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Patterns of *Hericium coralloides* growth with competitive fungi

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Growth and morphological patterns of cultures were examined for two strains of *Hericium coralloides* during competitive colonisation of different nutrient media. The nutrient chemical composition of the medium was found to play an important role in the manifestation of antagonistic potencies of cultures. On the nutrient-poor Czapek medium with cellulose, radial growth of the monoculture was very slow. However, in triple confrontation cultures, the rate of substrate colonisation increased, and a positive effect on *H. coralloides* growth was observed. On all the examined media, *Fomes fomentarius* was consistently antagonistic to *H. coralloides*. The less suitable the medium for *H. coralloides* growth, the greater inhibitory effect was observed, but only in the combination of *H. coralloides* and *F. fomentarius*. This effect was observed for both strains of *Hericium*. *Schizophyllum commune* displayed both an antagonistic and a stimulating influence on *H. coralloides*, depending on the medium used and the strain of *Hericium*. The morphology of cultures *H. coralloides* 2332 and 2333 on media of different compositions in dual confrontation cultures was typical of the strains, but the colony growth was mostly uneven.

The obtained results will be used to reintroduce native strains of *Hericium coralloides* into the ecosystem of Hutsulshchyna NNP. The interrelations between different fungi should be taken into account for successful colonisation of natural substrate.

Key words: fungal interactions, direct confrontation, antagonism, xylotrophic fungi.

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Předmětem studie je růst a morfologie kultur dvou kmenů *Hericium coralloides* v průběhu kolonizace různých živných médií v podmínkách kompetice s jinými druhy hub. Chemické složení a obsah živin v médiu hraje důležitou roli v projevu antagonistických schopností konkrétních kultur. Na živinami chudém Czapkově médiu s celulózou byl radiální růst monokultury *H. coralloides* velmi pomalý, avšak v kulturách s konfrontací tří druhů vzrostla rychlost kolonizace substrátu a byl pozorován pozitivní vliv na růst korálovce. *Fomes fomentarius* vykazuje konzistentně antagonismus vůči

H. coralloides na všech použitých médiích; větší antagonistický efekt byl pozorován na médiích méně příznivých pro růst korálovce. Tento efekt byl pozorován u obou použitých kmenů *H. coralloides*, ale pouze v kombinaci s *F. fomentarius*. Naproti tomu *Schizophyllum commune* může mít jak antagonistický, tak i stimulační vliv na *H. coralloides* v závislosti na použitém médiu a kmeni korálovce. Při duální konfrontaci na médiích různého složení se ukazuje typická morfologie kmenů *H. coralloides* 2332 a 2333, ale růst kultur je většinou nerovnoměrný.

Získané výsledky budou využity při reintrodukci původních kmenů *Hericium coralloides* v ekosystému NPP Huculščyna, kde je vhodné vzít v potaz vzájemné vztahy mezi různými houbami pro úspešnou kolonizaci přirozeného substrátu.

INTRODUCTION

Hericium coralloides (Scop.) Pers. is a rare fungus in many countries, listed in the Red Lists of Bulgaria, Croatia, Denmark, France, Germany, Latvia, Lithuania, Macedonia, the Netherlands, Norway, Poland, Romania, Serbia, Sweden, Switzerland, the UK and India (The Global Fungal Red List Initiative on-line). This fungus is also listed in the Red Data Book for certain regions of the Russian Federation (Garibova 2012) and in the Red Data Book of Ukraine (Didukh 2009).

The main conservation measures on the territory of Ukraine is the protection of its gene pool in culture collections (ex-situ method) and of fungal populations in nature reserves and nature parks (in-situ conservation method). In Hutsulshchyna National Nature Park (NNP), Kosiv, Ukraine, a re-situ technology of cultivation of rare mushrooms in nature has been developed and successfully tested (Petrichuk et al. 2017, Pasailiuk et al. 2018). The reproduction of native strains of rare fungal species in nature is a measure to protect fungal diversity. To avoid the situation of an ecological explosion of re-introduced fungal species and to preserve population dynamics of rare fungal species in a particular territory, a study was initiated using native fungi in pure culture.

Strains 2332 and 2333 of *H. coralloides* are native to the Hutsulshchyna NNP. They are maintained in the Collection of Cultures of Cap Mushrooms (IBK) of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (Lomberg et al. 2015), and in the laboratory of ecological monitoring of Hutsulshchyna NNP. This fungus is included in the database of the World Federation for Culture Collections (WFCC), according to the World Data Centre for Microorganisms (2017) and Bisko et al. (2016).

Since 2013, reintroduction experiments have been conducted with the strains 2332 and 2333, to prevent the extinction of *H. coralloides* in the Park.

Competition drives community development in decaying woody resources, with interactions occurring at a distance, following physical contact, and through specialised relationships such as mycoparasitism. Outcomes of combative interactions range from replacement, where one mycelium displaces another, to dead-

lock, where neither combatant captures the territory from the other. A range of intermediate outcomes (i.e. partial or mutual replacement) lie between these extremes (Hiscox et al. 2018).

At the same time, xylotrophic fungi can occupy ecological niches which develop during wood decay. In this process, fungi in a previous ecological niche generate material more bioreceptive for wood decay fungi, thereby creating another ecological niche (Alfieri et al. 2016).

We often observed *H. coralloides* fructifying on decomposed wood in its last stages of decay (Pasailiuk 2018). Taking into account that this fungus grows in natural beech forests on large dead trees, its reintroduction plots should possess similar features.

We previously found that successful inoculation of beech logs with *H. coralloides* required absence of other wood-decaying fungi in the log (Petrichuk et al. 2017). Therefore, we assumed that there is an antagonism between *H. coralloides* and other widely distributed xylotrophic fungi of the same ecological niche, able to grow on beech wood. Analysis of wood decay fungi on beech logs in the forests of Hutsulshchyna NNP (Pasailiuk 2018) and observations of fruitbodies (started in 2013) partially support this assumption. Beech logs colonised by *Fomes fomentarius* and *Schizophyllum commune* are not suitable for *H. coralloides* (Petrichuk et al. 2017). For the fifth year after inoculation of the logs we have not detected any fruitbodies of this rare fungus on them.

The presence of antagonism between cultures *H. coralloides* 2332, *S. commune*, and *F. fomentarius* was confirmed in consistent and dual cultivation on a reference standard PDA nutrient medium. Furthermore, *H. coralloides* 2332 was found to be less susceptible to the inhibitory effect of other fungal cultures when *H. coralloides* culture was inoculated 10 days before *S. commune* and *F. fomentarius* using a sequential cultivation method in Pasailiuk (2017).

Additionally, we were interested in determining how altering the substrate conditions (i.e. nutrient medium) would affect the ability of *H. coralloides*, *S. commune*, and *F. fomentarius* cultures to suppress each other's growth in an antagonistic interaction experiment. Furthermore, the degree of inhibition of various *H. coralloides* strains by common xylotrophic fungi remains questionable. If antagonism of *H. coralloides* 2332 is strain-specific, as observed on PDA, then it would be logical to use other native strains of the fungus which are less susceptible to the possible inhibitory effects of widespread xylotrophic fungi for implementation of the re-situ technology in nature. Therefore, our aim was to study the growth characteristics of *H. coralloides* 2332 and 2333 in direct confrontation with *S. commune* and *F. fomentarius* on nutrient media of different compositions.

MATERIAL AND METHODS

Source of the fungi. The fungi studied, *Schizophyllum commune* 1763, *Fomes fomentarius* 1528, and *Hericium coralloides* 2332 and 2333, 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).

Nutrient media and culturing fungi. Fungal isolates were cultivated at 22 ± 1 °C on three different nutrient media, 1. Malt Extract Agar – MEA (pH 6.0), 2. Wort Agar – WA (8° by Baling, pH 6.0), and 3. Czapek's non-agarised medium ($NaNO_3$ 2.0 g/l, KH_2PO_4 1.0 g/l, KCl 0.5 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l, $FeSO_4 \cdot 7H_2O$ 0.01 g/l, pH 5.0) using α-cellulose (Whatman No. 101 filter paper at 500 mg), as the sole source of carbon (Dreval & Boyko 2011). Culture growth was measured every 2–3 days. The radial growth rate was calculated according to Lomberg & Solomko (2012). We studied the growth and morphology of the monoculture colonies which were grown on agar disks (diameter of 5 mm) using the mycelium of seven-day cultures of each species as the inoculum.

Determination of antagonist activity. The antagonist activity on MEA, WA and Czapek media with cellulose was studied according to Camporota (1985) and Bouziane et al. (2011). We used the method of direct confrontation, in which the fungal culture is placed in the same Petri dish containing 15 ml of MEA, WA or Czapek medium with cellulose and two or three pieces of the inoculum (diameter of 5 mm). One strain of *Hericium coralloides* (*Hc*) and *Fomes fomentarius* (*Ff*) and/or *Schizophyllum commune* (*Sc*) each was positioned in a diametrically opposite manner at a distance of 2 cm from the edge of the Petri dish and 5 cm apart (culture combinations: *Hc* + *Ff*; *Hc* + *Sc*; *Hc* + *Ff* + *Sc*). Monocultures of each type of fungus on the corresponding medium served as a control. Control and experimental plates were incubated in a thermostat chamber at 22 ± 0.1 °C.

Experimentally we determined the best days of the observation for different media to present in the paper. Mycelial growth was assessed on the 5th (on WA), 10th (on MEA) and 23rd (on Czapek + cellulose) days by measuring the diameter of the colony. Inhibition of *Hericium* was quantified as the percentage of mycelial growth inhibition using the following formula: $\mathbf{I}\% = (1 - \mathbf{Cn/Co}) \times 100$, where **Cn** is the average diameter of the *Hericium* colonies in the presence of *Fomes* and/or *Schizophyllum*, and **Co** is the average diameter of *Hericium* colonies in the control (Camporota 1985, Bouziane et al. 2011). If the resulting value is negative, its absolute value is given as stimulation of *H. coralloides* growth in Tabs. 2–4. We stored one Petri plate in the thermostat chamber during each culture transfer until a teleomorph was formed to verify the identity of the fungal colonies obtained in the surface culture.

The typology of the fungi interaction. The typology of fungi interactions in dual culture was based on Barinova et al. (2008): type I – culture stops its growth after contact with the colony of another fungus, type II – both cultures stop their growth with following lysis of their mycelia, type III – both cultures stop their growth after contact, type IV – both cultures stop their growth without contact of their colonies and an inhibition zone is formed, type V – both cultures slow their growth down before contact without developing an inhibition zone, type VI – inhibition of one organism by another after contact with following mycelial lesion of colony of the inhibited organism in contact zone, type VII – same as VI, but without contact between the colonies, type VIII – inhibition of one organism by another before contact of the colonies, followed by overgrowing of the inhibited organism's colony. Macro-morphological features of the mycelium were described according to the standard methods proposed by Stalpers (1978) on the 8^{th} (on WA), 15^{th} (on MEA) and 30^{rd} (on Czapek + cellulose) days.

Data analysis. The results were processed using Statistica 8.0 (StatSoft Inc., Tulsa, Oklahoma, USA). All experiments were conducted using four biological replicates, df = 27; x ± y represents mean ± standard deviation in all cases.

RESULTS AND DISCUSSION

Radial growth rate of colonies cultivated on different nutrient media and their morphology

The use of media of different chemical compositions made it possible to compare and establish the role of antagonistic interrelationships of confronting cultures. The patterns of monoculture growth on various media served as a visual example to compare growth rate and morphology, as well as specificity of the confrontations. First, we determined the rate of monoculture radial growth on nutrient media of different compositions. In doing this, we observed dependence of these values on the type of nutrient medium (Tab. 1).

Tab. 1. Radial growth rate of colonies (Vr, mm/day) cultivated on different nutrient media at 22 ± 0.1°C.

Species	Strain	Nutrient medium		
		MEA	WA	Czapek + cellulose
Hericium coralloides	2332	1.40 ± 0.2	2.60 ± 0.3	0.09 ± 0.01
	2333	1.07 ± 0.2*	1.80 ± 0.2*	0.08 ± 0.02
Schizophyllum commune	1768	3.85 ± 0.3	6.07 ± 0.5	1.25 ± 0.01
Fomes fomentarius	1528	3.21 ± 0.4	9.00 ± 0.7	1.10 ± 0.01

* Values are significantly lower in the case of *H. coralloides* 2332, $P \leq 0.05$.

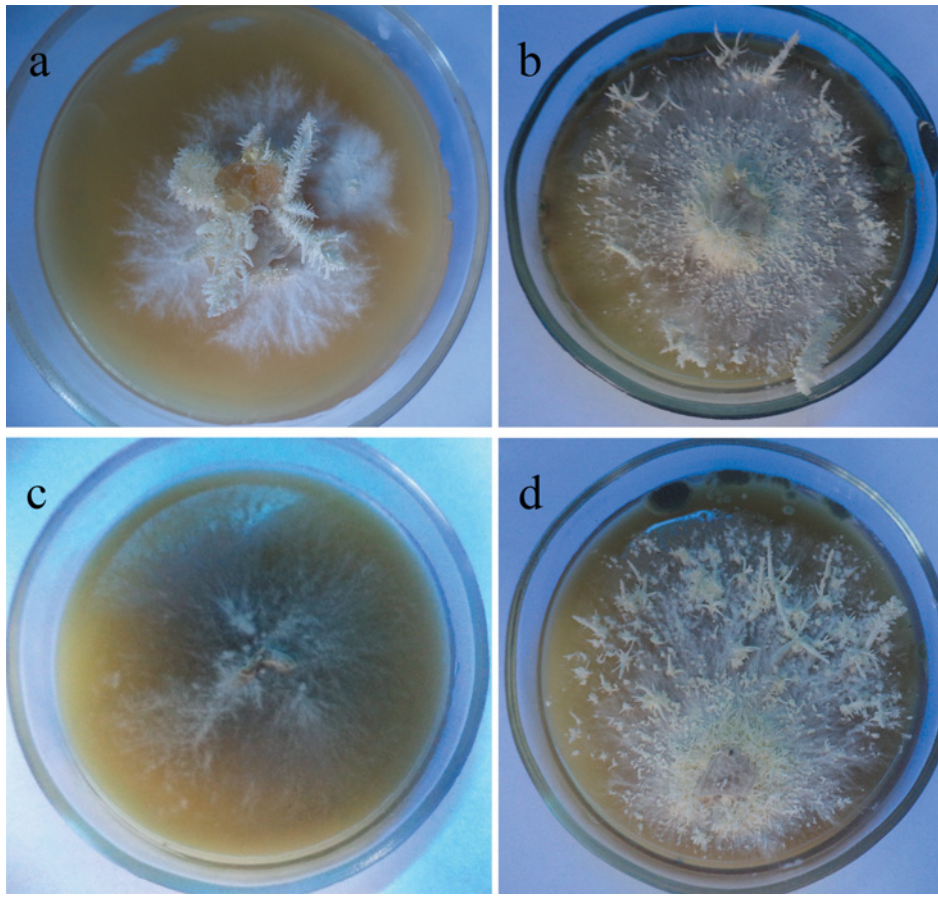

Fig. 1. Mycelial colonies of *Hericium coralloides* 2332 (a, b), *H. coralloides* 2333 (c, d): **a** – Day 25 on MEA, **b** – Day 30 on WA, **c** – Day 30 on MEA, **d** – Day 33 on WA at 22 ± 0.1 °C.

The smallest amount of colony radial growth rate among the studied species was recorded on the Czapek + cellulose medium. Thus, this polysaccharide substrate was not suitable as the sole carbon source for cultivation of the studied xylotrophic fungi. However, the cultures did not lose their vitality during the time of the experiment.

Hericium coralloides 2333 grew slowly on all three media. The maximum growth rate of this strain (1.8 mm/day) was recorded on WA. Therefore, *H. coralloides* was considered to belong to a group of very slowly growing mushrooms (less than 2 mm/day). In contrast, the cultures of *S. commune* and *F. fomentarius* grew much faster on the tested media. For example, on Day 5 of the experiment, *F. fomentarius* colonised the entire medium surface in the Petri plates.

We adjusted the time of observing the interaction experiments to reflect the growth rates observed on the different media: for MEA, it was 10 days of the joint cultivation, for WA 5 days, and for Czapek+cellulose 23 days. Morphological characteristics of mycelial colonies were dependent on the composition of the nutrient medium. Strain 2332 of *H. coralloides* was white on WA, formed dense felt colonies on MEA reversing to yellow with age and displayed fluffy white colonies through which the environment was visible on Czapek + cellulose. Teleomorphs were observed on MEA and WA (Fig. 1 a and b, respectively).

On MEA and WA, *H. coralloides* 2333 (Fig. 1.c, d) developed feathery colonies with mycelial bundles of hyphae growing radially from the centre. On the Czapek + cellulose medium it formed white, fluffy colonies through which the medium was visible.

Effect of interaction of medium and other fungi on *Hericium coralloides* growth

Co-culturing of *H. coralloides* together with other xylotrophic fungi changed the growth patterns of all individuals. We found *Fomes fomentarius* to be an unambiguous antagonist for both *H. coralloides* strains in all cases. However, inhibition values for mycelial growth depended on the type of culture medium on which the experiment was performed, the combinations of confronting cultures, and on the *H. coralloides* strain. For example, the dual culture of *Hericium* + *Fomes* showed significant inhibition values of *Hericium* strain growth on MEA of 54.5 and 43.2%, respectively. Thus, the growth rate of both strains decreased almost two-fold (Tab. 2).

Tab. 2. Growth of *Hericium coralloides* cultivated with confronting cultures on MEA, Day 10.

Combination of confronting cultures	Colony diameter of *H. coralloides* strains (cm)				Interaction type			
	Experiment		Control		Inhibition (%)		Stimulation (%)	
	2332	2333	2332	2333	2332	2333	2332	2333
Hericium + *Fomes* + *Schizophyllum*	2.50 ± 0.3*	2.50 ± 0.3	3.30 ± 0.4	2.60 ± 0.4	24.24 ± 2.1	5.30 ± 0.4	–	–
Hericium + *Fomes*	1.50 ± 0.2*	1.50 ± 0.2*			54.50 ± 5.3	43.18 ± 3.7	–	–
Hericium + *Schizophyllum*	2.10 ± 0.2*	3.40 ± 0.4			36.36 ± 3.2	–	–	28.78 ± 2.0

* Difference is significant compared with the control, $P \leq 0.05$.

Among the studied cultures, we observed the fastest growth rate on WA, which was found to be the most favourable for all the cultures used in the experiment (Tab. 1). We also observed significant inhibition of *H. coralloides* growth by *F. fomentarius* on WA, but the inhibition values were significantly lower for

strains 2332 and 2333 at 25% and 35%, respectively (Tab. 3). The inhibitory effect of *F. fomentarius* on both *H. coralloides* strains was maximal (100%) on Czapek + cellulose (Tab. 4).

Tab. 3. Growth of *Hericium coralloides* cultivated with confronting cultures on WA, Day 5.

Combination of confronting cultures	Colony diameter of *H. coralloides* strains (cm)				Interaction type			
	Experiment		Control		Inhibition (%)		Stimulation (%)	
	2332	2333	2332	2333	2332	2333	2332	2333
Hericium + Fomes + Schizophyllum	2.25 ± 0.3*	1.80 ± 0.2	3.35 ± 0.4	2.30 ± 0.3	32.83 ± 3.3	21.74 ± 2.1	–	–
Hericium + Fomes	2.50 ± 0.2*	1.50 ± 0.2*			25.37 ± 3.1	34.78 ± 2.0	–	–
Hericium + Schizophyllum	3.15 ± 0.4	1.40 ± 0.2*			6.00 ± 0.5	39.13 ± 4.0	–	–

* Difference is significant compared with the control, $P \leq 0.05$.

Tab. 4. Growth of *Hericium coralloides* cultivated with confronting cultures on Czapek + cellulose, Day 23.

Combination of confronting cultures	Colony diameter of *H. coralloides* strains (mm)				Interaction type			
	Experiment		Control		Inhibition (%)		Stimulation (%)	
	2332	2333	2332	2333	2332	2333	2332	2333
Hericium + Fomes + Schizophyllum	8.0 ± 0.9*	5.8 ± 0.7*	4.0 ± 0.5	3.8 ± 0.5	–	–	100 ± 3	52 ± 3
Hericium + Fomes	0	0			100 ± 2	100 ± 2	–	–
Hericium + Schizophyllum	8.0 ± 0.8*	5.4 ± 0.7*			–	–	100 ± 2	42 ± 4

* Difference is significant compared with the control, $P \leq 0.05$.

The inhibitory effect of *F. fomentarius* was reduced on media favourable for the growth of *H. coralloides*, in which we observed faster growth of its monoculture (Tab. 1). Conversely, on the nutrient-poor Czapek + cellulose medium, the antagonistic effect of *F. fomentarius* was markedly intensified up to complete inhibition of *Hericium* growth. Comparison of the rate of radial growth of both *Hericium* strain monocultures on different media with its growth inhibition on different media in the presence of *F. fomentarius* provided a correlation coefficient of –0.8, demonstrating a strong negative correlation between these values. Consequently, *F. fomentarius* was clearly antagonistic to *H. coralloides* for all the dual variants on the tested media. The less suitable the medium for *H. coralloides* growth, the greater the inhibitory effect of *F. fomentarius* was.

We checked whether such an inhibitory effect was species-specific, i.e. inherent only to *F. fomentarius* – a fungus with ecological and trophic characteristics similar to *H. coralloides*. On WA also *S. commune* inhibited growth of *H. coralloides*. We observed an inhibitory effect on *H. coralloides* 2332 and 2333 in the *Hc + Sc*

combination on WA (6 and 39%, respectively; Tab. 3) and on *H. coralloides* 2332 on MEA (Tab. 2). However, culturing *H. coralloides* with *S. commune* in dual or triple culture on MEA and Czapek + cellulose medium stimulated the growth of one or both *H. coralloides* strains. Thus, interaction with a competitive fungus can lead to growth stimulation of *H. coralloides*. A stimulation effect in the presence of antagonists on agar media was also shown for *H. cirrhatum* and *H. erinaceus* (Boddy et al. 2011). Thus, interaction of *Hericium* species with cultures of xylotrophic fungi reveals not only 'fungus wars' but also advantageous competition.

We observed a distinct influence of medium composition (or its nutritional value) on the interaction pattern of the studied fungal species. The inhibitory effect was reduced on the medium that was the least suitable for all species, i.e. a stimulation effect was observed on the Czapek + cellulose medium (Tab. 4). An opposite pattern was observed on MEA, where enhanced growth of *H. coralloides* colonies was observed compared with the Czapek + cellulose medium. Growth on MEA showed both antagonism (36% for *H. coralloides* 2332) and stimulation (29% for *H. coralloides* 2333; Tab. 2). For WA, on which the growth of *H. coralloides* was maximal, we observed an inhibitory effect in the presence of *S. commune* (Tab. 3). Thus, the interaction of *H. coralloides* with *S. commune* was accompanied by growth inhibition and stimulation, and the effects of confrontation between cultures could not be explained by different rates of substrate colonisation by these cultures.

In our previous experiments we described fungi which inhibit *H. coralloides* on the PDA medium. Interestingly, *H. coralloides* was found to be significantly less susceptible to the inhibitory effect of other fungal cultures if inoculated 10 days before other cultures (Pasailiuk 2017). This can be explained that more vigorous fungi colonised the substrate more rapidly, preventing the growth of a slow-growing culture of *H. coralloides* largely through secondary metabolites (Hiscox et al. 2018). Boddy et al. (2011) report similar observations in natural ecosystems. *Hericium* species were rarely replaced by heart rot fungi if they were latently present in the sapwood. The prolonged occupancy of dead wood by *Hericium* species is likely to be related to their combative ability. This can explain successful inoculation of beech logs with *H. coralloides* when no wood-decaying fungi were already present in the log, as we found in our previous experiments. We observed fructification of *H. coralloides* only on logs where no other fungi were found (Petrichuk et al. 2017). This can also explain unsuccessful reintroduction. The inoculation of wood logs which already bear fruitbodies with fungi antagonistic to *H. coralloides* in pure culture would be rather unsuccessful (Petrichuk et al. 2017).

However, simultaneous inoculation of beech logs with mycelium of *H. coralloides* and its stimulators on agarised media might increase the chance to obtain *H. coralloides* fruitbodies in nature. We will study this assumption in tests to follow.

In the triple composition of *Hc* + *Ff* + *Sc* on MEA (Tab. 2), we observed an added antagonism in which the inhibition values toward *Hericium* for two competing fungi were several times lower than the inhibition rate in binary combinations on this medium. Interestingly, the significant effect of stimulation was related to *H. coralloides* 2332 and 2333 (100 and 52%, respectively; Tab. 4) on the Czapek + cellulose medium.

We assume that there is a stimulating effect on *H. coralloides* growth on the Czapek + cellulose medium during co-cultivation with *S. commune* and in the triple culture combination due to the characteristics of cellulose decomposition by the enzymatic systems of these fungi. Since all three species are white rot fungi (Jankovský et al. 2002, Parihar et al. 2012, Větrovský et al. 2013) with an endo- or exoglucanase system, they are capable of decomposing cellulose. Simultaneously, the improved growth values of the *H. coralloides* culture under the conditions of competition, as compared to the control values, might be due to the active production of enzymes and utilisation of decomposition products for the growth of these fungi under conditions of intense competition on a low-nutrient substrate. The production of substances of antagonistic nature was in this case probably disadvantageous for the energy production during the time frame of the experiments (Hiscox et al. 2015). However, this process is species-specific and potentially results in either synergism or antagonism in dual or triple confrontation cultures.

Colony morphology of direct confrontation

The colony morphology of both *Hericium* cultures was similar in monoculture to the dual and triple cultures, while the growth of colonies was mostly uneven. According to the typology of Barinova et al. (2008), we recorded termination of colony growth after contact (type III) in a confronting culture for *H. coralloides* 2332 with *Schizophyllum* on MEA and WA, with *Ff* + *Sc* on MEA and WA, and with *Fomes* on WA. The separation zone between confronting cultures was clear (Figs 2.a, c, d, e, f). For the *Hc* + *Ff* combination on MEA only *H. coralloides* displayed a complete termination of growth combined with subsequent growth of *F. fomentarius* mycelium (type VIII; Fig. 2.b).

Similar growth patterns on MEA were observed with all confronting variants for *H. coralloides* 2333. Termination of colony growth after contact was characteristic of the variants *Hc* + *Sc* and *Hc* + *Ff* + *Sc*, which showed clear zones of separation (Fig. 2.g, i). In the *Hc* + *Ff* combination, only *H. coralloides* showed growth cessation, while the mycelium of *F. fomentarius* continued to expand as in the type VIII culture interaction (Fig. 2.h). The growth of *H. coralloides* 2333 under confrontation conditions on WA showed a clear separation zone between cultures only for the variant with *S. commune* (Fig. 2.j). For the other variants,

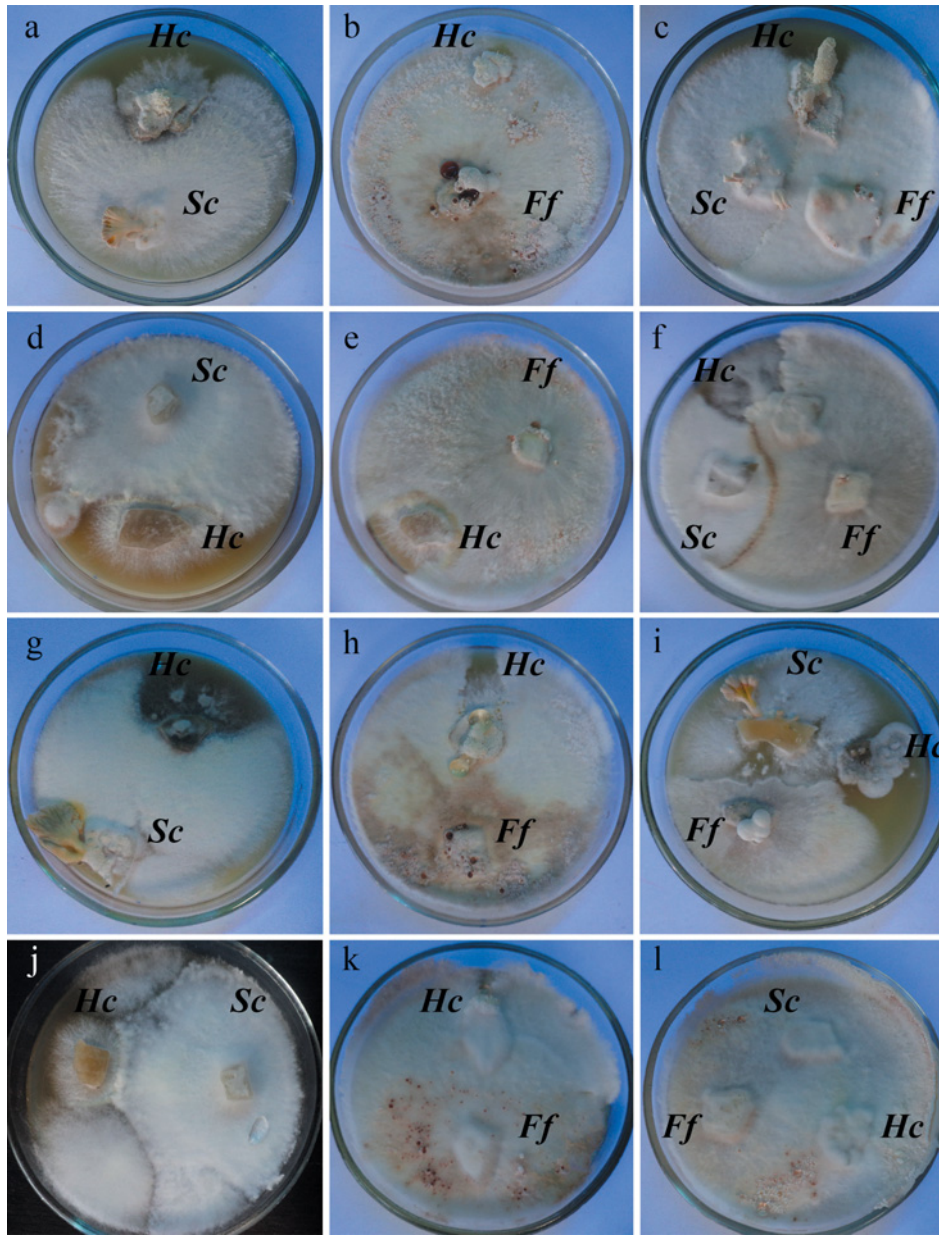

Fig. 2. Direct confrontation of *Hericium coralloides* (*Hc*) 2332 (a–f) and 2333 (g–l), *Fomes fomentarius* (*Ff*) and *Schizophyllum commune* (*Sc*) cultures in different combinations: **a, b, c** – Day 15 on MEA; **d, e, f** – Day 8 on WA; **g, h, i** – Day 15 on MEA; **j, k, l** – Day 8 on WA.

we observed increased size in *F. fomentarius* colonies (Fig. 2.k, l), a VIII type of interaction. For all combinations on Czapek + cellulose, we observed an interaction similar to type IV: double or triple growth inhibition with formation of an inhibition zone, and no visible contact between the confronting cultures (Fig. 3).

Fig. 3. Direct confrontation of *Hericium coralloides* 2332, *Fomes fomentarius* and *Schizophyllum commune* cultures, Day 30 on Czapek + cellulose medium.

However, given that the co-culture of *H. coralloides* with two xylotrophic fungi was accompanied by a stimulating effect, we believe that the used typology does not reflect the morphological nuances of the confrontation on the Czapek + cellulose medium. This phenomenon might represent a technical issue, e.g. the fungal colonies were not able to colonise the entire surface of the medium in the Petri plate due to slow growth. Thus, this type of experiment could not last longer than 36 days due to complete consumption of the nutrition in the substrate by the cultures. Therefore, we might have failed to assess the interaction type properly because of their slow growth rates on exhausted substrates and the loss of vigour before contact between the colonies in surface cultivation.

While growth rates change in confronting cultures, the macromorphological features of the fungal colonies remain the same as in monocultures. Consequently, in antagonistic/stimulating interactions, the studied cultures formed typical colonies on nutrient media of different compositions just as in monocultures.

We observed that *F. fomentarius* consistently exhibited clear antagonism towards both *H. coralloides* strains regardless of the type of medium. Moreover, an enhanced inhibitory effect of interaction was observed for less suitable culture media in *H. coralloides*. The results of the present study are consistent with our early observations of *H. coralloides* 2332 growth on PDA interacting with widely distributed xylotrophic fungi (Pasailiuk 2017). In the natural environment, the chances for *Hericium* to colonise a substrate simultaneously with *Fomes* might be very low. This can be supported by the study of Wald et al. (2004) demonstrating similarity of *H. coralloides* ecological strategies in dual cultures with more than 20 fungal species on agarised media and on wood.

Considering the significant differences between the results obtained on substrates with different carbon sources, the role of the chemical structure of the nutrient medium in the development of the xylotrophic fungal mycelium should be stressed.

CONCLUSIONS

Fomes fomentarius acts as an antagonist towards *H. coralloides* cultures regardless of the *Hericium* strain medium. The less suitable the medium used for *H. coralloides* growth, the stronger were the inhibitory effects of *Fomes* on both *Hericium* strains. *Schizophyllum commune* showed both antagonistic and stimulation effects towards *H. coralloides*, depending on the nutritional profile of the medium and strain of *H. coralloides*.

The chemical composition of the nutrient medium plays an important role in the manifestation of the antagonistic potencies of the cultures. We found that WA was the most cost-efficient medium for the cultivation of monocultures, as the radial growth rate on this medium was the highest of all media assessed for the experimental strains.

On the nutritionally poor Czapek + cellulose medium, radial growth rate values were, as expected, lower in monocultures. However, under co-culture conditions (namely in triple confrontation experiments), the substrate colonisation rate of *H. coralloides* increased, and a stimulating effect was observed.

On all nutrient media, the growth of *H. coralloides* 2333 was slower than that of *H. coralloides* 2332, hence its culture was associated with more demanding cultivation conditions. The morphology of the *H. coralloides* 2332 and 2333 strains on media of various compositions was typical in terms of their growth both in monocultures as well as under confrontation conditions. However, in combined cultures, the growth was usually uneven. On MEA and WA, *H. coralloides* 2332 formed dense, white, felty colonies reversing to yellow with age, and white, cottony, transparent colonies on Czapek + cellulose. On MEA and WA, *H. coralloides* 2333 formed strand-like, feathery colonies, with mycelial strands growing radially from the centre. White cottony and transparent colonies were formed on the Czapek + cellulose medium.

Among the variants, we observed complete growth inhibition of type III for *H. coralloides* 2332: *Hc* + *Sc* on MEA and WA; *Hc* + *Ff* + *Sc* on MEA and WA; *Hc* + *Ff* on WA. A clear delimitation zone was observed between the confronting cultures of *Hc* + *Ff* on MEA, while only *H. coralloides* displayed halted growth combined with subsequent growth of *F. fomentarius* mycelium (type VIII). The same types of colony morphology were observed under conditions of confrontation for cultures of *H. coralloides* 2333 on MEA and for *H. coralloides* 2332 on both media.

For *H. coralloides* 2333 on WA, we observed a clear separation zone only in the confrontation with *S. commune*. For other variants, expanding growth of *F. fomentarius* mycelium was observed.

The obtained results are important for further *H. coralloides* reintroduction in natural ecosystems. In particular, the use of beech logs with any colonisation by *F. fomentarius* should be avoided. Regarding our observation of *H. coralloides* growth stimulation by *S. commune* on Czapek medium with cellulose, further studies of their interaction on beech logs might be promising. This will allow for establishing if such stimulation is possible under natural conditions.

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