ANTIFUNGAL ACTIVITY OF SOME METABOLITES OF HIGHER FUNGI (BASIDIOMYCETES) – AN OVERVIEW

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ABSTRACT

A series of compounds of different chemical structures showing antifungal activity were isolated from higher fungi (Basidiomycetes) fruit bodies. Among the microflora against which the examined metabolites showed effective activity, there are pathogenic organisms for people as well as for animals: *Candida albicans*, *Candida tropicalis*, *Rhodotorula rubra*, *Aspergillus fumigatus* and pathogens attacking plants: *Penicillium chrysogenum*, *Botrytis cinerea*, *Alternaria solani*, *Fusarium culmorum*, *Trichoderma lignorum* and *Verticillium dahliae*.

Searching for fungal metabolites having antifungal activity creates possibilities of using them against a range of fungal pathogens of clinical, agronomic and environmental significance.

KEY WORDS: Basidiomycetes, antifungal activity, antifungal-agents-metabolism.

Among physiologically active higher fungi (Basidiomycetes) metabolites, many have different types of biological activity (Semerdzieva 1984; Grzybek 1992; Kohlmünzer 1992, 1996). Compounds having antifungal activity from different chemical groups, constitute a distinguished group (Table 1). Chemical structures of the most important secondary metabolites isolated from various higher fungi that show antifungal activity are presented in Figures 1-4. Natural product derivatives of β -methoxyacrylic acid: mucidin, oudemansins and strobilurins (Fig. 1) are produced by a number of sa-

protrophic higher fungal species and were isolated from basidiomycete the family Crepidotaceae and Tricholomataceae. Musilek et al. (Musilek 1965; Musilek et al. 1969; Subik et al. 1974) isolated from growing on decaying beech-trees *Oudemansiella mucida* a compound of policetide structure – mucidin, showing antifungal activity against opportunistic human pathogen *Candida albicans* as well as dermatophytes from genus *Trichophyton*, *Epidermophyton* and *Microsporum* (Kejda 1976). This antibiotic was introduced to Czech pharmacopoeia as a component of a preparation named Mucider-

TABLE 1. Antifungal substances detected from fruit bodies of higher fungi (*Basidiomycetes*).

Species	Active component	Authors
<i>Aleurodiscus mirabilis</i> (Berk. Curt/Hohn.)	aleurodiscal	Lauer et al. (1989)
<i>Caloporus dichrous</i> (Fr./Ryv.)	caloporoside	Weber et al. (1994)
<i>Crepidotus fulvotomentosus</i> Peck	strobilurin E	Weber et al. (1990a)
<i>Cyathus striatus</i> (Huds. Pers./Willd.)	striatins	Anke et al. (1977)
<i>Cyphellopsis anomala</i>	strobilurins D, F	Weber et al. (1990b)
<i>Favolaschia pustulosa</i>	9-methoxystrobilurin, oudemansin L	Wood et al. (1996)
<i>Hyphodontia</i> sp.	hyphodontal	Erkel et al. (1994)
<i>Lentinellus cochleatus</i> (Pers.: Fr./Karst.)	lactaran	Wunder et al. (1996)
<i>Marasmius scorodoni</i> (Fr./Fr.)	scorodonin	Anke et al. (1980)
<i>Marasmius alliaceus</i> (Jacq.: Fr./Fr.)	alliaccols A, B	Anke et al. (1981)
<i>Merulius tremellosus</i> Fr.	merulidial	Quack et al. (1978); Gianetti et al. (1986)
<i>Oudemansiella mucida</i> (Schrad.: Fr./Hoehnel)	mucidin, oudemansin	Musilek et al. (1969); Anke et al. (1979)
<i>Oudemansiella radicata</i> (Relhan.: Fr.)	oudemansin X	Anke et al. (1990)
<i>Pisolithus arhizus</i> (Scop. Pers./Rauschert)	pisolithins A, B	Kope et al. (1991)
<i>Pleurotus griseus</i>	pleuromutilin	Kohlmünzer (1992); Molitoris (1994)
<i>Psathyrella subatrata</i>	tamulin	Kohlmünzer (1992)
<i>Punctularia atropurpurascens</i>	phlebiacauranol aldehyde	Anke et al. (1987)
<i>Strobilurus tenacellus</i> (Fr./Sing)	strobilurin	Anke et al. (1977)
<i>Xerula</i> sp.	strobilurin C, oudemansin B	Anke et al. (1983)

Fig. 1. Chemical structures of metabolites of higher fungi-derivatives of β -methoxyacrylic acid.

min (SPOFA). From *Strobilurus tenacellus* (Pers.: Fr./Sing.) derivatives of mucidin – strobilurin A and B were isolated (Anke et al. 1977). The structure of strobilurin A was determined and described 2 years later (Anke et al. 1979; Nadrchalova et al. 1979; Sedmera et al. 1981). Further derivatives: strobilurin C was isolated from *Xerula* species (Anke et al. 1983), strobilurin E from *Crepidotus fulvotomentosus* Peck

Fig. 2. Diterpenoids metabolites of higher fungi – tamulin ($\text{R}=\text{COCH}_2\text{SCH}_2\text{CH}_2\text{NEt}_2$) – pleuromutilin ($\text{R}=\text{COCH}_2\text{OH}$).

(Weber et al. 1990a), strobilurin D and F from *Cyphellopsis anomala* (Weber et al. 1990b) and strobilurin M from tropical basidiomycete *Mycena* sp. (Daferner et al. 1998). Oudemasin A was isolated from *Oudemansiella mucida* (Schrader: Fr./Hoehnel) (Anke et al. 1979) and *Oudemansiella radicata* (Relh.: Fr./Sing.) (Anke et al. 1990). Its structure was described in 1979 (Anke et al. 1979). The antibiotic exhibits strong antifungal properties and inhibits respiration in fungi, cells of the ascitic form of Ehrlich carcinoma and rat liver mitochondria. Oudemasin B was isolated from *Xerula* species (Anke et al. 1983), oudemasin X from *Oudemansiella radicata* (Anke et al. 1990) and oudemansin L from *Favolaschia pustulosa* (Jung./Sing.) (Wood et al. 1996). Oudemansin L showed antifungal activity against *Candida albicans* (Minimal Inhibitory Concentration MIC = 6 μM). Strobilurin C and oudemansin B isolated from cultures of *Xerula longipes* and *Xerula melanotricha* (Anke et al. 1983) inhibit the growth of a wide variety of saprophytic and phytopathogenic fungi.

Like strobilurins A, B, and oudemasin A they were potent inhibitors of respiration. The mechanism of antifungal activity of derivatives of β -methoxyacrylic acid is concerned with binding to a specific site on cytochrome b and inhibiting mitochondrial respiration (Clough 1993). Oudemansin A and strobilurin A are inhibitors mitochondrial cytochrome c reductase (Brandt et al. 1988, Kraiczky et al. 1996). Volatile substances from *Marasmius alliaceus* (Jacq.: Fr.) from the family Tricholomataceae, totally inhibited growth of *Aspergillus fumigatus*, *Penicillium chrysogenum* as well as spore formation in *Trichoderma lignorum*. They significantly limited growth of pathogenic for humans fungi such as *Candida albicans*, *Rhodotorula rubra* and fitopathogenic fungi *Fusarium culmorum*, *Trichoderma lignorum*, *Aspergillus gypsophilae* and *Thielaviopsis* sp. (Lamer-Zarawska et al. 1988). Earlier Anke et al. (1981) isolated from this fungus alliacols A and B showing antifungal, antibacterial activity and strongly inhibiting DNA synthesis in cells of the ascitic form of Ehrlich carcinoma. Both alliacols A and B react with cysteine to form adducts with strongly reduced biological activities. Growth of the pathogenic fungus *Candida albicans* was also inhibited by extracts from *Piptoporus betulinus* (Kohlmunzer et al. 1983). From the belonging to the same family *Marasmius scorodonius* (Fr./Fr.) (Anke et al. 1980) was isolated another antifungal metabolite – scorodonin. The antibiotic inhibits the growth of bacteria, yeasts, and filamentous fungi. Scorodonin in cells of the ascitic form of Ehrlich carcinoma the incorporation of thymidine and uridine into DNA and RNA strongly inhibited. The more common fungal components include di- and sesquiterpenoids. Diterpenoids of high fungistatic activity – tamulin and pleuromutilin (Fig. 2) were isolated from *Psathyrella* genus and *Pleurotus griseus* (Kohlmunzer 1992).

Tiamulin inhibited ATPase (Marthinet et al. 1998). Three sesquiterpenoids showing activity against *Fusarium oxysporum*, *Mucor miehei*, *Nematospore coryli*, *Paecilomyces variotii*, *Rhodotorula glutinis* and *Saccharomyces cerevisiae* (MIC = 50–100 $\mu\text{g}/\text{cm}^3$), were isolated from *Lentinellus cochleatus* (Pers.: Fr./Karst.) (Wunder et al. 1996) and named as deoxylactarorufin A, blennin A and blennin C (Fig. 3). There are potent inhibitors of leukotriene biosynthesis in rat basophilic leukemia (RBL-1) cells and human peripheral blood leukocytes (PBL). Other sesquiterpenoid – hyphodontal (Fig. 3) was isolated from fungus *Hyphodontia* sp. (family Corticiaceae) and showed activity against *Nematospore coryli*, *Saccharomyces cerevisiae* and *Nadsonia fulvescens* (Erkel et al. 1994). Hyphodontal strongly inhibits the growth of several yeasts and is a non-competitive inhibitor of avian myeloblas-

Fig. 3 Chemical structures of sesquiterpenoids isolated from higher fungi.

tosis virus and Moloney murine leukemia virus reverse transcriptases. Antifungal activity was also found by sesquiterpenoids isolated from other fungi (Gianetti et al. 1986; Anke et al. 1989; Lauer et al. 1989). Three metabolites from *Cyathus striatus* (Huds. Pers./Willd.) – striatins A, B and C (Fig. 4) showed high activity against microfungi (fungi imperfecti) (Anke et al. 1977). From fungus *Pisolithus arhizus* two antifungal compounds: p-hydroxybenzoylformic and /R/-/p-hydroxymandelic acids were obtained (Kope et al. 1991).

From *Caloporus dichrous* (Fr./Ryv.) a derivative of salicylic acid – caloporoside was isolated (Weber et al. 1994). Caloporoside and caloporoside analogs are inhibitors of phospholipase c (Weber et al. 1994; Tatsuta et al. 1996). Salicylic acid derivatives such as merulic acid (isolated from *Merulius tremellosus* and *Phlebia radicata*) can have influence on mammals brain cells and neuron transmitters of GABA type (similarly like muscimol *Amanita muscaria*) Pujol et al. established antifungal activity in extracts from fruit bodies cultures of other common fungi like *Lycoperdon perlatum* (Pers./Pers.) and *Oudemansiella platyphylla* (Pers.: Fr./Mos.) (Pujol et al. 1990). Extracts from these fungi showed activity against human pathogens *Candida albicans*, *Candida tropicalis* and *Aspergillus fumigatus*. Extracts from *Pholiota spumosa* (Fr./Singer.) as well as *Lycoperdon perlatum* showed activity against *Botrytis cinerea*. *Lycoperdon perlatum* contained also growth inhibitors of such fitopathogens as *Alternaria solani* and *Verticillium dahliae*.

Fig. 4 Chemical structures of striatins A, B and C isolated from higher fungi.

There number of substances isolated from fungi showing antifungal activity is much greater, but due to their great toxicity or significant instability they are not taken into consideration (Sonnenbichler et al. 1994).

The discussed active substances belong to different chemical groups and except for tamulin they do not contain nitrogen. They exhibit activity against pathogens attacking human, animal and vegetable organisms.

The presented overview indicates that there exist great possibilities in nature which could be used as alternatives to chemical fungicides in both medicine and agrotechnics.

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AKTYWNOŚĆ PRZECIWGRZYBICZA NIEKTÓRYCH METABOLITÓW GRZYBÓW WYŻSZYCH (BASIDIOMYCETES) – PRZEGLĄD BADAŃ

STRESZCZENIE

Z owocników grzybów wyższych (Basidiomycetes) zostało wyodrębnionych szereg związków o różnicowanych strukturach chemicznych, wykazujących aktywność przeciwgrzybiczą. Wśród mikroflory względem której badane metabolity wykazywały skuteczne działanie znajdują się zarówno mikroorganizmy chorobotwórcze dla ludzi jak i zwierząt: *Candida albicans*, *Candida tropicalis*, *Rhodotorula rubra*, *Aspergillus funigatus* i patogeny atakujące rośliny: *Penicillium chrysogenum*, *Botrytis cinerea*, *Alternaria solani*, *Fusarium culmorum*, *Trichoderma lignorum* czy *Verticillium dahliae*.

Dokonany przegląd dowodzi celowości dalszych badań w tym zakresie.

Poszukiwanie metabolitów grzybowych działających przeciwgrzybiczo stwarza potencjalne możliwości wykorzystania ich zarówno w medycynie klinicznej, ochronie środowiska jak i agrotechnice.

SŁOWA KLUCZOWE: Basidiomycetes, aktywność przeciwgrzybicza, przeciwgrzybicze metabolity – metabolizm.