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Pigment production in incompatibility zones of Trametes versicolor is in correlation with the laccase activity of the dikaryons involved

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A correlation between the extracellular laccase activity (estimated by a drop test using ABTS) and the presence of a dark pigmented zone in the pairing of different *Trametes (Coriolus) versicolor* dikaryons in vitro was studied. Altogether 24 dikaryotic strains from different substrates and distant localities of three European countries were paired to each other and the pairings were checked for the presence of dark pigment in the contact zone. Using the χ^2 test, a positive correlation between the laccase activity and the presence of pigment was found.

Key words: Basidiomycetes, *Trametes versicolor*, laccase, pigment, χ^2 test

Tomšovský M. a Homolka L. (2003): Produkce pigmentu v nekompatibilních zónách outkovky pestré (Trametes versicolor) souvisí s lakázovou aktivitou přilehlých dikaryonů. – Czech Mycol. 55: 155–160

Závislost mezi aktivitou extracelulární lakázy (zjišťovanou kapkovacím testem pomocí ABTS) a tvorbou tmavé pigmentované zóny byla sledována při párování dikaryotických kmenů outkovky pestré – *Trametes (Coriolus) versicolor* – in vitro. Celkem 24 dikaryotických kmenů sebraných na různých substrátech a pocházejících ze tří evropských zemí bylo vzájemně pářováno ve všech kombinacích. Následně byla zjišťována přítomnost pigmentu v kontaktní zóně vytvořené mezi kmeny. Závislost mezi tvorbou pigmentu a lakázovou aktivitou byla byla potvrzena pomocí χ^2 testu.

INTRODUCTION

Pairing tests between different dikaryotic basidiomycete strains in vitro often take place in experimental studies on fungal ecology. These studies describe fungal ecological strategies in interactions of the respective strain with other individuals. A combat between two fungal individuals (of the same or a different species) can

result in either a deadlock (no fungus is able to invade the other) or in replacement of one individual by the other. Interspecific interactions of various basidiomycetes were described in several studies (Boddy and Rayner 1983, Holmer et al. 1997, Iakovlev and Stenlid 2000, White and Boddy 1992).

Trametes versicolor (L.: Fr.) Pilát, a common circumglobal polypore species, is often used in such experiments. The following interactions between *T. versicolor* and other basidiomycete species were found: *T. versicolor* replaced *Phanerochaete magnoliae* (Ainsworth and Rayner 1991) or *Phlebia rufa* (Boddy and Rayner 1983) and the species reached a deadlock with *Phlebia radiata* (Boddy and Rayner 1983, White and Boddy 1992). On the other hand, *T. versicolor* was often replaced by *Lenzites betulina*, which is able to destroy *Trametes* mycelium (Rayner et al. 1987).

During pairing between dikaryons in vitro, pigmented zones are often formed. These zones resemble the narrow, dark zones that separate genetically different individuals in wood (Rayner and Todd 1977). The formation of pigmented zones is a result of enzymatic action: darkening of the fungal tissues results from oxidation of phenolic substances by extracellular phenoloxidases (such as laccase) and peroxidases. These enzymes produced by basidiomycetes decompose nutrient substrates. The products of oxidation are usually *o*-quinones, which are highly unstable and undergo polymerisation to yield dark melanin-like pigments (Collins et al. 1963). Depending on the cultivation conditions and interactions with antagonists, the activity of the respective enzymes changes. Li (1981) compared phenoloxidase activity in pigmented zone lines with that found in adjacent mycelial tissues of *Phellinus weirii*. The zone lines exhibited stronger enzymatic reactions than the adjacent tissues.

The aim of the study was to find out if there is any correlation between the production of dark pigment and laccase activity of different *T. versicolor* dikaryotic strains.

MATERIAL AND METHODS

The strains used were obtained by isolation from the fresh sporocarps under sterile conditions. The sporocarps of *Trametes versicolor* from 15 different hardwood species of 13 genera were collected in distant localities in the Czech Republic, Bulgaria and Montenegro (former Yugoslavia) (Tab. 1). The strains were maintained on MEGA medium (malt extract 1 %, glucose 1 %, agar Difco 1.6 %) and incubated at 23 °C. All cultures are deposited in the Culture Collection of Basidiomycetes (CCBAS), Prague, Czech Republic. The sporocarps are deposited in the herbarium of the National Museum (PRM), Prague, Czech Republic.

Pairings between the strains were made by placing mycelial plugs (9 mm diameter), cut from the margins of actively growing colonies, approximately 3 cm apart in the centre of Petri dishes (6 cm diameter) containing MEGA medium.

Table 1. Ecological characteristics and laccase activity of the tested *Trametes versicolor* strains.

Strain	Substrate	Country	Locality	Latitude N	Longitude E	Laccase activity	Herbarium number
V01	*Rosa* sp.	Czech Republic	Havlíčkův Brod	49°36' 27.43"	15°35' 25.94"	++++	PRM 900581
V02	*Prunus domestica*	Czech Republic	Mírovka	49°33' 43.78"	15°36' 56.99"	+++	PRM 900612
V03	*Alnus glutinosa*	Czech Republic	Velemín	50°32' 18.49"	13°56' 56.99"	++++	PRM 900592
V04	*Fagus sylvatica*	Czech Republic	Mt. Milešovka	50°33' 20.85"	13°56' 55.00"	+++	PRM 900600
V05	*Corylus avellana*	Czech Republic	Srbsko	49°55' 52.20"	14°06' 56.76"	++++	PRM 900594
V06	*Fagus sylvatica*	Czech Republic	Srbsko	49°55' 52.20"	14°06' 56.76"	+++	PRM 900587
V07	*Fagus sylvatica*	Czech Republic	Prague	50°04' 08.30"	14°25' 16.94"	++++	PRM 900608
V08	*Malus domestica*	Czech Republic	Havlíčkův Brod	49°36' 24.17"	15°35' 20.21"	++	PRM 900580
V09	*Vitis vinifera*	Czech Republic	Brno	49°13' 31.95"	16°35' 13.34"	++++	PRM 900591
V10	*Fagus sylvatica*	Czech Republic	Brumov-Bylnice	49°02' 42.79"	18°02' 06.67"	++++	PRM 900614
V11	*Robinia pseudacacia*	Czech Republic	Prague	50°02' 27.45"	14°26' 53.66"	+++	PRM 900595
V12	*Carpinus betulus*	Czech Republic	Nové Město nad Metují	50°20' 58.94"	16°09' 27.00"	++++	PRM 900604
V13	a hardwood	Czech Republic	Jílové	49°54' 50.88"	14°30' 11.27"	+++	PRM 900602
V14	*Fagus sylvatica*	Czech Republic	Žofín primeval forest	48°40' 00.13"	14°42' 32.76"	+	PRM 900583
V15	*Acer platanoides*	Czech Republic	Potštejn	50°04' 13.81"	16°18' 44.53"	+	PRM 900599
V16	*Fagus* sp.	Montenegro	Lovčen Mts.	42°25' 35.0"	18°51' 00"	+++	PRM 900613
V17	*Fagus* sp.	Montenegro	Bjelasica Mts.	42°54' 00"	19°37' 00"	++++	PRM 900576
V18	*Fagus orientalis*	Bulgaria	Rhodopes Mts.	42°01' 30"	24°15' 00"	++++	PRM 900575
V19	*Quercus* sp.	Bulgaria	Tulovo primeval forest	42°34' 59"	25°33' 00"	++++	PRM 900609
V20	*Quercus* sp.	Bulgaria	Tulovo primeval forest	42°34' 59"	25°33' 00"	+++	PRM 900577
V21	*Betula* sp.	Czech Republic	Albeř	49°01' 35.64"	15°09' 10.02"	++++	PRM 900593
V22	*Salix* sp.	Czech Republic	Mirochov	49°00' 24.84"	14°56' 37.42"	++	PRM 900607
V23	*Corylus avellana*	Czech Republic	Bečov nad Teplou	50°05' 03.38"	12°49' 22.36"	+++	PRM 900579
V24	*Alnus incana*	Czech Republic	Šilhéřovice	49°53' 24.31"	18°16' 18.31"	++++	PRM 900597

The cultures were incubated in darkness at 23 °C for three weeks. The dishes were then checked for the presence of the pigmented zone.

Laccase activity was estimated by a spot test using ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) according to Niku-Paavola et al. (1990). The mycelium was removed from the 2-week old cultures on Petri dishes (9 cm diameter) by scraping off with a scalpel; then 3 ml of a fresh staining ABTS solution (5 mg/ml distilled H_2O) was poured over the agar plate, which was then incubated for 6 hours. The colour intensity (indicating extracellular laccase activity) was classified using a four-point scale as follows: weak (+), medium (++), strong (+++), very strong (++++).

The correlation between pigment production in pairings and laccase activity of the strains paired was estimated by the χ^2 test, which is the most common test for significance of relationship between categorical variables (according to Lepš 1996).

RESULTS AND DISCUSSION

The results of the pairings are summarised in Table 2. Altogether 126 pairings (60 %) out of 210 resulted in antagonism accompanied by pigment production. The remaining 84 pairings (40 %) exhibited antagonism without pigmentation. In case of pigment production, 37 % of pairings formed pigment only in a small part of the contact zone between the mycelia, and 63 % pairings formed pigment in the entire zone. All pairings between the same strains resulted in complete fusion of isolates.

Intensity of the enzymatic reaction of the paired isolates (Tab. 1) was classified as weak in 2, medium in 2, strong in 8, and very strong in 12 out of 24 strains. Relations among the four above-mentioned values of laccase activity and three values of pigment occurrence (no pigment; pigment in the part of the zone; pigment in the entire zone) were then tested. The χ^2 test revealed a significant relation between the tested variables ($\chi^2 = 47.61$, df = 6, $P<0.05$). Positive correlation between laccase activity and presence of pigment was found at *Trametes versicolor* pairings. These results do not correspond with those of Iakovlev and Stenlid (2001), who did not find any relation between pigmentation and laccase activity. The above-mentioned authors studied interspecific matings of several basidiomycete species (*Antrodiella citrinella, Flammulina velutipes, Fomitopsis pinicola* etc.). It is likely that pigment production is also influenced by other factors. For example, the presence of cadmium in the media can induce a dark pigmentation in *T. versicolor* (Baldrian and Gabriel 1997). Todd and Rayner (1978) found that pigment production diminishes with increased relatedness of the isolates. To decrease the effect of relatedness, isolates from distant localities were tested in this study. In three cases the couples of strains (V01, V08; V05, V06; V19, V20) were collected in the same locality, but all three couples were genetically distinct

Tab 2. Pairing among different dikaryotic isolates of *Trametes versicolor* (V01-V24). ○: antagonism; △: antagonism accompanied by pigment production, △N: pigment producted in small part of contact zone; ●: complete fusion of isolates.

	V24	V23	V22	V21	V20	V19	V18	V17	V16	V15	V14	V13	V12	V11	V10	V09	V08	V07	V06	V05	V04	V03	V02	V01
V01	△N	○	△N	△N	△N	△	○	○	△	○	○	△	○	○	△	△	○	○	△	○	○	△	○	●
V02	○	○	△N	○	△N	○	△	○	△	△	△	○	△	△	△	△	△	△	△	△	△	△	●	
V03	△N	△	○	△	△N	△	△	○	△N	△	△	△	○	△	△	△	○	○	△	△	△	●		
V04	△N	○	△	○	△N	△N	○	○	△	△	○	△	○	○	△	△	○	△	○	○	●			
V05	△	○	△	△	△	△N	△	○	△N	△	○	△	○	○	△	△	△	△	○	●				
V06	○	○	○	△N	○	△N	△	△	○	△N	○	○	○	○	○	○	○	○	●					
V07	△N	△N	△N	△N	△N	△N	△N	○	△N	△N	○	○	○	○	△	△	○	●						
V08	○	○	○	○	○	△N	○	○	△N	△N	○	○	△	○	○	○	●							
V09	△	△N	△	△	△	△	△N	○	△	△	△	△	△	△	○	●								
V10	○	△N	△N	△N	△N	△	△	○	○	△	△N	△	○	△	●									
V11	△N	△	△	○	△N	△N	○	△N	△N	△N	○	○	△	●										
V12	○	△	△N	△	○	△N	△	○	△N	△N	△	△	●											
V13	○	○	○	○	○	△N	△N	○	○	○	△	●												
V14	△N	○	○	○	○	△N	○	○	△N	△N	●													
V15	△N	△N	△N	△	○	△N	○	△N	△N	●														
V16	○	△N	○	△	○	△	△	○	●															
V17	△	○	○	△	○	○	△	●																
V18	○	○	△N	△N	△N	△N	●																	
V19	△N	△N	△N	△N	△N	●																		
V20	○	△N	○	○	●																			
V21	○	△N	△N	●																				
V22	○	○	●																					
V23	○	●																						
V24	●																							

and their pairings turned out as expected (i.e., contact zones were formed). The communication between different fungal individuals is a complex process requiring further investigations.

Finally, we believe that this study can help understanding intraspecific ecological processes.

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