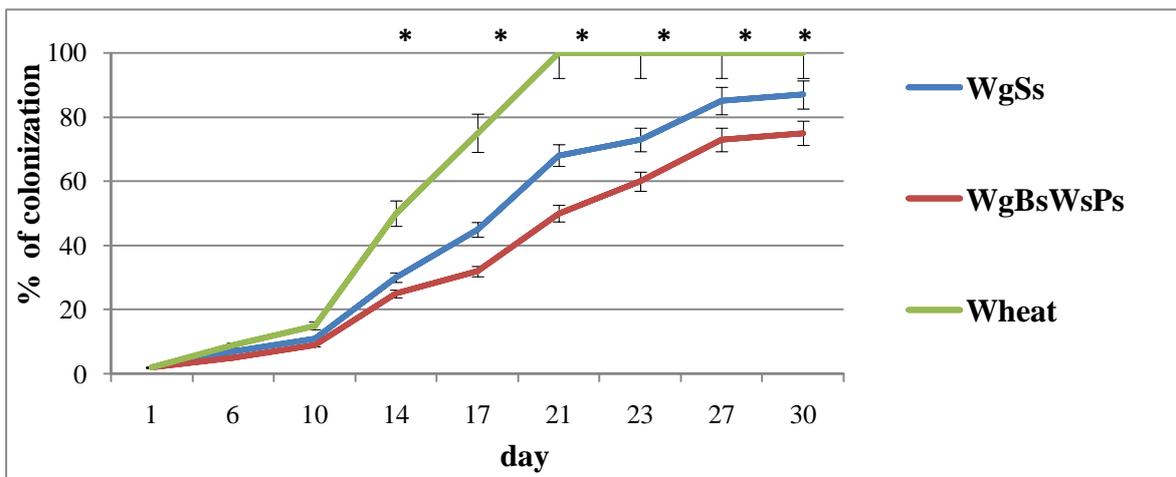


Fig. 3 – Colonization rate of *Polyporus umbellatus* strain 2511 on various substrates.



Fig. 4 – *Polyporus umbellatus* strain 2510 incubated on different substrates for 28 days. a WgSs substrate. b WgBsWsPs substrate. c Wheat substrate.



Fig. 5 – *Polyporus umbellatus* strain 2511 incubated on different substrates. a WgSs substrate after incubation for 28 days. b WgBsWsPs substrate after incubation for 28 days. c Wheat substrate incubated for 19 days.

Substrate, colonized with mycelium, was not firmly attached to the jar wall (Fig. 6a, b, c), as it was in the flasks. Therefore its removing from the flasks (Fig. 6f) was hard, but its removing from the jars (Fig. 6d, e) was easy, not damaging the mycelium structure and did not take long time, thus minimizing the mycelium exposure to the exogenous environmental factors.



Fig. 6 – *Polyporus umbellatus* mycelium on the combined substrates. a, b, c Mycelium in the jars. d, e Mycelium taken out from the jars. f Mycelium taken out from the flask.

Morphology and growth rate of *Polyporus umbellatus* on plant leaf and WsOl substrates

Polyporus umbellatus 2510 and 2511 are growing better on leaf substrate than on WsOl substrate. The level mycelial colonization of WsOl substrate was only 45–50% even after 30 days of cultivation (Figs 7, 8). Color of mycelial colonies on the leaf substrate was white or milky-white, covered with distinct spidery hyphae, while the center of the colony was more wooly (Fig. 9a, c). The mycelial colonies on the WsOl substrate was like a white cottage cheese (Fig. 9b, d).

Primordia and fruiting bodies of *Polyporus umbellatus* formed on plant substrates

Strain 2511 of *P. umbellatus* colonized the leaf substrate and WsOl substrate and its primordia and young fruiting bodies were developed on the day 18 and 38 accordingly (Fig. 10).

The fruiting bodies were formed on top of the bags on two substrate types (Fig. 10c, d). Spherical primordia with well-visible small numerous branches were formed at the perforation

point of the bags (Fig. 10a, b). Young fruiting bodies had milky white color and became light gray while mature. The maximum size of the fully formed fruit bodies did not exceed 2.5×3.5 cm, weighing 60–70 g each, only 1–2 fruiting bodies per bag (at the perforation site) were formed. The fruiting bodies on top of the bags were composed of numerous caps with a barely noticeable stems. Caps had a common base, the size of fruit bodies didn't exceed 6–7 cm (basis) and 3–4 cm (high) (Fig. 10c). Within the same bag with leaf substrate we observed their simultaneous formation at both locations, i.e. at the perforation site and on the top of bag.

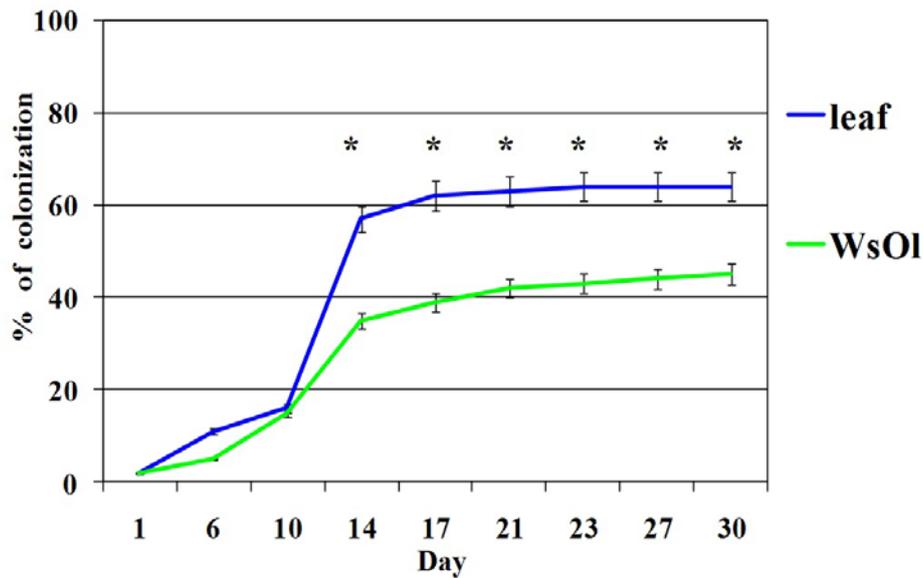


Fig. 7 – Colonization of plant substrates by the mycelium of *Polyporus umbellatus* 2510.

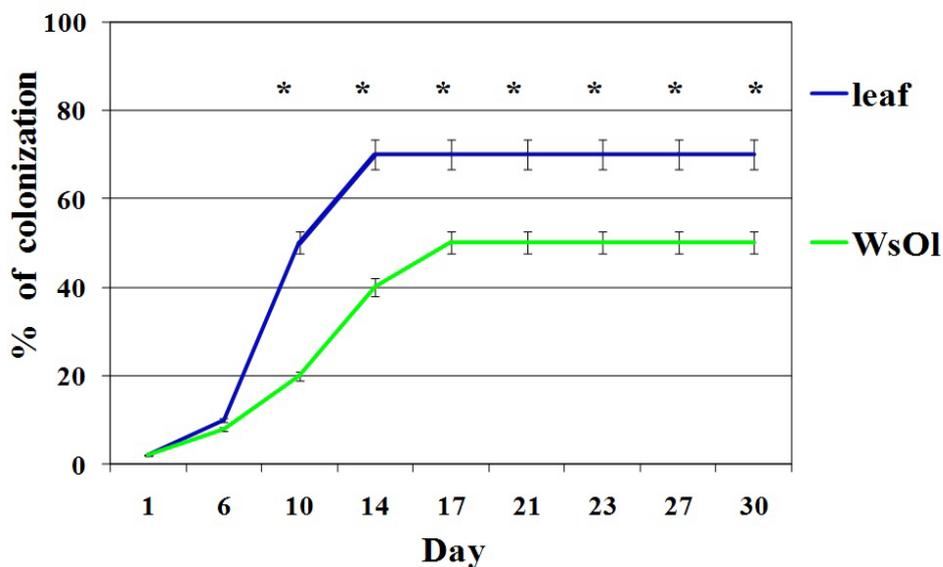


Fig. 8 – Growth rate of *Polyporus umbellatus* 2511 on plant substrates.

On the WsOI substrate the light gray spherical primordia of *Polyporus umbellatus* 2511 were formed only at the top of the package (Fig. 10d). Their lower part was lacking any patterns, with bushy branches on the top. The fruiting of *P. umbellatus* 2511 on WsOI substrate occurred only in 50% of cases, in such cases only one primordium was observed within one bag. Strain 2510 was not fruiting on any used substrate.

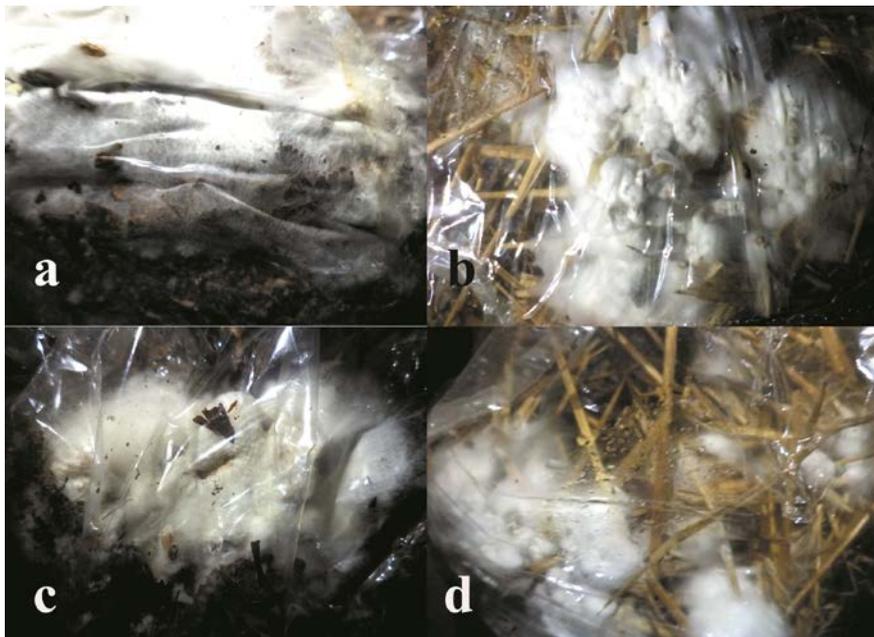


Fig. 9 – *Polyporus umbellatus* mycelium. a, b Strain 2511. c, d Strain 2510. a, c Leaf substrate. b, d WsOI substrate at day 23.

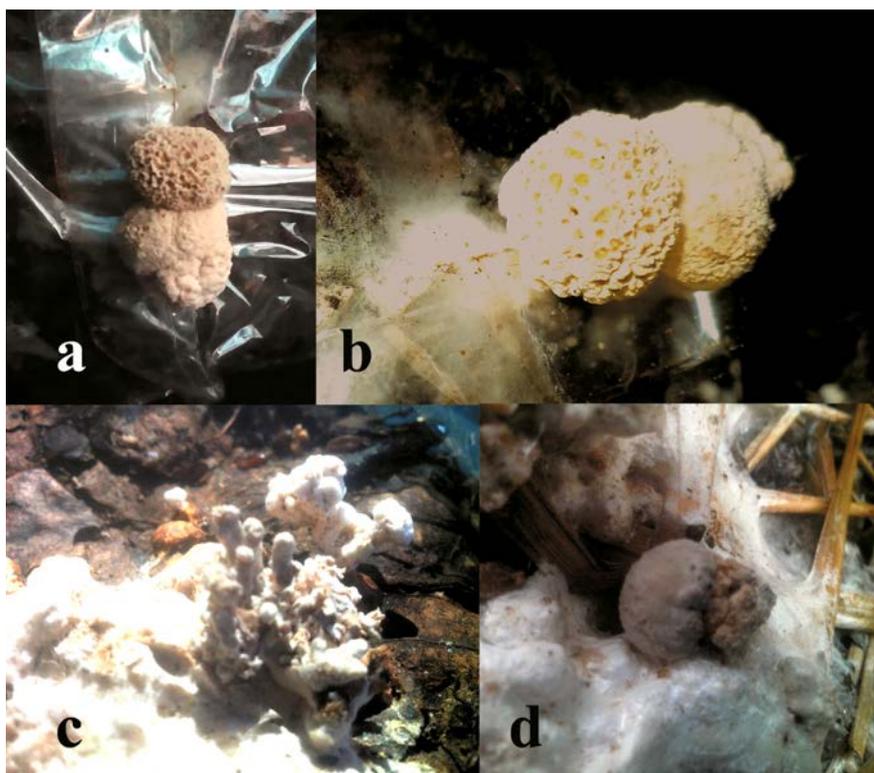


Fig. 10 – The primordia and fruiting bodies of *Polyporus umbellatus* strain 2511. a, b, c Mycelial colonization of the leaf substrate at day 18. d Mycelial colonization of the WsOI substrate at day 38.

Discussion

The ecological distribution of the edible and medicinal mushroom *P. umbellatus* in Central Europe was studied. Main motivation of this investigation was to describe the potential for commercial cultivation of this species (Kunca & Pavlik 2019). Xing et al. (2013) illustrated that exposure to low temperatures induced the *P. umbellatus* sclerotial morphogenesis during

cultivation in a sawdust substrate. The low temperature treatment enhanced reactive oxygen species in mycelia, which may be important in triggering sclerotial differentiation in *P. umbellatus*. We demonstrated the *P. umbellatus* strain 2511 grew well at 22–26°C on the combinations of plant substrates. Kunca & Pavlik (2019) reported that *P. umbellatus* predominantly grew in acidic soils (pH 4.5–4.9). Leaf and WsOl substrates have acidic pH, this feature is important for *P. umbellatus* fruiting. However, *P. umbellatus* strain 2510 was not forming the fruiting bodies, which might be a sign of the culture degeneration. Also, the fruiting bodies of *P. umbellatus* strain 2511 had lighter weight than those found in natural habitats (2.5 to 3 kg) (Petrichuk & Pasailiuk 2015). Mycelium of both *P. umbellatus* strains obtained on WgBsWsPs could be stored for six months at 21°C, without drops of exudate and yellowing of mycelium. Interestingly, the texture and degree of grinding of the substrate's components has a little influence on mycelial growth as it was observed for other fungi (Pasailiuk et al. 2018, 2019).

This work to find out the optimal weight ratio of the substrates and modifying them to continue. We do not exclude that modifying may change the time of overgrowth of substrates by mycelium of the fungus. We also see the prospect of future research in the application of the obtained knowledge for the remediation of the fungus in natural conditions by the *re-situ* method to preserve the specie in nature. Interesting, growing of *P. umbellatus* on the combination of plant substrates do not needs the *Armillaria mellea* mycelium like burying method or root inoculation method (Choi et al. 2003). Nevertheless, the obtained results are valuable because the sequence of affordable and relatively inexpensive plant substrates were found (fallen leaves are an almost inexhaustible resource), on which the fruiting of *P. umbellatus* 2511 can be achieved under laboratory conditions.

Conclusion

We obtained fructification of *P. umbellatus* on several plant substrates in axenic culture, without adding of *A. mellea*. However, one of two studied strains didn't fruit on any used substrate. Obtained fruitbodies were significantly smaller and not well developed comparing to the harvested in the natural habitat ones.

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