ACTA MYCOLOGICA
Vol. 34 (2): 315–327
1999

The search for lectin isolated from the mycelial cultures of *Laetiporus sulphureus*

GRAŻYNA KOŃSKA[1], JEAN GUILLOT[2], STANISŁAW KOHLMÜNZER[1], MIREILLE DAMEZ[2], and KINGA RAKOCZY[1]

[1] Department of Pharmaceutical Botany, Faculty of Pharmacy, Jagiellonian University
9 Medyczna, PL-30-688 Kraków, Poland
[2] Department of Botany and Mycology, Faculty of Pharmaceutical Sciences,
University d'Auvergne
28 H. Dunant, F 63100 Clermont-Ferrand, France

Końska G., Guillot J., Kohlmünzer S., Damez M., Rakoczy K.: *The search for lectin isolated from the mycelial cultures of Laetiporus sulphureus.* Acta Mycol. 34 (2): 315–327, 1999.

This study proved the presence of lectin in mycelial cultures of *Laetiporus sulphureus*. Lectin was excreted into the medium and its erythroagglutinating activity was not high. No active lectin was detected in hyphae using both extraction and immunofluorescence method. Comparative studies based on immunological methods indicated that the lectin synthesised in vitro differed from the lectin produced in fruit-bodies.

Key words: Mycelium, *Laetiporus sulphureus*, lectin, mycelial cultures.

INTRODUCTION

Higher fungi are a rich, but still little known source of biologically active substances.

Lectins are an interesting group of compounds, which are characterised by exceptionally high affinity for sugars, including those constituting glycopeptide and glycolipid cellular receptors. In many disciplines of biology and medicine, lectins are being used more and more commonly as tools in the studies of the changes in the cell membrane surface in different physiological and pathological states of the cell.

Furthermore, there are reports on the possibility of using lectins produced by such species of fungi as *Agaricus bisporus* (Lange) Imbach, *Laccaria amethystina* (Bolt.) Murr., *Russula nigricans* (Bull.) Fr., in microbiological and parasitological diagnostics (Payne, Capbell, Patchett and Kroll 1992; Gueugnot, Guillot, Damez and Coulet 1984; Petavy, Guillot and Coulet 1975).

Galβ1-3GalNAc-specific lectin of *Agaricus bisporus* was used in the studies of membrane receptors on human blood cells and lymphocytes in patients suffering from chronic leukaemia (Green, Fleisher and Waldmann 1981; Presant and Kornfeld 1972). Moreover, it was reported that this lectin inhibited proliferation of epithelial cell lines and expression of oncofoetal antigen (TF-antigen) (Yu, Fernig, Smith, Milton and Rhodes 1993). L-fructose-specific lectin obtained from *Aleuria aurantia* (Pers.: Fr.) Funck. fruit-bodies was used in the isolation of glycoproteins from human erythrocyte membranes by affinity chromatography (Yazawa, Furukawa and Kochibe 1984), separation of oligosaccharide fractions of human immunoglobulin G (Harada, Kamei, Tokumoro, Yui, Koyama, Kochibe, Endo and Kobata 1987), identification of glycoproteins involved in axonal transport in nervous cells (Ohlson and Karlson 1983), and isolation and characterization of tumour-associated antigens (Yazawa, Kochibe and Asao 1990).

N-acetyllactosamine-specific lectin isolated from *Laetiporus sulphureus* (Bull.: Fr.) Murr. fruit-bodies (Końska, Guillot, Dusser, Damez and Botton 1994) was employed to investigate the changes in the location of surface receptors on human breast cancer cells depending on cancer type and stage of carcinomatous process (Końska, Guillot, De Latour and Fonck 1998). A purified extract from *L. sulphureus* carrying a lectins activity also in embryological studies. They showed the changes in expression and character of glucoconjugates in embryonic cells during morphogenesis (e.g. urogenital morphogenesis) (Didier, Didier, Guillot, Croisille and Thiery 1990).

Fruit-bodies developing under natural conditions are the main source of fungal lectins, which undoubtedly seriously limit the possibility of obtaining material for the study. Therefore, research aimed at the development of biotechnological methods of lectin production have been undertaken both using mycelial cultures (Banerjee, Ghosh and Sengupta 1982; Musilek, Ticha, Volc and Kocourek 1990) and genetic engineering (Fukumori, Takeuchi, Hagiwara, Ito, Kochibe, Kobata and Nagata 1989).

In the present study an attempt has been made to demonstrate the presence of active lectin in mycelial cultures of *Laetiporus sulphureus* (Fig. 1 A and B). In addition a comparative analysis of lectins isolated from the mycelium and fruit-bodies was conducted.

MATERIALS AND METHODS

Culture conditions. In vitro culture was started from inoculum which was a tiny fragment (0.5 cm^2) of hymenial part of *Laetiporus*

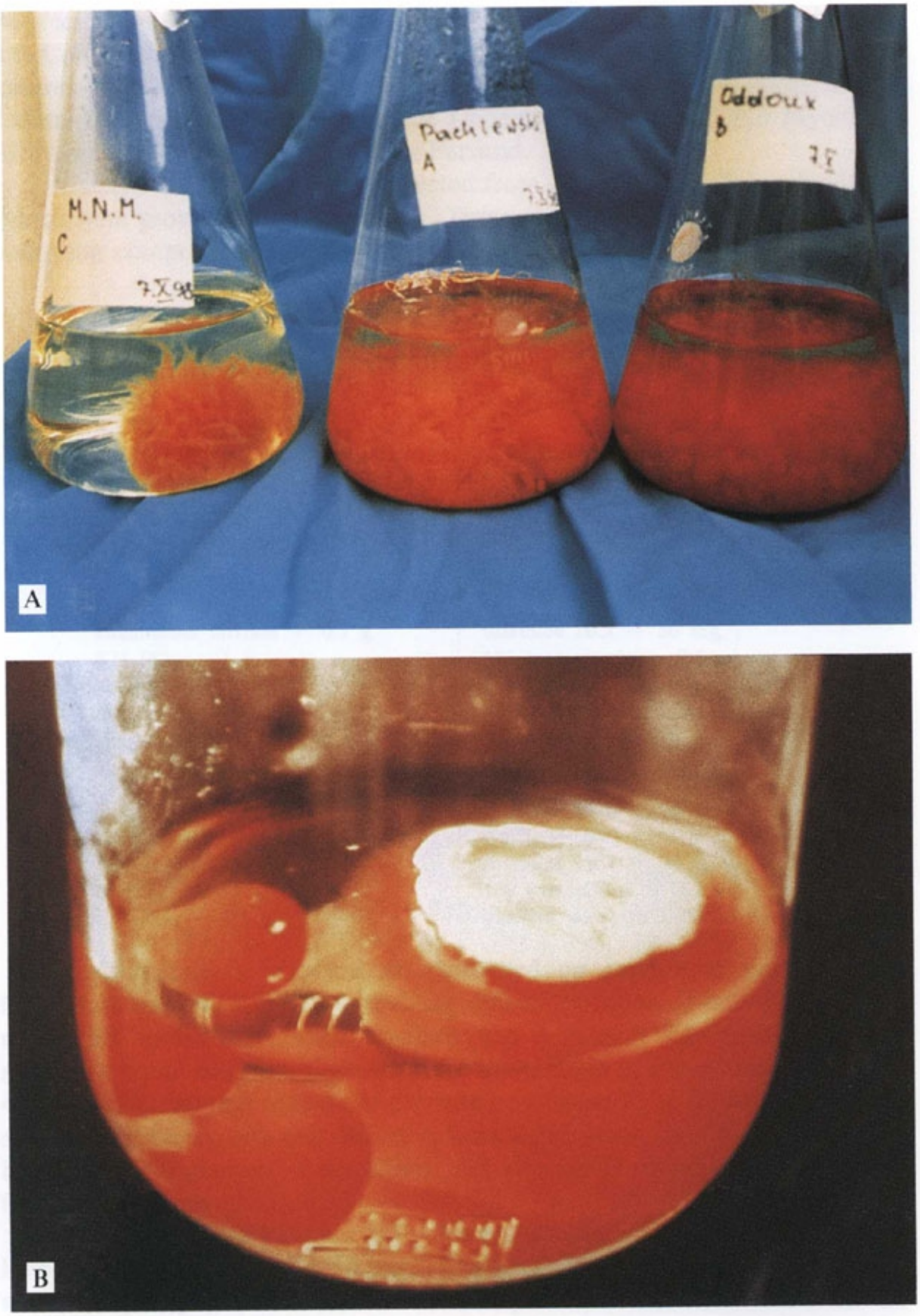

Fig. 1. *Laetiporus sulphureus* mycelium cultivated in vitro in MNM, Pachlewski's and Oddoux shaking media (A) and stationary culture (B)

sulphureus fruit-body collected in September 1997 in Clermont-Ferrand (France). Continuous cultures derived from this inoculum were run parallelly at two centres: the Department of Pharmaceutical Botany, Medical College, Jagiellonian University in Kraków and the Department of Botany and Mycology, University d'Auvergne in Clermont-Ferrand.

Material for the study was collected from the 18th and 19th subcultures which were grown in liquid medium. Three versions of the medium had the following composition:

Oddoux medium:

glucose – 10 g
malt extract broth – 5 g,
NH_4Cl – 0.5 g
$MgSO_4 \cdot 7H_2O$ – 0.5 g
KH_2PO_4 – 0.5 g,
L-asparagine – 1g,
1% $FeCl_3$ – 1.5 ml,
0.5% $MnSO_4 \cdot H_2O$ – 1.5 ml,
0.2% $ZnSO_4$ – 1.5 ml,
0.2% $CaCl_2 \cdot 6H_2O$ – 0.5 ml,
casein hydrolysate – 0.2 g,
Bacto yeast nitrogen base – 30 mg
adenine – 12 mg,
0.5% vitamin B_1 – 0.2 ml,
0.2% vitamin B_6 – 0.5 ml,
distilled water to 1000 ml;

Pachlewski's medium:

ammonium tartrate – 0.5 g,
KH_2PO_4 – 1 g,
$MgSO_4 \cdot 7H_2O$ – 0.5 g,
malt extract broth – 5 g,
glucose – 20 g,
thiamine HCl – 50 mg,
1% iron citrate – 0.5 ml,
0.2% $ZnSO_4 \cdot 7H_2O$ – 0.5 ml,
distilled water to 1000 ml;

MNM medium:

$(NH_4)_2HPO_4$ – 0.25 g,
malt extract broth – 3 g,
glucose – 10 g,
$MgSO_4 \cdot 7H_2O$ – 0.15 g,
KH_2PO_4 – 0.5 g,
NaCl – 0.025 g,
$CaCl_2$ – 0.05 g,
thiamine HCl – 0.1 g,
1% iron citrate – 1.2 ml,
distilled water to 1000 ml.

Small fragments of mycelium cultivated on solid agar medium were transferred to conical flasks containing 300-ml portions of one of the above mentioned media. The solid medium had a similar composition as the respective liquid medium. Cultures were maintained at room temperature for 41 days under varying conditions: (a) with or without light, (b) with or without shaking (stationary cultures). Before culture termination, its purity was verified by microscopic analysis of mycelium, and afterwards mycelia were separated from the medium.

Demonstration of the presence of active lectin in the medium. Protein fractions were precipitated with ammonium sulphate from the medium separated after termination of the culture and neutralized to pH 6.5–7.0. Protein fractions were sedimented at + 4°C for at least 48 hours. Subsequently the precipitate was filtered and dissolved in a small amount of distilled water. The obtained solutions were dialysed using membranes with pore diameter of 8,000–15,000 Da against

distilled water first, to remove ammonium sulphate completely. Then the solutions were concentrated about 10 times using polyethylene glycol and equilibrated (dialysed) against phosphate buffer PBS (pH 7.2). Agglutinating activity of the obtained solutions was determined with the use of bromelin-treated human erythrocytes A, B, 0 (Rh+).

Erythrocyte preparation and agglutinating activity testing. Full blood was washed three times with PBS and centrifuged to obtain a suspension of pure erythrocytes. The erythrocyte suspension was mixed with 0.4% bromelin solution at volume ratio of 1:1 and then incubated at room temperature for 0.5 h and washed with PBS.

Preliminary tests of agglutinating activity consisted in mixing of equal volumes of the tested extracts and erythrocyte suspension on porcelain plates. If positive reaction was obtained (at least 25% of erythrocytes agglutinated), agglutination titre was determined. For this purpose, the method of geometric dilutions of the tested fractions in PBS was used. To each dilution, a drop of 4% erythrocyte suspension was added and after 15, 30 and 45 min, the highest dilution of the tested extract which yielded agglutinating reaction was determined. This dilution was considered as agglutination titre.

Identification of the lectin studied. Comparative identification of the lectin from in vitro culture and that found in fruit-bodies was based on immunological analysis of both lectins. The method of Ouchterlony (1953) was used.

Production of anti-lectin antibody: To be able to employ this method, rabbit antibodies against lectin from *L. sulphureus* fruit-bodies were developed first. One milliliter of rabbit full blood was collected on heparin from auricular artery, erythrocytes were centrifuged and washed three times with PBS. Subsequently, 4% erythrocyte suspension was mixed with geometrical dilutions of pure lectin solution. Four consecutive lectin dilutions yielding the weakest agglutinating reaction, together with agglutinated erythrocytes, were combined, centrifuged and erythrocytes obtained in that way in the form of tiny agglutinates were suspended in 1 ml of sterile PBS and injected to the rabbit's auricular vein. The procedure was repeated every seven days for consecutive 21 weeks. Following 5 weeks, approximately 10 ml samples of full blood were collected from auricular vein also in 7-day intervals, and plasma samples obtained by centrifugation were stored in 0.5 ml portions at −20°C.

Antibody diffusion and precipitation reaction in agar gel (acc. to Ouchterlony 1953). Agar was poured onto glass plates and after its setting 6 wells about 3 mm deep and 8 mm in diameter were cut out around a circle with a circumference of 3 cm, and one well was cut out in the circle centre. The central well contained serum of the immunised rabbit, while remaining wells were filled with: lectin from fruit-bodies, mycelial extract and protein fractions of the medium. Antibody diffusion in the gel was run at +4°C for 48 h in humidified chamber. Gel plate

was then placed in 5% solution of sodium citrate for 75 min and subsequently washed many times with physiological saline. Gels were stained with Amido black for 8 min and destained by many washings with 5% acetic acid until clear background was obtained.

Demonstration of the presence of active lectin in mycelium was based on the testing of agglutinating activity of mycelial extract isolated directly from the culture and on searching lectin molecules in hyphae using immunofluorescence method.

Testing of extract agglutinating activity. Half of the obtained mycelium was first treated enzymatically to break partially an integrity of chitin hyphal wall to facilitate extraction in water environment, while the other half of the mycelium was extracted omitting enzyme addition.

For this purpose 1.0 g of mycelium was filtered and mixed with a mixture of chloroform and methanol (2:1) and washed many times with PBS. Subsequently, the mycelium was suspended in 2 ml of PBS and treated with lyticase solution from *Arthrobacter luteus* (0.25 U/1 g of mycelium fresh weight) at 25°C for 15 h and again repeatedly washed with PBS.

The mycelium was then homogenized in PBS at weight ratio of 1:4, shaken at room temperature for at least 3 h and centrifuged. The activity of the extract was determined in a reaction with bromelin-treated human erythrocytes A, B, O Rh+.

Immunofluorescence method: The cultured mycelium, washed out of the medium with PBS was transferred to PBS, containing 2% bovine serum albumin (PBS-BSA) and added to serial geometrical dilutions (in PBS-BSA) of immunised rabbit serum. After one-hour incubation the samples were washed three times with PBS. The control contained rabbit serum without antibodies against lectin from *L. sulphureus* fruit-bodies. In the next step, mycelium was left for an hour in contact with goat antibody against rabbit Ig labeled with FITC diluted at a ratio 1:100. After washing twice with PBS-BSA hyphae were observed under fluorescence microscope.

RESULTS

Morphological and microscopic analysis of the cultured mycelium. *L. sulphureus* mycelium cultivated in vitro both on solid medium and in stationary liquid culture developed as various forms:

aerial mycelium – forming a kind of floury coating on medium surface, creamy-orange in colour. Microscopic analysis showed not very compact hyphal net, masked by numerous, oval or roundly spores with dimensions of $6.0-9.0 \times 6.0-7.5$ μm, which constituted a major part of this mycelial form (Fig. 2 A).

submerged mycelium – appearing as gelatinous mass, intensely orange in colour caused by lengthy hyphal threads bearing chlamydospores at their

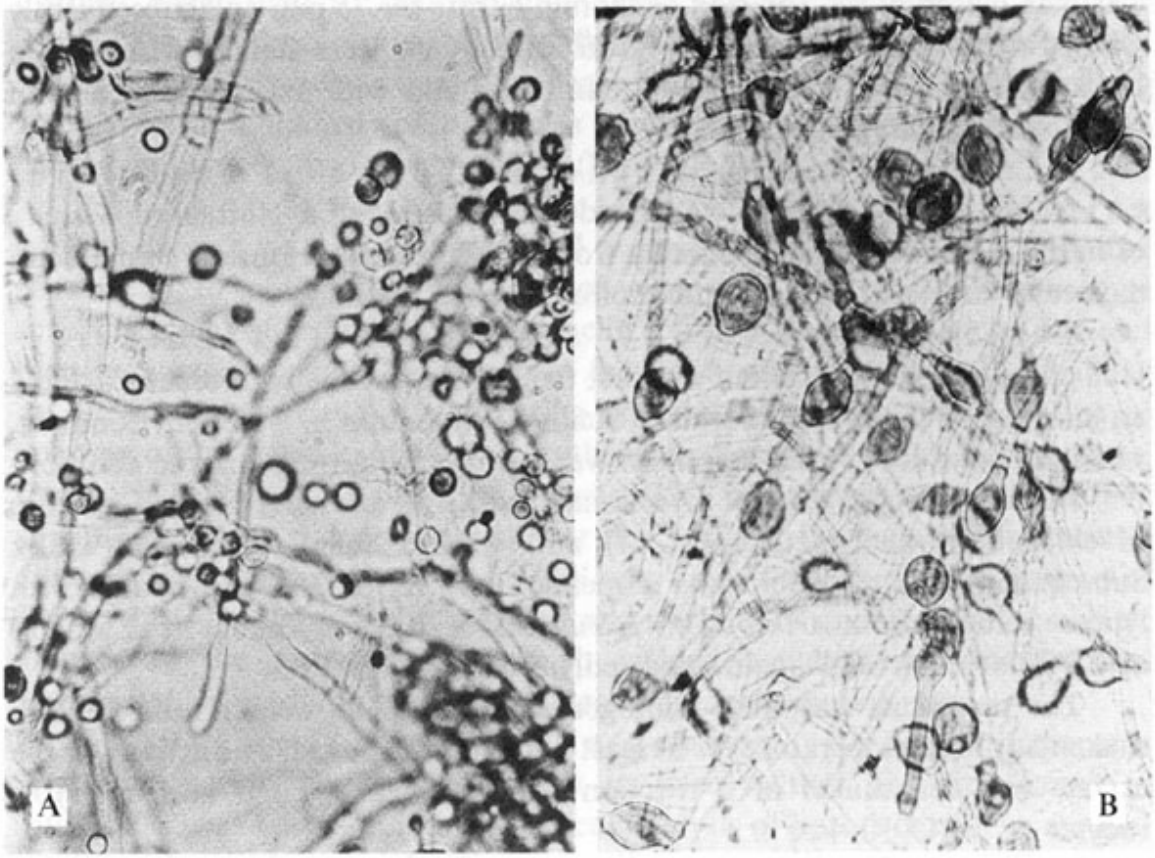

Fig. 2. *Laetiporus sulphureus* mycelium cultivated in vitro; aerial mycelium with oval spores (A) and submerged mycelium with chlamydospores (B)

ends with dimensions of 12–19 × 7.5–13.5 μm, oval- or club-shaped, with strongly thickened walls (Fig. 2 B). Only this mycelial form developed in shaking liquid cultures.

Characteristics of both mycelial forms confirmed earlier observations (N o b l e s 1965). First signs of mycelial growth in shaking culture appeared 4–7 days after culture initiation. The growth in stationary culture was delayed and both mycelial forms were observed (submerged and aerial). Limited exposure to light inhibited initial stage of mycelial growth and stain synthesis, the latter being especially noticeable. Medium composition influenced both mycelial growth rate (final mycelium mass) and intensity of orange stain synthesis. The most advantageous conditions for obtaining large mycelium mass were liquid culture on Pachlewski's and Oddoux medium with exposure to light and medium aeration (Fig. 1 A, Fig. 3).

A n a l y s i s o f a c t i v e l e c t i n c o n t e n t. In all the cases mycelial extracts studied yielded negative results of erythroagglutinating reaction. However, protein fractions precipitated from the medium after culture termination caused agglutination of bromelin-treated human erythrocytes to a different degree, depending on culture conditions, while proteins from the medium before culture did not give such a reaction. No specificity for blood

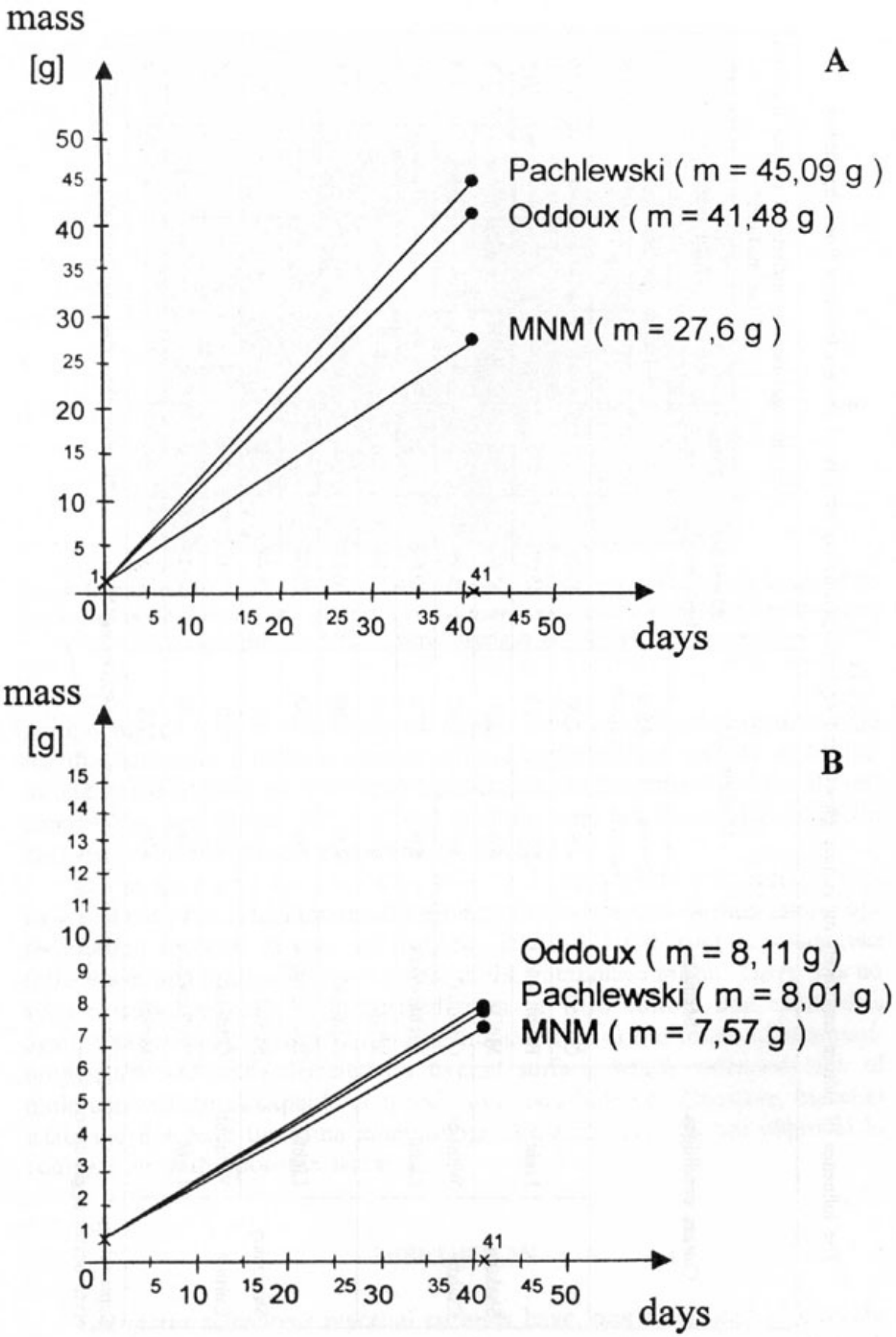

Fig. 3. Increments in mycelial mass in MNM, Pachlewski's and Oddoux shaking media with unlimited exposure to light (A) and stationary conditions with limited exposure to light (B)

Table 1

The influence of culture conditions on culture growth and erythroagglutinating activity of protein fractions from the medium

Culture conditions		Medium	Medium pH		Mycelium fresh weight (g)	Erythroagglutinating activity of protein fractions of the medium*				
			Before culture	After culture		Before culture	After culture			
							Incubation time (min)			Titre
							15	30	45	
Shaking Culture	Light	Oddoux'	4.5	2.00	41.48	0	++++**	++++	++++	1/16
		Pachlewski's	5.0	2.10	45.09	0	++++	++++	++++	1/16
		MNM	6.4	2.50	27.66	0	++++	++++	++++	1/8
	Without Light	Oddoux'	4.5	2.00	25.99	0	++++	++++	++++	1/8
		Pachlewski's	5.0	2.15	40.69	0	++++	++++	++++	1/16
		MNM	6.4	2.40	16.06	0	+++	+++	+++	1/4
Stationary Culture	Light	Oddoux'	4.5	1.90	10.38	0	+	++	+++	1/8
		Pachlewski's	5.0	1.90	10.04	0	++	+++	+++	1/8
		MNM	6.4	2.2	8.44	0	+	++	++	1/4
	Without Light	Oddoux'	4.5	2.0	8.11	0	++	+++	+++	1/8
		Pachlewski's	5.0	1.90	8.01	0	++	+++	+++	1/8
		MNM	6.4	2.0	7.57	0	+	+	++	1/4

Explanation: * – in comparison with bromelin-treated human erythrocytes; ** + – 25% of erythrocytes agglutinated; ++ – 50% of erythrocytes agglutinated; +++ – 75%; ++++ – 100%

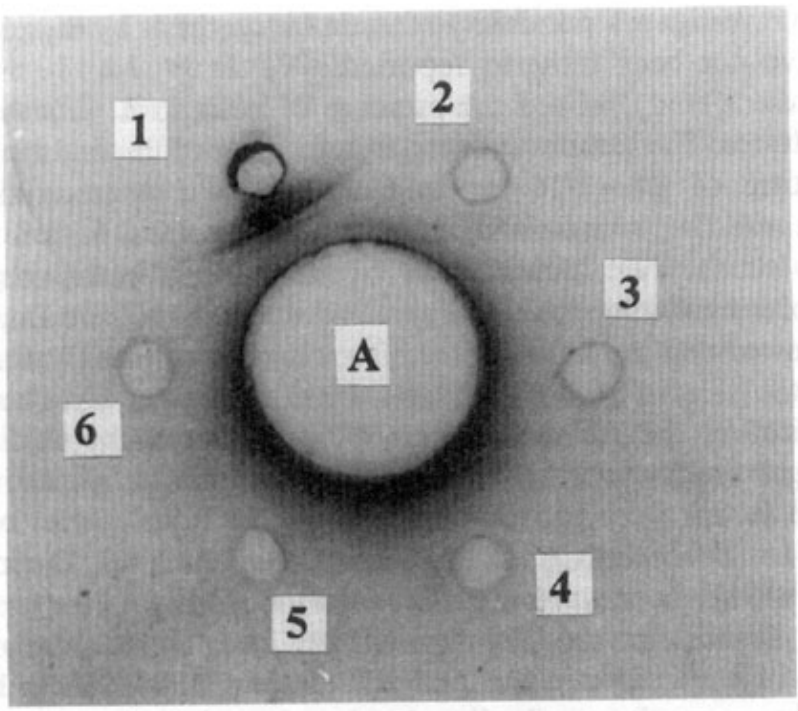

Fig. 4. Immunoprecipitation positive reaction in agar gel between antibodies from serum of the immunized rabbit (A) and lectin isolated from *Laetiporus sulphureus* fruit-bodies (1) and negative reaction with lectin derived from in vitro mycelium (2–3) and cultures media (4–6)

group antigens A, B, H was observed. Higher intensity of erythroagglutinating reaction indicated increased content or/and agglutinating activity of lectins, isolated from cultures run with shaking and unlimited exposure to light. Poorer composition and higher pH of MNM medium were less favourable for lectin synthesis. The results are presented in Table 1.

Comparative analysis of mycelial and native lectin. The immunodiffusion method showed a distinct immunoprecipitation reaction in agar gel between lectin isolated from *L. sulphureus* fruit-bodies and antibodies from serum of the immunized rabbit. There was no such reaction between lectin derived from in vitro culture and antibodies against native lectin (isolated from fruit-bodies) (Fig. 4). No traces of immunofluorescence were also detected on hyphal surface, which indicated lack of molecular structures capable of specific antibody binding. Therefore, mycelial lectin did not have the same immunological nature and was not identical in comparison with sporome lectin.

DISCUSSION

Laetiporus sulphureus mycelial cultures have long been used in biotechnology to obtain various metabolites (Nour el Dein and Abdallach 1967; Villanueva, Barbier and Lederer 1967).

Attempts to investigate a possibility of lectin biosynthesis by mycelia cultivated in vitro have not been hitherto reported.

The present study proved the presence of lectin in *L. sulphureus* in vitro mycelial cultures. The lectin was excreted into the medium and its content was not high (titre of 1/8–1/16 for bromelin-treated human erythrocytes) in comparison with the lectin isolated from fruit-bodies (K o ń s k a et al. 1994). No active lectins were detected in hyphae, using both extraction and immunofluorescence methods. Comparative studies based on immunological methods showed that the lectin derived from in vitro cultures was not identical with the lectin isolated from fruit-bodies. Until now, there have been only few reports indicating the isolation of active lectins from mycelial cultures of various fungal species, which had the same character as respective sporome lectins. Such lectins were proved to be present in three species of the genus *Lactarius*: *L. deliciosus* (G u i l l o t, G i o l l a n t, D a m e z and D u s s e r 1991), *L. deterrimus* (G i o l l a n t, G u i l l o t, D a m e z, D u s s e r, D i d i e r and D i d i e r 1993) and *L. salmonicolor* (G u i l l o t, G i o l l a n t, D a m e z and D u s s e r 1994). These lectins were recognised by respective antibodies developed against their native counterparts. Active mycelial lectins with very similar sugar-specificity to those derived from fruit-bodies have been isolated from mycelial cultures of *Kuehneromyces mutabilis* (M u s i l e k, T i c h a, V o l c and K o c o u r e k 1990) and *Fomes fomentarius* (P a r d o e, U h l e n b r u c k, A n s t e e and R e i f e n b e r g 1969). It has also been shown that *Volvariella volvacea* fruit-bodies and mycelium cultivated in vitro contain VVL lectin exhibiting immunomodulating activity (S h e, N g and L i u 1998).

On the other hand, there are some reports on mycelial synthesis of lectins differing from those lectins produced in fruit-bodies. Such lectins were found e.g. in *Pholiota squarrosa* (M u s i l e k et al. 1990) and, as indicated in this paper, in *L. sulphureus*. Mycelial lectin synthesis gradually disappears during primordia formation when other lectins characteristic of fruit-bodies succeed them. This holds true for *Pleurotus cornucopiae* (K a n e k o, O g u r i, K a t o and N a g a t a 1993; O g u r i, A n d o and N a g a t a 1996) but it is probably common in many other species in which no active mycelial lectins were detected, such as *Flammulina velutipes* (M u s i l e k et al. 1990). In *Agrocybe aegerita* mycelial cultures, only one lectin was found, which had similar characteristics as anti-A-specific lectin derived from fruit-bodies. A non-specific lectin from fruit-bodies of this species was not detected in mycelium, the only moment of its appearance being primordia formation (T i c h a, D u d o v a and K o c o u r e k 1985).

The above data show that there are no consistent views on the character of lectins synthesized in different developmental stages in fungi, as well as on the place and time of their production. This problem directs our attention to another very important issue, i.e. biological role of lectins in organisms which synthesize them. The following theories are worth mentioning:

a) recognition of appropriate partners in the course of ectomycorrhiza (Guillot et al. 1994);
b) fruit-body formation (Oguri et al. 1996);
c) mechanism of parasitic fungi penetration into host organism (Botton and Guillot 1987).

Regardless of the problems connected with elucidation of the role of fungal lectins, a possibility of obtaining by biotechnological methods of substances with potential therapeutic properties is a very positive and encouraging phenomenon. The examples are lectins from *Volvariella volvacea* with immunomodulating activity (She et al. 1998) or from *Tricholoma mongolicum* exhibiting hypotensive action (Wang, Ooi, Ng, Chiu and Chang 1996) and antiproliferative activity against tumour cells in vitro (Wang, Ng, Liu, Ooi and Chang 1995). In spite of the fact that lectin obtained from *L. sulphureus* mycelial cultures differs of that from fruit-bodies, probably also in terms of its sugar-specificity (relevant studies are in progress), its detailed, physico-chemical characterization and possible usefulness in glucoconjugate studies will be a subject of further research.

REFERENCES

Banerjee P. C., Ghosh A. K., Sengupta S. 1982. Hemagglutinating activity in extracts of mycelia from submerged mushroom cultures. Appl. Envir. Microbiol. 44 (4): 1009–1011.

Botton B., Guillot J. 1987. Purification d'une lectine a partir du basidiomycete *Rigidoporus lignosus*, parasite de l'Hevea. Implication possible de la molecule dans la differenciation des palmettes du champignon. IV eme Reunion du Reseau Mycologie, Lyon.

Didier E., Didier P., Guillot J., Croisille Y., Thiery J. P. 1990. Expression and distribution of carbohydrate sequences in chick germ cells: a comparative study with lectins and the NC-1/HNK-1 monoclonal antibody. Int. J. Dev. Biol. 34: 421–431.

Fukumori F., Takeuchi N., Hagiwara T., Ito K., Kochibe N., Kobata A., Nagata Y. 1989. Cloning and expression of a functional fucose-specific lectin from an orange peel mushroom, *Aleuria aurantia*. Febs Letters 250 (2): 153–156.

Giollant M., Guillot J., Damez M., Dusser M., Didier P., Didier E. 1993. Characterization of a lectin from *Lactarius deterrimus*. Plant. Physiol. 101: 513–522.

Green C. W., Fleisher T. A., Waldmann T. A. 1981. Suppression of human T and B lymphocyte activation by *Agaricus bisporus* lectin. J. Immun. 126 (2): 580–586.

Gueugnot J., Guillot J., Damez M., Coulet M. 1984. Identification and taxonomy of human and animal leishmanias by lectin-mediated agglutination. Acta Tropica 1: 135–140.

Guillot J., Giollant M., Damez M., Dusser M. 1991. Isolation and characterization of a lectin from the mushroom *Lactarius deliciosus*. J. Biochem. 109: 840–845.

Guillot J., Giollant M., Damez M., Dusser M. 1994. Intervention des lectines fongiques dans les evenements precoces de reconnaissance arbre/champignon au cours de la formation des ectomycorhizes. Acta Bot. Gallica 141 (4): 443–447.

Harada H., Kamei M., Tokumoro Y., Yui S., Koyama F., Kochibe N., Endo T., Kobata A. 1987. Systematic fractionation of oligosaccharides of human immunoglobulin G by serial affinity chromatography on immobilized lectin columns. Analyt. Biochem. 164: 374–381.

Kaneko T., Oguri S., Kato S., Nagata Y. 1993. Developmental appearance of lectin during fruit body formation in *Pleurotus cornucopiae*. J. Gen. Appl. Microbiol. 39: 83–90.

Końska G., Guillot J., De Latour M., Fonck Y. 1998. Expression of Tn antigen and N-acetyllactosamine residues in malignant and benign human breast tumors detected by lectins and monoclonal antibody 83D4. Internal. J. Oncol. 12: 361–367.

Końska G., Guillot J., Dusser M., Damez M., Botton B. 1994. Isolation and characterization of an N-acetyllactosamine-binding lectin from the mushroom *Laetiporus sulphureus*. J. Biochem. 116: 519–523.

Musilek M., Ticha M., Volc J., Kocourek J. 1990. Studies on lectins. LXXI. Lectins-Biology, Biochemistry, Clinical Biochemistry, Sigma Chem. Comp., St. Louis, Missouri USA. 7: 53–56.

Nobles M. K. 1965. Identification of cultures of wood-inhabiting hymenomycetes. Can. J. Bot. 43: 1097–1139.

Nour el Dein M. S., Abdallah N. M. 1967. Biosynthesis of fat in submerged culture of *Polyporus sulphureus*. Z. Allg. Mikrob. 7 (1): 29–32.

Oguri S., Ando A., Nagata Y. 1996. A novel developmental stage-specific lectin of the basidiomycete *Pleurotus cornucopiae*. J. Bacteriol. 178 (19): 5692–5698.

Ohlson C., Karlsson J-O. 1983. Glycoproteins of axonal transport: polypeptides interacting with the lectin from *Aleuria aurantia*. Brain Research 264: 99–104.

Ouchterlony O. 1953. Antigen-antibody reactions in gels. IV. Types of reactions in coordinated systems of diffusion. Acta Path. Microbiol. Scand. 32: 231–240.

Pardoe G. I., Uhlenbruck G., Anstee D. J., Reifenberg V. 1969. On the specificity of broad spectrum agglutinins. III. Z. Immunitatsforsch. Allergie Klin. 139: 468–485.

Payne M. J., Capbell S., Patchett R. A., Kroll R. G. 1992. The use of immobilized lectins in the separation of *Staphylococcus aureus*, *Escherichia coli*, *Listeria* and *Salmonella* spp. from pure cultures and foods. J. Appl. Bact. 73: 41–52.

Petavy A. F., Guillot J., Coulet M. 1975. Interet des lectines dans l'identification des Candida. Bull. Soc. Mycol. Med. 19: 225–228.

Presant C. A., Kornfeld S. 1972. Characterization of the Cell Surface Receptor for the *Agaricus bisporus* Hemagglutinin J. Biol. Chem. 247 (21): 6937–6945.

She Q. B., Ng T. B., Liu W. K. 1998. A novel lectin with potent immunomodulatory activity isolated from both fruiting bodies and cultured mycelia of the edible mushroom *Volvariella volvcacea*. Biochem. Biophys. Res. Commun. 247 (1): 106–111.

Ticha M., Dudova V., Kocourek J. 1985. Studies on Lectins. W. de Gruyter et Co., Berlin–New York, Germany. 112 (4): 505–514.

Villanueva V. R., Barbier M., Lederer E. 1967. Sur la biosynthese d'acides triterpeniques par *Polyporus sulphureus*. Bull. Soc. Chim. Biol. 49 (4): 389–393.

Wang H. X., Ng T. B., Liu W. K., Ooi V. E., Chang S. T. 1995. Isolation and characterization of two distinct lectins with antiproliferative activity from the cultured mycelium of the edible mushroom *Tricholoma mongolicum*. Int. J. Pept. Protein Res. 46 (6): 508–513.

Wang H. X., Ooi V. E., Ng T. B., Chiu K. W., Chang S. T. 1996. Hypotensive and vasorelaxing activities of a lectin from the edible mushroom *Tricholoma mongolicum*. Pharmacol. Toxicol. 79 (6): 318–323.

Yazawa S., Furukawa K., Kochibe N. 1984. Isolation of fucosyl glycoproteins from human erythrocyte membranes by affinity chromatography using *Aleuria aurantia* lectin. J. Biochem. 96: 1737–1742.

Yazawa S., Kochibe N., Asao T. 1990. A simple procedure for isolation of tumor-associated antigens by affinity chromatography using fucose-specific *Aleuria aurantia* lectin. Immunolog. Invest. 19 (4): 319–327.

Yu L., Fernig D.G., Smith J.A., Milton J.D., Rhodes J.M. 1993. Reversible Inhibition of Proliferation of Epithelial Cell Lines by *Agaricus bisporus* (Edible mushroom) Lectin. Cancer Research 53: 4627–4632.

Poszukiwanie lektyn w hodowli grzybni *Laetiporus sulphureus*

Streszczenie

Udowodniono obecność lektyny w hodowli grzybni *Laetiporus sulphureus* (Bull.: Fr.) Murr. oraz wpływ warunków hodowli na jej syntezę. Lektyna ta jest wydzielana do pożywki, a jej aktywność erytroaglutynacyjna nie jest wysoka w porównaniu z aktywnością lektyny pochodzącej z owocników. Na poziomie grzybni, przez ekstrakcję oraz za pomocą metody immunofluorescencji nie odnaleziono cząsteczki lektyny. Dzięki zastosowaniu metody immunodyfuzji w żelu agarowym stwierdzono, że lektyna grzybniowa różni się od lektyny z owocników.