INHIBITION OF GROWTH AND SPORULATION OF *PENICILLIUM EXPANSUM* BY EXTRACTS OF SELECTED BASIDIOMYCETES

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ABSTRACT

A screening of antifungal activity of 33 Basidiomycetes strains was realized with their extracts. The influence of aqueous extracts of fruit-bodies of the tested fungi after addition of spores or hyphae of *Penicillium expansum* on growth and sporulation of the fitopathogen in vitro was estimated. The species: *Hydnum repandum* L.: Fr., *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, *Coprinus comatus* (Müll: Fr.) S.F. Gray, *Coprinus atramentarius* (Bull: Fr.) Fr. and *Lactarius turpis* (Weinm.) Fr. reduced the mycelial growth and inhibited sporulation of this pathogen.

KEY WORDS: Basidiomycetes, antifungal agents, *Penicillium expansum*, growth inhibitor.

INTRODUCTION

Higher plants are a rich source of antifungal metabolites of different chemical structures, belonging to all major classes of secondary compounds: terpenoids (e.g. iridoids, sesquiterpenoids, saponins), nitrogen – and/or sulphur – containing constituents (e.g. amines, amides, alkaloids), aliphatics (e.g. fatty acids and especially long – chain alkanes) and aromatics (e.g. phenolics, flavonoids, stilbenes, bibenzyls, xanthenes and benzoquinones).

Over a 12 years span (1982-1994) some 250 new antifungal substances were characterized in plants (Grayer and Harborne 1994). Also antifungal compounds isolated from higher fungi (Basidiomycetes) fruit-bodies were identified as diterpenoids (e.g. tamulin, pleuromutilin), sesquiterpenoids (e.g. hyphodontal, blennins, deoxylactarorufin, striatins) or derivatives of β -methoxyacrylic acid (e.g. mucidin, oudemansins, strobilurins) (Florianowicz 1999). The antifungal tests of the latter compounds, found in higher fungi fruit-bodies were active against a number of human and animals fungal pathogens as *Candida albicans*, *C. tropicalis*, *Rhodotorula rubra*, *Aspergillus fumigatus* and plant pathogenic fungal species as *Penicillium chrysogenum*, *Botrytis cinerea*, *Alternaria solani*, *Fusarium culmorum*, *Trichoderma lignorum* or *Verticillium dahliae*. Data on the activity of higher fungi metabolites against *Penicillium expansum* – a common pathogen in the natural environment – is unavailable in the literature. The aim of the study was to determine in vitro the influence of aqueous extracts from fruit-bodies of commonly found in Poland selected species of Basidiomycetes on growth of the patulinogenous species of *Penicillium expansum*.

MATERIALS AND METHODS

Plant material

Fruit-bodies of two cultivated higher mushrooms species purchased from the producer and 31 wild-grown species collected in 1996-1997 from the districts of Łódź, constituted the study material. The species list of all the examined material is presented in Table 1. The aqueous extracts were obtained from fruit-bodies, frozen directly after collection and stored at the temperature of -20°C. Five grams of a fruit-body were homogenised at 10 000 g for 4 min in several cubic centimetres of volume of phosphate buffer (0.1 mol/dm³, pH 7.0) and after filling the sample up to the volume of 50 cm³, left for night at the temperature of 4°C for maceration. After centrifugation the supernatant was concentrated in a vacuum evaporator down to 1/3 of the volume, before autoclaving (20 min at 121°C).

Strain and culture media

Patulinogenous strain of *Penicillium expansum* No 729, obtained from the Research Institute of Pomology and Floriculture in Skierniewice, stored on potato-dextrose agar (PDA), was used in this study. Suspension of spores contained about 10⁶ colony forming units (cfu) per milliliter was obtained by washing out ten-day old culture growing at 25°C on Sabouraud agar medium (Sabouraud 2% maltose broth, Merck). The spores were removed from the slants with physiological saline solution with 0.02% of Tween 80.

TABLE 1. Growth and sporulation of *Penicillium expansum* on PDA medium supplemented with aqueous fungal extracts.

Species	Number of colonies per dish	Percent of reduction ^{x)}	Days			
			3	5	7	9
			Percent of sporulated colonies			
<i>Agaricus bisporus</i> (Lange) Sing.	30	74	0	31	97	100
<i>Agaricus campestris</i> L.	44	62	0	30	72	76
<i>Calvatia utriformis</i> (Bull.: Pers.) Jaap	41	65	0	29	71	78
<i>Cantharellus cibarius</i> Fr.	33	72	0	60	100	100
<i>Cantharellus cinereus</i> Pers.: Fr.	60	49	0	42	58	100
<i>Clitocibe gibba</i> (Pers.: Fr.) Kummer	117	0	0	17	34	42
<i>Coltricia perennis</i> (L.: Fr.) Murr.	67	43	0	100	100	100
<i>Coprinus atramentarius</i> (Bull.: Fr.) Fr.	33	72	0	30	100	100
<i>Coprinus comatus</i> (Müll.: Fr.) S.F. Gray	29	75	0	20	90	90
<i>Cortinarius armillatus</i> (Fr.) Fr.	38	67	0	5	24	32
<i>Cortinarius coerulescens</i> Fr.	60	49	0	18	24	25
<i>Cortinarius variegatus</i> Fr.	37	68	0	5	24	32
<i>Craterellus cruncupioideus</i> (L.) Pers.	52	55	0	23	48	62
<i>Fomes fomentarius</i> (L.: Fr.) Kickx	37	68	0	81	100	100
<i>Ganoderma applanatum</i> (Pers.) Pat.	60	49	0	27	92	100
<i>Hydnum repandum</i> L.: Fr.	27	77	0	100	100	100
<i>Lactarius quietus</i> (Fr.) Fr.	30	74	0	71	100	100
<i>Lactarius torminosus</i> (Schaeff.: Fr.) S.F. Gray	64	45	0	100	100	100
<i>Lactarius turpis</i> (Weinm.) Fr.	20	83	0	50	93	100
<i>Leccinum aurantiacum</i> (Bull.) S.F. Gray	37	68	0	41	95	100
<i>Leccinum scabrum</i> (Bull.: Fr.) S.F. Gray	44	62	0	100	100	100
<i>Lycoperdon perlatum</i> Pers.: Pers.	35	70	0	29	31	51
<i>Macrolepiota procera</i> (Scop.: Fr.) Sing.	61	48	0	20	90	90
<i>Paxillus involutus</i> (Batsch: Fr.) Fr.	57	51	0	100	100	100
<i>Pleurotus ostreatus</i> (Jacq.: Fr.) Kummer	25	78	17	17	100	100
<i>Pluteus cervinus</i> (Schaeff.: Fr.) Kummer	42	64	0	50	60	71
<i>Ramaria abietina</i> (Pers.: Fr.) Quelet	50	57	0	20	100	100
<i>Russula ochroleuca</i> (Pers.) Fr.	71	39	0	14	27	35
<i>Scleroderma citrinum</i> Pers.	60	49	0	33	43	63
<i>Sparassis crispa</i> (Wulf.): Fr.	39	67	100	100	100	100
<i>Tylopilus felleus</i> (Bull.: Fr.) P. Karst.	60	49	0	17	92	97
<i>Trametes versicolor</i> (L.: Fr.) Quelet	44	62	0	50	52	93
<i>Xerocomus badius</i> (Fr.) Kühn. ex Gilb.	30	74	0	39	77	81
Control	117		0	53	67	84

Data are means of five replicate experiments, significant at $p < 0.05$.

^{x)} given in% of control.

Effect of fungal extracts in PDA medium on growth and sporulation of *Penicillium expansum*

The influence of fungal extracts on pathogen growth in vitro was estimated by two methods.

Tests for growth of colonies were conducted on Petri dishes (9 cm diameter) with 10 cm³ of densified PDA medium containing 5 cm³ of fungal extract on which subsequently 1 cm³ of diluted spore suspension (10² cfu/dish) were inoculated. For 9 consecutive days of incubation in the dark at the temperature of 25°C, linear growth was determined by measuring the diameter of the colonies. The results were evaluated in relation to a control sample without fungal extracts.

In the second method, PDA disks six mm in diameter, overgrown by three-day old mycelia of the pathogen tested, were placed in the centre of Petri dishes (5 cm in diameter) containing 1.5 cm³ of densified PDA medium and 1 cm³ of fungal extract. Growth of mycelium was assessed after 9 days of incubation by measuring the diameter along two parallel lines.

Statistical evaluation of results

The U-Mann-Whitney Test was performed for statistical analysis.

RESULTS AND DISCUSSION

The results of assessment of *Penicillium expansum* growth and rate of spores production in laboratory conditions on PDA medium with addition of aqueous extracts from fruit-bodies of 33 species of Basidiomycetes are presented in Table 1. The results of observation indicate that there is much difference in antifungal activity of the tested extracts in relation to the pathogen. A significant number of them limited colony growth and development of *Penicillium expansum* and some of them stimulated its growth. After 9 days cultivation, both the no. of colonies as well as their size were mostly limited by extracts of *Lactarius turpis*, *Hydnum repandum*, *Coprinus atramentarius* and *Coprinus comatus*. Morphological observation has shown a change in colour (into grey – beige) and diminution of the colony size of *Penicillium expansum*, which was noticed for each series of inoculation. Simultaneously the radial growth of three-day old mycelium after 9 days incubation on PDA medium supplemented with the fungal extracts, in relation to the control group, was investigated (Table 2, Fig. 1). Inhibition of pathogen growth in samples with addition of *Hydnum repandum* extracts was 44.5% relative to

TABLE 2. Effect aqueous extracts of selected basidiomycetes on mycelium growth of *Penicillium expansum* on PDA medium.

Species	Days of culture											
	3			5			7			9		
	Diameter of mycelium (mm)											
	Range min.-max	Mean	Median	Range min.-max	Mean	Median	Range min.-max	Mean	Median	Range min.-max	Mean	Median
<i>Agaricus bisporus</i>	9-11	10.1	10.0	17-25	20.2	20.0	21-24	22.9	21.0	22-24	23.1	22.5
<i>Agaricus campestris</i>	9-11	10.0	10.0	18-26	20.6	20.0	20-24	21.0	20.5	21-24	22.6	22.0
<i>Calvatia utriformis</i>	13-14	13.5	13.5	17-28	20.7	20.0	20-27	23.6	23.5	22-35	27.6	26.5
<i>Cantharellus cibarius</i>	8-11	9.2	10.0	12-15	13.0	14.0	12-22	18.7	22.5	18-25	20.1	22.5
<i>Cantharellus cinereus</i>	9-11	10.1	10.0	13-17	15.0	15.0	15-19	17.2	17.0	19-22	21.0	21.5
<i>Clitocybe gibba</i>	16-20	16.8	16.5	18-21	19.2	19.0	19-26	22.8	23.5	25-28	27.3	27.5
<i>Coltricia perennis</i>	8-14	10.6	10.5	13-19	15.5	15.0	18-24	20.8	21.5	22-29	26.3	26.0
<i>Coprinus atramentarius</i>	9-13	11.3	11.5	12-16	14.5	15.0	16-21	18.5	18.5	21-26	23.1	22.5
<i>Coprinus comatus.</i>	9-13	11.3	12.0	14-19	16.5	16.0	19-24	20.7	20.5	20-25	22.6	21.0
<i>Cortinarius armillatus</i>	12-17	13.2	13.0	17-23	23.6	18.0	21-26	23.6	23.5	29-33	30.7	31.0
<i>Cortinarius coerulescens</i>	10-12	11.0	11.0	13-17	15.3	16.0	19-23	21.2	21.5	24-30	26.6	26.0
<i>Cortinarius variecolor</i>	10-15	12.3	12.0	15-20	17.9	18.0	24-30	27.1	27.5	26-31	28.6	28.5
<i>Craterellus crnucopioides</i>	12-15	13.6	13.5	16-19	17.4	17.0	21-26	23.5	24.0	25-28	26.6	27.0
<i>Fomes fomentarius</i>	10-13	11.0	12.5	19-23	19.9	22.5	28-38	28.4	31.5	29-39	29.3	32.0
<i>Ganoderma applanatum</i>	12-18	15.0	15.5	20-26	22.9	22.0	21-28	24.6	24.5	23-30	26.7	26.5
<i>Hydnum repandum</i>	8-11	10.1	10.0	12-20	15.0	14.5	15-20	17.0	16.5	15-20	17.1	17.0
<i>Lactarius quietus</i>	10-13	11.5	11.5	15-21	16.7	16.0	18-31	23.0	21.0	20-33	24.6	23.5
<i>Lactatrius torminosus</i>	11-14	12.6	12.5	15-22	18.8	19.5	18-28	23.4	24.0	21-28	25.5	26.0
<i>Lactarius turpis</i>	8-9	8.6	9.0	10-17	13.5	12.5	10-17	13.9	13.0	13-20	17.0	16.0
<i>Leccinum aurantiacum</i>	13-18	14.2	15.5	16-22	18.0	20.0	24-27	23.2	25.5	29-32	27.8	30.0
<i>Leccinum scabrum</i>	10-17	13.8	13.5	14-20	17.9	18.5	21-25	22.8	22.5	24-29	26.3	25.5
<i>Lycoperdon perlatum</i>	13-17	14.5	14.0	18-21	19.1	19.0	19-27	25.8	26.5	27-34	30.1	30.0
<i>Macrolepiota procera</i>	11-14	11.4	12.5	15-18	16.1	16.0	21-25	22.1	22.0	24-27	25.1	25.0
<i>Paxillus involutus</i>	9-11	10.1	10.0	13-14	13.6	14.0	14-20	16.5	16.0	19-23	21.1	21.5
<i>Pleurotus ostreatus</i>	9-15	12.2	12.5	15-21	17.5	17.0	17-23	19.7	19.0	20-25	22.5	21.0
<i>Pluteus cervinus</i>	13-15	14.0	14.0	16-18	17.0	17.0	21-28	23.9	23.0	24-31	27.0	26.5
<i>Ramaria abietina</i>	7-11	9.6	10.5	10-16	14.4	16.0	15-24	20.0	21.0	20-26	23.8	22.5
<i>Russula ochroleuca</i>	8-14	10.6	10.5	13-19	15.6	15.5	18-24	20.6	20.5	22-29	26.1	26.5
<i>Scleroderma citrinum</i>	11-15	12.9	12.5	17-22	18.7	18.0	19-31	24.2	23.5	23-31	26.7	26.5
<i>Sparassis crispa</i>	10-13	11.4	11.0	13-17	14.9	15.0	17-24	20.5	20.5	22-29	25.5	25.0
<i>Tylopilus felleus</i>	10-18	12.1	12.0	15-23	17.1	17.0	22-30	24.3	22.5	25-36	28.4	27.5
<i>Trametes versicolor</i>	8-11	9.7	10.0	12-17	14.9	15.0	23-32	26.9	26.5	24-30	27.2	26.5
<i>Xerocomus badius</i>	12-14	13.4	13.0	18-22	20.0	20.0	23-30	25.4	25.0	24-30	26.2	26.0
Control	15-18	16.0	16.0	20-22	21.0	21.0	24-27	25.3	25.5	25-27	26.0	26.0

Data are means of eight replicate experiments.

mould growth on medium without extract. For *Pleurotus ostreatus* inhibition of pathogen growth was 29.5%, while for *Coprinus comatus* and *Coprinus atramentarius* it was respectively 25 and 24.5%. It has been reported that inhibition of hyphal mould growth causes also a delay of its sporulation. Possibility of easy obtaining of *Coprinus comatus* from the natural environment and commercial cultures (Vaaddrager 1978; Lelley 1983; Sadowska 1987) wide possibilities of increased crops of this species (Fritsche 1988) as well as autolytic changes (Bush 1974; Michalczyk et al. 1991) indicate that *Coprinus comatus* is an interesting biological active fungal species. The isolation and characterization of antifungal compounds from *Coprinus comatus* is currently under investigation.

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Fig. 1. Growth of *Penicillium expansum* mycelium after 9 days cultivated on PDA medium supplemented with fungal extracts: A) *Lactarius turpis*, B) *Hydnum repandum*, C) *Pleurotus ostreatus*, D) *Coprinus comatus*, K) Control sample.

INHIBICJA WZROSTU I ZARODNIKOWANIA *PENICILLIUM EXPANSUM*
PRZEZ EKSTRAKTY WYBRANYCH GRZYBÓW WYŻSZYCH
(BASIDIOMYCETES)

STRESZCZENIE

Badano wpływ wodnych ekstraktów z owocników 33 gatunków grzybów wielkoowocnikowych (Basidiomycetes) na wzrost i zarodnikowanie *Penicillium expansum* w hodowli in vitro. Gatunki: *Hydnum repandum* L.: Fr., *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, *Coprinus comatus* (Müll: Fr.) S.F. Gray, *Coprinus atramentarius* (Bull.: Fr.) Fr. and *Lactarius turpis* (Weinm.) Fr. redukowały wzrost grzybn i opóźniały zarodnikowanie patogena.

SŁOWA KLUCZOWE: Basidiomycetes, przeciwgrzybicze metabolity, *Penicillium expansum*, inhibitory wzrostu.