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COMPARISON OF THE MYCELIAL GROWTH OF SOME *PHELLINUS* SPP. ISOLATES ON DIFFERENT AGAR MEDIA

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ABSTRACT: The aim of this paper was to examine and compare the mycelial growth of *Phellinus* spp. The following isolates were used: *P. igniarius* (marked 1 and 2), *P. pini* (3 and 4), *P. pomaceus* (5 and 6), *P. robustus* (7 and 8) and *P. torulosus* (9). The growth of the isolates was checked on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Wheat Agar (WA) and Sawdust-Wheat Agar (SWA) in order to confirm their suitability for *Phellinus*. The isolates were incubated at 25°C. The results for the mycelial growth (G) (understood as the average values of the diameters of colonies after ten days of incubation) and short descriptions of the cultures on MEA are presented. The mycelial growth of each isolate was the weakest on MEA (G = 37.3 mm), while it was more abundant on the other media (G > 50 mm). *P. pomaceus* (G = 64.7 mm) and *P. igniarius* (G = 59.8 mm) had the highest G. In the case of *P. pomaceus* and *P. igniarius*, intraspecies differences were observed on all of the media.

KEY WORDS: *Phellinus* spp., fungal cultures, wheat medium, sawdust-wheat medium.

Introduction

Mushrooms from the genus *Phellinus* (Quél., 1886) (Hymenochaetaceae, Basidiomycota) are bracket fungi whose fruit bodies are found on trees. Species from this genus are found in Poland (Wojewoda 2003). They can infect living woody plants, and as parasitic fungi, they are threats to their hosts. However, their fruit bodies are useful in the treatment of many diseases. They are well-known sources of bioactive compounds that have health-promoting effects (Dai et al. 2010). For example, they are rich in antitumor and immunostimulating polysaccharides (Wasser 2002, Wang et al. 2012).

Due to their medicinal value as well as their destructive activities on trees, it is important to determine the preferences and the growth of their mycelium of *Phellinus* spp. Studies have demonstrated that the hyphae of *Phellinus* can be cultivated on agar media (for example, Hur 2008, Guo et al. 2009, Balaeş & Tănase 2012). The most frequently used media are prepared from common ingredients such as malt extracts. Natural ingredients and their decoctions are rarely used and it is worth examining whether they are better or worse media than the standard ones. For instance, the wood of a host tree can be an efficient source of nutritional components for agar media for bracket fungi.

Research to investigate the preferred media conditions for *Phellinus linteus* (Berk. & M.A. Curtis) Teng, 1963 (Kim et al. 2002, Jo et al. 2006, Hur 2008), *Phelinus igniarius* (L.: Fr.) Quél., 1886 (Jung et al. 1997, Guo et al. 2009, Balaeş & Tănase 2012), *Phelinus pini* (Brot.: Fr.) A. Ames, 1913 (Kim et al. 2002) and *Phelinus gilvus* (Schwein.) Pat. 1900 (Rew et al. 2000, Jo et al. 2006) has been performed. However, *Phelinus torulosus* (Pers.) Bourd. & Galz., 1925 (Gilbertson & Burdsall 1972), *Phelinus pini* (Worrall 1991, Kim et al. 2002), *Phelinus pomaceus* (Pers.) Maire, 1933 (Balaeş & Tănase 2012) and *Phelinus robustus* (P. Karst) Bourd. & Galz., 1925 are not common subjects in studies that are focused on mycelial growth. Previous studies have only been focused on one (rarely two) of the species of *Phellinus* that were selected for this study.

The objective of this study was to compare the growth of cultures and to determine the suitability of four media for five *Phellinus* species. This study presents new data about *Phellinus* mycelial growth and the potential of Sawdust-Wheat Agar (SWA) and Wheat Agar (WA) as alternative media for cultivating mushrooms.

Material and Methods

Fruit bodies

Nine isolates of five *Phellinus* species from nine localities were collected: 1 and 2 – *P. igniarius* from two specimens of *Salix alba* L., 1753; 3 and 4 – *P. pini* from two specimens of *Pinus sylvestris* L., 1753; 5 and 6 – *P. pomaceus* from specimens of *Prunus domestica* L., 1753 and *Prunus domestica* subsp. *syriaca*; 7 and 8 – *P. robustus* from two specimens of *Quercus robur* L., 1753; 9 – *P. torulosus* from a dead trunk of *Quercus* sp. L., 1753. The numbers of the isolates are the same as the numbers of their localities (Tab. 1). For a more detailed list of the samples, the isolates and the localities see Stala et al. (2015).

The fungal isolates

The fruit bodies were broken down and 2 mm-diameter discs of the plectenchyma were removed from the zone next to the hymenial layer. The discs were placed on Petri dishes with Difco PDA. The incubation temperature was 25°C and incubation was continued until vegetative mycelia were obtained. In the next step, the vegetative mycelia were used for the inoculation of the media.

Culture media

Four different agar culture media were prepared in order to determine mycelial growth:

1. Potato Dextrose Agar (PDA) – a 1 dm^3 decoction from 200 g of peeled potato tubers, 3 g glucose, 22 g agar, (pH = 5.8);
2. Malt Extract Agar (MEA) – 10 g malt extract, 0.1 g calcium carbonate, 22 g agar, (pH = 6.0);
3. Wheat Agar (WA) – a 1 dm^3 decoction from 200 g wheat grains (boiled until the first grains burst), 3 g glucose, 22 g agar, (pH = 6.2);
4. Sawdust-Wheat Agar (SWA) – a 1 dm^3 decoction from 100 g wheat grains and 150 g beech and oak sawdust in a volume ratio of 1:1, 3 g glucose, 22 g agar, (pH = 5.6).

The agar media were sterilized in an autoclave for 30 minutes at 121°C. There were 20 cm^3 of the media in each 9-cm Petri dish.

Inoculated discs (5 mm in diameter) of the nine isolates were cut out of the PDA media and transferred into the center of the Petri dishes. This part of the experiment was repeated eight times for the four media being investigated, and therefore, 288 colonies were obtained. The fungi were incubated in the dark with the humidity level set at 80-85% for ten days at 25°C after which the diameters of the colonies were measured to a 1 mm accuracy. The diameter of a mycelium was determined in three directions and then the average value was calculated. The results are presented as the mycelial growth (G).

Statistical analysis

All of the results are expressed as the mean ± SD (Standard Deviation). The differences between the species were determined using the ANOVA rank Kruskal-Wallis test. The differences between the isolates of the same species were determined using the Mann-Whitney *U* test. Statistical analysis of the effects of the type of media on mycelial growth was performed using the one-way ANOVA. The effects of the isolates and the type of media on mycelial growth were determined using the two-way ANOVA. In the case of significant differences, the post hoc test (NIR test) was used. P-values below 0.05 were regarded as statistically significant. Statistical data analysis was performed using Statistica 13 software.

Results

Differences between the mycelial growth of the species

Significant differences were observed in the mycelial growth of *Phellinus* (Tab. 2). The greatest differences were found between *P. pomaceus* (G = 64.7 mm) and *P. pini* (G = 33.2 mm), and then between *P. igniarius* (G = 59.8 mm) and *P. pini* (G = 33.2 mm). Species are arranged in the order of their decreasing mycelial growth – *P. pomaceus*, *P. igniarius*, *P. torulosus*, *P. robustus* and *P. pini* (Fig. 1).

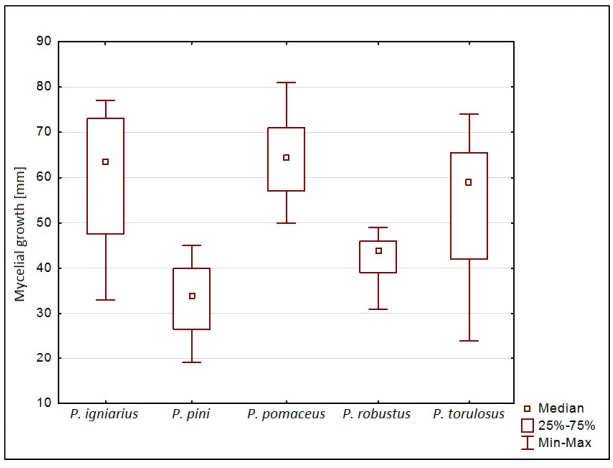

Fig. 1. Mycelial growth of *Phellinus* spp. on agar media, 25-75% represent the interquartile range

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Differences between the mycelial growth of the isolates of the same species

In the case of *P. pomaceus* and *P. igniarius*, significant intraspecies differences were observed on all of the media (Tab. 2). *P. pomaceus* isolate no. 5 had a larger growth (G = 72.1 mm) than isolate no. 6 (G = 57.2 mm), and the mycelial development of the *P. igniarius* isolate no. 1 was larger (G = 62.8 mm) than isolate no. 2 (G = 56.8 mm).

There were no significant differences between the *P. pini* or *P. robustus* cultures. *P. torulosus* was not investigated because only one isolate was used.

Effects of the types of media on mycelial growth

Each of the *Phellinus* species was influenced by the type of media in a statistically significant manner (Tab. 2). The mycelial growth of each isolate was weak on MEA, while on WA, SWA and PDA, it was much higher and no significant differences between them were found (Fig. 2). The media are arranged in order of their decreasing suitability – WA (G = 57.0 mm), SWA (G = 55.4 mm), PDA (G = 52.0 mm) and MEA (G = 37.3 mm). Differences were observed between the morphological features of the *Phellinus* species on MEA (Tab. 3).

Effects of the isolates and the type of media on mycelial growth

Each *Phellinus* species was influenced by the isolate as well as the type of media. In addition, the interaction between the medium and the isolate affected the mycelial growth in each of the species that were studied, with the exception of *P. torulosus* for which only one isolate was used. *P. igniarius* isolates no. 1 and 2 had differences between their media preferences. SWA was a better medium (G = 67.0 mm) for isolate no. 2 than for isolate no. 1 (G = 60.1 mm).

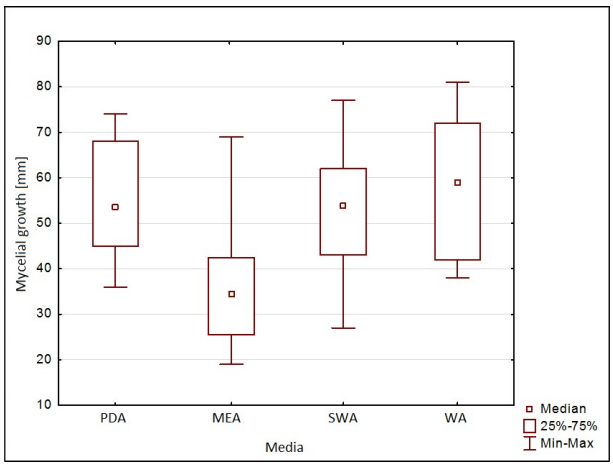

Fig. 2. Mycelial growth of *Phellinus* spp. on different media. PDA: Potato Dextrose Agar, MEA: Malt Extract Agar, WA: Wheat Agar, SWA: Sawdust-Wheat Agar, 25-75% mean the interquartile range

Discussion

Differences between the mycelial growth of the species

Significant differences were observed between *Phellinus* mycelial growth, especially between *P. pini* and *P. pomaceus*. To date, studies have been dedicated to one (or rarely two) of the species that were examined during this research (Worrall 1991, Kim et al. 2002, Hur 2008, Hur et al. 2008). A similar relationship between other *Phellinus* from Europe and Asia was described by Hur et al. (2008).

A study on two species was published by Balaeş and Tănase (2012). There were significant differences between the mycelial growth of *P. igniarius* and *P. pomaceus* on MEA on which the growth of *P. igniarius* was slower than the growth of *P. pomaceus*. This is in agreement with our observation.

Differences between the mycelial growth of the isolates of the same species

The isolates of *Phellinus* can have intraspecies differences in mycelial growth

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and these were observed between the isolates of *P. igniarius* and *P. pomaceus*.

The growth of *P. igniarius* and *P. pomaceus* on MEA was investigated by Balaeş & Tănase (2012). The time required for the entire 9 cm plate to be covered was investigated (*P. igniarius* – 90 mm/28 days; *P. pomaceus* – 90 mm/21 days). The growth was slower than in this study (*P. igniarius*: isolate no. 1 G = 74.9 mm, isolate no. 2 G = 53.9 mm; *P. pomaceus*: isolate no. 5 G = 71.1 mm, isolate no. 6 G = 60.6 mm).

The most conspicuous difference was observed between the results for the *P. torulosus* that was cultivated on MEA for 14 days by Gilbertson and Burdsall (1972) (6-8 mm/14 days) and for the *P. torulosus* on the MEA that was used in this study (G = 26.0 mm). In our study, the growth of the mycelium of *P. torulosus* was five times faster. In contrast to the results mentioned above, cultivation on MEA had better growth than in this study only once. This was observed between the *P. pini* growth in this study (isolate no. 3 – G = 21.8 mm; isolate no. 4 – G = 23.9 mm) and in the study carried by Worrall (1991) in which the radial growth of the mycelium was examined (11 mm/7 days).

Intraspecies differences in mycelial growth on PDA were also observed for *P. linteus* strains (Hur 2008, Hur et al. 2008).

All of the differences mentioned above may be related to the individual features of each isolate.

Effects of the media types on mycelial growth

The type of media is one of the most important factors that have an effect on mycelial development. The influence of media on *Phellinus* has been widely described (Worrall 1991, Kim et al. 2002, Jo et al. 2006, Hur 2008, Hur et al. 2008), but no one has used media that were based on a decoction of wheat and sawdust. The efficient growth of the *Phellinus* mycelium and the lack of significant differences between the growth on the agar media that were prepared from the decoctions of natural ingredients (sawdust, wheat grains, potatoes) showed the high degree of the suitability of WA, SWA and PDA for *Phellinus* cultures.

Generally, MEA is the most common media that is used to cultivate *Phellinus*, but this study indicates that there are more efficient media for its growth. Therefore, it can be concluded that WA, SWA and PDA may be alternatives to MEA. MEA was the least efficient media for all of the isolates.

Effects of the isolates and the type of media on mycelial growth

This study and earlier studies have confirmed that the media type as well as the selection of the isolates has an impact on mycelial growth (Harper & Kennedy 1986, Angwin 1989, Tura et al. 2009). However, it is not common to investigate whether there is a special (simultaneous) effect of both of these on mycelial growth. At this stage of the research, we can say that although there are statistically significant effects between them, their detailed description requires further studies.

Concluding remarks

1. MEA is often used as a basic medium for fungal cultures. Here it was shown that *Phellinus* mycelia had the smallest growth on this medium and that MEA was the least adequate medium,
2. An alternative for the cultivation of *Phellinus* could be media that are prepared based on decoctions from natural sources (for example, sawdust and wheat which were used in this study) and that they can be used as substitutes for MEA.

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Streszczenie

Celem pracy było określenie i porównanie wzrostu grzybni *Phellinus* spp. Użyto dziewięciu izolatów z gatunków: *P. torulosus*, *P. robustus*, *P. pomaceus*, *P. igniarius* i *P. pini*, które uprawiano na czterech różnych pożywkach agarowych, aby określić ich przydatność dla rodzaju *Phellinus*. Zastosowano pożywki: ziemniaczano-glukozową (PDA), maltozową (MEA), pszenną (WA) i trocinowo-pszenną (SWA). Po 10 dniach inkubacji w 25°C zmierzono średnice kolonii. Artykuł prezentuje dane dotyczące wzrostu grzybni (G) i krótkie opisy koloni na MEA. Wszystkie izolaty wykazały mały wzrost na MEA i duży wzrost na WA, SWA i PDA. Wzrost grzybni wszystkich izolatów na poszczególnych pożywkach wyniósł odpowiednio: MEA G = 37,3 mm, PDA G = 52,0 mm, SWA G = 55,4 mm, WA G = 57,0 mm. Największy wzrost grzybni odnotowano u *P. pomaceus* (G = 64,7 mm) i *P. igniarius* (G = 59,8 mm). Różnice wewnątrzgatunkowe we wzroście grzybni zaobserwowano u izolatów *P. pomaceus* i *P. igniarius.*

Table 1. List of isolates and their localities

Isolates	Species	Localities	Host
1	*P. igniarius*	Kiekrz, Wielkopolska Voivodeship	*Salix alba*
2	*P. igniarius*	Czarnowąsy, Opole Voivodeship	*Salix alba*
3	*P. pini*	Brynica, Opole Voivodeship	*Pinus sylvestris*
4	*P. pini*	Brynica, Opole Voivodeship	*Pinus sylvestris*
5	*P. pomaceus*	Starościn, Opole Voivodeship	*Prunus domestica* ssp. *syriaca*
6	*P. pomaceus*	Brynica, Opole Voivodeship	*Prunus domestica*
7	*P. robustus*	Grabczok, Opole Voivodeship	*Quercus robur*
8	*P. robustus*	Brynica, Opole Voivodeship	*Quercus robur*
9	*P. torulosus*	Protection area around Łężczok Nature Reserve, Silesia Voivodeship	*Quercus*sp.

Table 2. Average mycelial growth (G) of *Phellinus* spp. ± Standard Deviation

Isolates	Species	G [mm] Media			
		PDA	MEA	SWA	WA
1	*P. igniarius*	74.9 ± 1.6	41.0 ± 1.8	60.1 ± 1.8	75.4 ± 1.4
2	*P. igniarius*	53.9 ± 1.6	35.2 ± 1.8	67.0 ± 1.4	70.8 ± 1.4
3	*P. pini*	29.0 ± 1.3	21.8 ± 1.7	43.1 ± 1.4	40.1 ± 1.6
4	*P. pini*	29.5 ± 1.6	23.9 ± 1.4	38.0 ± 1.6	40.0 ± 1.6
5	*P. pomaceus*	71.1 ± 1.4	67.0 ± 1.3	71.2 ± 1.7	79.1 ± 1.4
6	*P. pomaceus*	60.6 ± 1.7	52.8 ± 1.8	53.6 ± 1.4	61.9 ± 1.4
7	*P. robustus*	45.9 ± 1.4	33.2 ± 1.7	46.9 ± 1.4	43.9 ± 1.4
8	*P. robustus*	44.0 ± 1.3	34.9 ± 1.4	47.0 ± 1.3	43.1 ± 1.4
9	*P. torulosus*	59.0 ± 1.3	26.0 ± 1.3	72.0 ± 1.3	58.5 ± 1.4

G: mycelial growth, PDA: Potato Dextrose Agar, MEA: Malt Extract Agar, WA: Wheat Agar, SWA: Sawdust-Wheat Agar

Table 3. Morphology of *Phellinus* spp. colonies after 10 days of incubation on Malt Extract Agar

Features of mycelial colonies	*Phellinus* species				
	P. igniarius	*P. pini*	*P. pomaceus*	*P. robustus*	*P. torulosus*
Photographs	Fig. 3 (a, b)	Fig. 3 (c, d)	Fig. 3 (e, f)	Fig. 3 (g, h)	Fig. 3 (i)
Form	Circular	Circular	Circular	Irregular, circular	Circular
Margin	Entire	Entire. Margin may grow into the medium	Entire or irregular, creeping on the wall of the dish	Entire, lobate	Filamentous
Elevation	Slightly raised to raised, especially in the ring zone, lower toward the margin	Flat to slightly raised, lower only in the ring zone.	Raised, lower in the ring zone	Flat or slightly raised, slightly raised in the ring zone	Raised, clearly higher in the middle
Surface	Knobby, irregular, wrinkled, denser in the middle, and then cottony, fluffy to filamentous on the edge with a transparent secretion in the ring zone	Cloddy, compact, felty with a slightly fluffy margin	Compact, knobby in the middle with a smooth transition zone and a knobby margin	Denser and knobby in the ring zone, then loose and fluffy with a delicate, loose and fluffy margin	Felty and loose
Color	Amber-cream to milk-white in the middle, then light yellow, light ochraceous in the transition zone to white and milk-white at the margin	Light ochraceous to dark ochraceous with a darker ring in the middle and with a milk-white margin	Milk-white with a clear light brown or ochraceous ring	Milk-white to cream in the middle with a brownish yellow ring and white or cream margin	Concentric zones: milky yellow in the middle to light ochraceous with a white margin

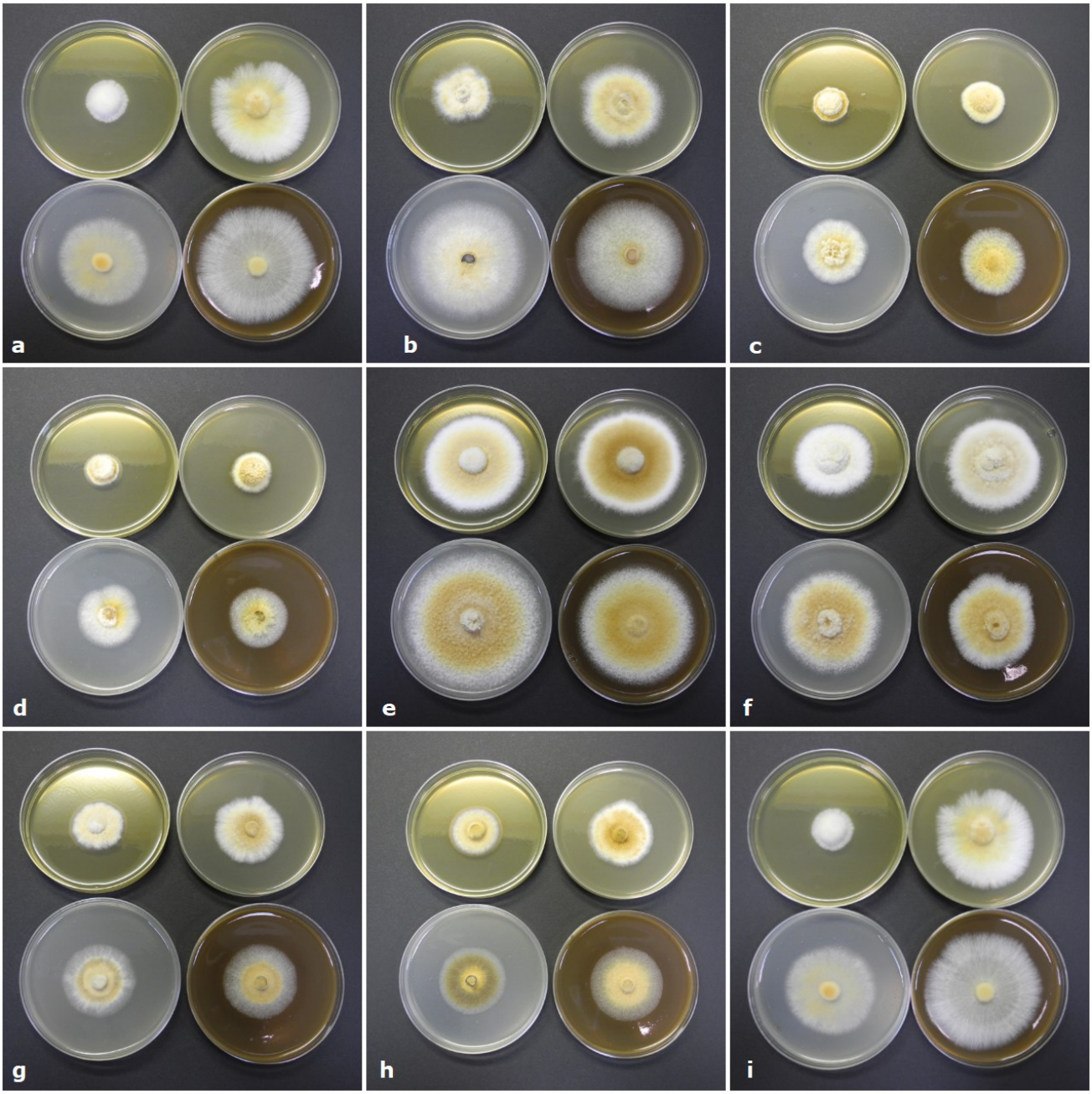

Fig. 3. Cultures on different media: a – *Phellinus igniarius*, isolate no. 1; b – *P. igniarius*, isolate no. 2; c – *P. pini*, isolate no. 3; d – *P. pini*, isolate no. 4; e – *P. pomaceus*, isolate no. 5; f – *P. pomaceus*, isolate no. 6; g – *P. robustus*, isolate no. 7; h – *P. robustus*, isolate no. 8; i – *P. torulosus*, isolate no. 9. The media are arranged as follow: Malt Extract Agar (top left), Potato Dextrose Agar (top right), Wheat Agar (bottom left), Sawdust-Wheat Agar (bottom right).

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