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Colloquium "Fungi as Model Organisms in Research and Biotechnology", Olomouc September 14th, 1999

The colloquium was organized by the joint Commission for Experimental Mycology of the Czechoslovak Microbiological Society and the Czech Scientific Society for Mycology. The purpose of the colloquium was to provide a platform for a broad discussion on the use of fungi as model organisms in both basic and applied research. The programme of the colloquium was divided into four parts dealing with the following topics: phytopathology, biotechnology and ecology, physiology and biochemistry, and medical mycology. Each topic was opened with a plenary lecture (30 min.), followed by short communications (10 min.) and accompanied by poster presentations. Besides four plenary lectures, 15 short communications and 21 posters were presented. In total 38 researchers took part in the colloquium, who discussed various questions of importance for experimental mycology until late in the evening. Abstracts of the contributions are given below.

Jiří Kunert and Václav Šašek

Structure and variability of plant pathosystems

Struktura a variabilita rostlinného patosystému

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Biological diversity (biodiversity) is defined as the total variability within all living organisms and the ecological complexes they inhabit. The diversity displayed among plant pathogens and their host plants is very large. More than 250,000 recently known vascular plants could be considered as potential hosts of pathogens (especially parasitic fungi). However, according to current estimates about 8.000 species of fungi are parasitic, i.e. cca 5% of known fungi (Allen et al. 1999). A plant pathosystem represents any subsystem of an ecosystem which involves parasitism or pathogenism. On the basis of origin there are two pathosystems, natural (wild pathosystem) and artificial (crop pathosystem). In general, these terms may be used at any system level (e.g. ecosystem, population, individual, tissue, physiological, molecular). Analysis of the pathosystem could be defined as the study of pathosystem structure and behaviour at all systems levels. Within the pathosystem, the host-pathogen interactions are governed by the laws applicable to different systems levels (Zadoks and Schein 1979). Both defined pathosystems must be considered as dynamic systems, however, with various level of stability.

As an example, in this paper some known and questionable features related to the pathosystem *Lactuca* spp. (lettuce) – *Bremia lactucae* (lettuce downy mildew) are summarized.

A wild plant pathosystem is autonomous. The control of this pathosystem is primarily based on communication between the three basic components: the host, the pathogen and the environment. Current knowledge of the structure and variation of this pathosystem is still limited (Burdon 1993). There is only rare information on the structure, variability and function of these interactions between naturally growing wild *Lactuca* spp. and *Bremia lactucae*. Some knowledge is available on host resistance and parasite virulence variation, resistance mechanisms and their genetics (Lebeda et al. 1999).

A crop pathosystem is more or less deterministic and has a fourth component which represents man – a highly influential factor in this system because under his control are: individual plants or plant populations (cultivars), the cultivation system and the control of pathogen populations. The stability of the crop pathosystem is extremely low because of the speed of co-evolutionary changes (Simms 1996). The interactions on various levels of biological hierarchy are known in more detail and illustrated by the host-parasite interaction *Lactuca sativa* – *Bremia lactucae*.

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Dynamics of enzymes during pathogenesis of Fusarium oxysporum on pea seedlings

Dynamika enzymů během patogeneze Fusarium oxysporum na rostlinách hrachu

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Plants have formed various defense mechanisms during their co-evolution with pathogenic fungi. Enzymes as biocatalyzators participate in all reactions of living organisms. Enzymes involved in plant defense responses have a direct antimicrobial activity or participate in synthesis of defense substances. Only a limited number of enzymes have been studied in relation to the infection process (Lebeda et al. 1999a). Thanks to an investigation of enzymes enhancing their activity during pathogenesis, new defense mechanisms have been found and utilized for practical purposes, e.g. as a markers in resistance breeding (Lebeda et al. 1999b).

In the host-pathogen interaction *Pisum sativum* - *Fusarium oxysporum* we have focused mainly on the role of enzyme metabolisms (peroxidase, catalase and superoxide dismutase) included in the active oxygen metabolism. Especially peroxidases, which occur in many isoforms in cells and extracellular matrix, are important for plant defense (Buonario and Montalbini 1993). They were found responsible for the process of lignification in healthy plants (Vidhyasekaran 1997) and probably play the same role in defense lignification of peas. According to our results the rhizodermis cells in infected pea roots show a high degree of lignification. Diamine oxidase and proteases have an important function in plant protein degradation. Diamine oxidase catalyze the oxidative deamination of biogenic amines and produce also an important secondary product, hydrogen peroxide (Peč and Frébort 1990). It is evident that hydrogen peroxide serves as a substrate for peroxidases in infected pea plants (Jančová et al., unpubl. results). It was found that diamine oxidase is also important in biochemical pathways including defense lignification and synthesis of phenolic compounds.

Our recent research is focused on a detailed comparative study of enzyme dynamics in susceptible and resistant genotypes of peas. However, practical use is also stressed on using of these data for the detection of efficient biochemical markers for disease resistance breeding.

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Suitable immunochemical methods for detection of Fusarium sp. in winter wheat

Vybrané imunochemické metody pro identifikaci Fusarium sp. v ozimé pšenici

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Fusarium culmorum is the dominant pathogen of winter wheat heads in the Czech Republic. The detections of pathogens were evaluated with PTA-ELISA, immunoprinting, dot blot and immunofluorescence, and realized in vitro, in artificially infected swelled grains and in seedlings. Inoculum was injected into swelled grains using syringe. Monoclonal and polyclonal antibodies were used in all methods. Antibodies were prepared and tested in the laboratories of RICP, Prague, Czech Republic. Specifity of antibodies was established by the reactions with antigens from other species of *Fusarium* (*oxysporum, graminearum, solani, avenaceum, tricinctum, moniliforme*) and with antigens from other pathogens of winter wheat (*Pseudocercosporella herpotrichoides, Gaeumannomyces graminis, Rhizoctonia solani*). The antibodies (mono- and polyclonal) were genus specific and did not cross-react with other fungal pathogens of winter wheat.

The tissues of host or mycelial mats were extracted in PBS pH 7,2 for ELISA and dot blot. A nitrocellulose membrane was used for immunoprinting and dot blot. Fluorescein was conjugated with special antibodies for immunofluorescence. The optimal method for the detection of pathogens in host tissues was dot blot. ELISA and immunoprinting were less suitable. The antibodies (mono- and polyclonal) were similarly specific and sensitive.

Relation between field resistance to the powdery mildew of cucurbits, development stage and plant habit of Cucurbita pepo L.

Vztah mezi polní odolností k padlí tykvovitých, vývojovým stadiem a habitem rostlin Cucurbita pepo L.

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The growth and development of plants are associated with changes on various levels of the plant organism, e.g. tissues, cells, intracellular structures, enzymes and hormones. These processes are linked very often with changes of reaction to pathogens. Changes of resistance to the powdery mildew of cucurbits during plant development have been studied on cucumber (*Cucumis sativus* L.) (Zijlstra and Groot 1992). The current study is aimed at a similar phenomenon in squash (*Cucurbita pepo* L.).

A set of 170 accessions of *Cucurbita pepo* L. genetic resources (PI introductions) was evaluated for the resistance to the powdery mildew of cucurbits (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*) under conditions of natural field infection. Plant genotypes were represented by local varieties and land races (zucchini, vegetable marrow and pumpkin morphotypes) with a combination of resistance genes and plant habit (i.e. bush and/or vine stem) resulting from a long-lasting natural evolutionary process. During vegetative growth, the infection degree was assessed four times by evaluating leaf surface covered by mycelia of powdery mildew. Disease infection progress on each accession was expressed as the Area below curve (ABC) and the resulting data were processed statistically.

The whole set of 170 *C. pepo* accessions was highly susceptible to the infection of powdery mildew (ABC = 3070.5), while two control cultivars (scallop morphotypes) were highly tolerant (ABC = 978.1). These results support the previously obtained information that the*C. pepo* morphotypes zucchini, vegetable marrow and pumpkin are highly susceptible to powdery mildew (Lebeda and Křístková 1994). Accessions with vine plant habit were more resistant to powdery mildew than bush types. Similarly, accessions with late female flowering were more resistant as compared to accessions with early pistillate flowering. Different plant habit, i.e. vine and/or bush type of stem and a beginning of female flowering existing among *Cucurbita pepo* genotypes reflect key physiological differences which could be connected with their resistance to pathogens. The ontogenetic stage of plants could thus influence their resistance to pathogens. It is probable that several genes are involved in conferring *C. pepo* resistance and that they are activated or suppressed during plant growth and development. These results could

confirm the hypothesis of Leibovich et al. (1996) that the resistance of *C. pepo* to powdery mildew is encoded oligogenically and becomes more susceptible to the infection during development the plants.

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Variability of interactions between morphotypes of squash (Cucurbita pepo L.) and two groups of obligate biotrophs – cucumber downy mildew (Pseudoperonospora cubensis) and powdery mildew of cucurbits (Sphaerotheca fuliginea, Erysiphe cichoracearum)

Variabilita interakcí morfotypů tykve obecné (Cucurbita pepo L.) a dvou skupin obligátních biotrofů – plísní okurkové (Pseudoperonospora cubensis) a padlí tykvovitých (Sphaerotheca fuliginea, Erysiphe cichoracearum)

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Cucumber downy mildew (*Pseudoperonospora cubensis* (Berk. et Curt.) Rostow) and powdery mildew of cucurbits (*Erysiphe cichoracearum* DC.: Fr. and *Sphaerotheca fuliginea* (Schlecht.: Fr.) Pollaci) are the most important foliar fungal pathogens of cucurbitaceous vegetables. Both microfungi are obligate biotrophs but they belong to different groups of fungi – Oomycota and Ascomycotina – with different phylogeny, metabolism and ecological requirements (Hawksworth, 1995). Squash (*Cucurbita pepo* L., family Cucurbitaceae) shows a broad variability of fruit shapes which corresponds with their place and way of development. The aim of this study was to compare the interaction of a group of morphotypes of *Cucurbita pepo* with both pathogens on the level of individual genotypes.

Eight commercial varieties of *C. pepo* representing all groups of morphotypes were evaluated for their resistance to downy and powdery mildew after artificial inoculation in a cultivation chamber. The infection degree of the downy mildew on the leaf discs was measured visually, the yield of conidia of powdery mildew from a leaf unit was counted in a hemocytometer. Data were processed statistically.

The morphotypes zucchini, cocozelle and vegetable marrow were highly resistant to the downy mildew, the morphotypes acorn, scallop, crookneck and straightneck were very susceptible. On the contrary, the morphotypes cocozelle, zucchini and pumpkin were susceptible to the powdery mildew and the morphotypes acorn and scallop showed a high level of resistance. These results support the previously obtained information about the negative correlation between the reaction to both pathogens on hundreds of accessions of different morphotypes of *C. pepo* (Lebeda and Křístková 1993, 1996). It is very probable that the interaction of *C. pepo* morphotypes with downy and powdery mildews is influenced by the place and way of their evolution. In contrast with some other cucurbitaceous vegetables (cucumbers, melons) (Munger 1996) the genes encoding resistance to both pathogens are probably not linked in *Cucurbita pepo*.

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The resistance of Venturia inaequalis to fungicides

Rezistence Venturia inaequalis vůči fungicidům

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Venturia inaequalis (Cooke) Winter (anamorph *Spilocaea pomi* Fr.) is a bitunicate Ascomycete species, which is provisionally placed in the order Dothideales Lindau, family Venturiaceae E. Müll. et Arx.

Venturia inaequalis only attacks representatives from the *Rosaceae* family, especially the genus *Malus* Mill.. This pathogen causes the economically most important disease of produced cultivars of *Malus domestica* Borkh. and *M. sylvestris* Mill. It can attack the wild and ornamental apples *M. baccata* Borkh., *M. dasyphylla* Borkh., *M. purpurea* (Barbier) Rehder and *M. floribunda* van Houtte too.

One hundred and twenty-six monoconidial strains of *Venturia inaequalis* were collected in 1995 and 1996 from orchards either treated or not treated with different

fungicides. Ninety strains of *V. inaequalis* were collected from commercial orchards in Horoměřice. The remaining 36 strains were obtained from the experimental orchard of RICP Ruzyně, that had been sporadically treated with DMI fungicides (Topas 1996), and from a private garden that had never been treated with fungicides.

All strains were tested to flusilazole and benomyl. Most of isolates, except three resistant ones, were highly sensitive to benomyl at a concentration of 0.1 μg/ml. For flusilazole, strains collected from treated orchards showed 2–3 fold increase in frequency in the category of 20 to 50% inhibition of growth. Resistant strains with an ED_{50} – value up to 1.668 μg/ml were obtained only from treated orchards. For both fungicides, the level of strain sensitivity did not depend on the cultivars the strains were collected from.

Fifteen selected strains were tested to fenarimol, dodine and difenoconazole. A broad spectrum of strain sensitivity was found for dodine and difenoconazole. The ED_{50} – value for difenoconazole fluctuated from 0.0023 μg/ml to 0.42 μg/ml with a resistance factor of 88.

The ED_{50} – value for dodine varied between 0.005 μg/ml and 23.2 μg/ml, with an excessive resistance factor of 9,283.

For fenarimol, only one strain was found to be partly sensitive. The remaining 14 strains were resistant with ED_{50} – values from 0.46 μg/ml to 26.37 μg/ml (resistance factor 659). The calculated resistance factor markedly exceeded the value of 100, meaning a high level of resistance. Absence of sensitive strains was clearly demonstrated by the variability factor, which was approximately 10 times lower than the resistance factor.

Co-evolution of host-parasite interactions: Case study Lactuca sativa – Bremia lactucae

Koevoluce interakce hostitel-parazit: příčinná studie Lactuca sativa – Bremia lactucae

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Co-evolution, i.e. reciprocal evolution in interacting species, has become the major paradigm under which a great deal of research is carried out on the evolution of interactions between plants and pathogens. These interactions represent a very dynamic and flexible system. In a host-parasite interaction that follows gene-for-gene relationships, the expression of resistance or susceptibility to a particular pathogen is conditional on the pathogen genotype, and the degree of pathogen

virulence is conditional on the host genotype (Crute and Pink 1996). The level of genetic variance in resistance and virulence can strongly influence population dynamics and equilibrium of the interacting species. Understanding the co-evolution of hosts and parasites is crucial to understanding their epidemiology and ecology (Simms 1996). The use of resistant cultivars often results in genetic changes in parasite populations. During the last decade there has been a growing appreciation of the importance of understanding the processes which bring about these changes, which is essential for the development of effective disease control strategies. Most of these studies were focused on powdery mildews and rusts. However, a limited number of these studies is concentrated on peronosporaceous fungi (Lebeda and Schwinn 1999).

Probably the best known system (in peronosporaceous fungi) from the viewpoint of co-evolution is represented by the interaction *Lactuca sativa – Bremia lactucae*. The microevolutionary shift on the level of host genotypes and pathogen races was well documented and summarized by Lebeda and Schwinn (1994). From the history of lettuce breeding and occurrence of new *Bremia lactucae* races, interesting data were derived. It was demonstrated that the durability of resistance of newly introduced lettuce cultivars with race-specific resistance was rather short. Recent more detailed population genetic studies showed (Lebeda and Zinkernagel 1998) that the *Lactuca sativa – Bremia lactucae* system is enormously changable. On the example of a German host-parasite population it was demonstrated that changes in the frequency of R-genes in the host population were followed by a substantial shift of v-factors in the pathogen population. Occurrence of new v-factors (e.g. v18), considerable increase of some v-factors (e.g. v3, v7, v11, v16), and a complexity of v-phenotypes in the pathogen population was recorded. The recent data on changes of virulence structure very well exemplified the process of host-pathogen co-evolution, however, also the enormous instability of race-specific R-genes (Lebeda and Zinkernagel 1999).

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Comparative study of Oidium lycopersici development on wild representatives of Lycopersicon spp.

Srovnávací studium vývoje Oidium lycopersici na planých zástupcích rodu Lycopersicon

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Oidium lycopersici (Cooke et Massee, 1888, emend. Nordeloos et Loerakker, 1989) is an obligate biotroph of Erysiphaceae, which causes serious damage mainly to glasshouse tomatoes. Although nearly all recent tomato cultivars are highly susceptible, valuable sources of resistance to this pathogen were found only among wild *Lycopersicon* species (Mieslerová and Lebeda 1999).

Totally seven more or less resistant accessions of wild *Lycopersicon* spp. and two newly released oidium resistant tomato lines (OR 4061, OR 960008 /Rijk Zwaan/) were used for a detailed histological study. The obtained data were compared with the results of a parallel experiment in a glasshouse. *L. esculentum* cv. Amateur served as a susceptible control. The Czech isolate (C-2) of *Oidium lycopersici* was used for inoculation. The methodology of microscopic evaluation was described by Lebeda and Mieslerová (1999). Leaf discs of tested plants were artificially inoculated with *O. lycopersici* (isolate C-2). In various intervals after inoculation (6 h, 24 h, 48 h and 72 h) the following data were recorded: % of germinated conidia, number and length of germ tubes, formation of papillae, hypersensitive response and after 9 days conidiophore production. The same set of accessions (5 plants per genotype) was placed in a glasshouse together with artificially inoculated plants (= spread rows) serving as a source of inoculum. In two-week intervals the infection degree (% max ID) was assessed based on powdery mildew occurrence and sporulation on leaves. The dynamics of the infection process was quantified as area below curve (ABC) based on ID.

Comparison of *O. lycopersici* development revealed reduced germ tube growth on resistant and moderately resistant accessions. Hypersensitive response was detected to a large extent in highly resistant and moderately resistant accessions of wild *Lycopersicon* species and in oidium resistant tomato lines. The highly resistant *L. hirsutum* (LA 1347) showed a different response not based on hypersensitivity.

In glasshouse experiments the responses of the studied accessions were slightly different. Only moderately susceptible and highly susceptible accessions showed some symptoms of disease development under infection pressure. Even if the highly susceptible control was completely destroyed, highly resistant accessions such as *L. hirsutum* (LA 1347, LA 1738), *L. hirsutum* f. *glabratum* (LA 2128) and the moderately resistant *L. chmielewskii* (LA 2663) and *L. parviflorum* (LA

1322) were without disease symptoms. Newly released oidium resistant tomato lines showed a slower rate of disease development (ABC = 2685 for OR 96 0008, resp. ABC = 1328 for OR 4061) in comparison with the highly susceptible control (ABC = 5919).

Regression analysis between ABC and length of *Oidium lycopersici* germ tubes 48 hpi of all tested accessions revealed a non-linear relationship. The linear model explained only 35.8% of variability in ABC. For example, in *L. parviflorum* (LA 1322) a relatively high rate of *O. lycopersici* germ tube development was detected in contrast with no symptoms of disease found in conditions of natural infection. Thus, glasshouse experiments could reveal other sources of resistance (field resistance).

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Microtubular arrangement in epidermal cells of lettuce and wild Lactuca spp. during infection process of Bremia lactucae

Uspořádání mikrotubulů v epidermálních buňkách salátu a planých druhů locik během infekce Bremia lactucae

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Cytological studies in several pathosystems have revealed the connection between cytoskeleton and plant cell response to pathogen attack, hypersensitive cell death included. During the infection process the cytoskeletal network of host cells undergoes dramatic reorganization, which extent differs in various plant-pathogen interactions (Škalamera and Heath 1998). The interaction between *Lactuca sativa*, the closely related *L. serriola* and *Bremia lactucae* confirms a "gene-for-gene" relationship, i.e. race-specificity. A similar reaction pattern and resistance mechanism was recognized also in *Lactuca saligna* and *L. virosa* (Lebeda and Pink 1998). The experimental set of nine genotypes of *Lactuca* spp. (three of *L. sativa*, two of *L. serriola*, *L. saligna* and *L. virosa*) used in this study represented various types of resistance reaction to *Bremia lactucae* isolate NL16.

Early stages of cell pathogenesis and alignment of microtubules (MTs) in epidermal cells of *Lactuca* spp. susceptible genotypes and those with different mechanisms of resistance were investigated 24 and 48 h after inoculation by *Bremia lactucae*. Visualization of MTs was based on immunofluorescence detection of α-tubulin (Sedlářová et al. 1999).

In uninfected cells the cortical array of MTs was organized into a net in transverse orientation with respect to the long axis. In infected and adjacent (*Lactuca virosa*) epidermal cells special configurations of cortical MTs occurred. In epidermal cells of genotypes susceptible to race NL16 (*L. sativa* cv. Cobham Green and UCDM 2, *L. serriola* LSE/18) the pathogen developed quickly and induced formation of a microtubular "basket" surrounding primary and secondary vesicles. Hypersensitive reaction was rare with only one cell participating.

In cells of genotypes with different mechanisms of resistance the pathogen developed slower (esp. *L. saligna*) but caused rapid changes of the microtubular net already during penetration. Later a microtubular basket appeared just as in the cells of susceptible genotypes, with the exception of *L. virosa*, where HR occurred immediately after penetration. The expression of hypersensitivity varied between genotypes. Cv. Mariska (*L. sativa*) and PIVT 1309 (*L. serriola*) were characterized by a prompt elimination of *Bremia lactucae* with aggregation of cytoplasm, and clustering and depolymerization of MTs in infected cells. In *L. saligna* (CGN 05147, CGN 05271) large changes occurred later after the development of primary infection structures and led to microtubular depolymerization during cell death. *L. virosa* genotypes (esp. CGN 04683) exhibited a very fast response to infection. Thick microtubular cables incidental to penetration and MTs depolymerizing later during HR in infected and 1–2 adjacent cells were detected. A correlation of speed and extent of microtubular reorganization with development of pathogen infection structures and susceptibility/resistance mechanism of the genotype was proved.

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Biological treatment of winter wheat seed against phytopathogens transferable by seed and soil

Biologická ochrana osiva ozimnej pšenice proti fytopatogénom prenosných osivom a pôdou

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In 1997 and 1998 the effect of biopreparations in comparison with a chemical preparation for the protection of seed and young plants against phytopathogens transferable by seed, soil and crop residues was tested in field experiments at Borovce near Piešťany. The test comprised these variants: control – non-treated seed; seed treated with Ekovert biopreparation; Trichomil biopreparation applied to straw and the seed treated chemically with Vitavax 200 FF. The incidence of *Fusarium* spp. and *Pseudocercosporella herpotrichoides* was monitored in 32 and 75 DC phase in plant samples – 25 plants × 4 replications. Winter wheat was represented by the Ilona variety.

At both evaluation phases the highly significantly lowest number of infected plants was found in the variants to which biopreparations were applied in the form of Ekovert dressing (28 plants and 24 plants in 1997 and in 1998) and in the form of Trichomil spraying treatment for straw (1997–32, 1998–33 infected plants), compared with the control (1997–60, 1998–57 infected plants), and with chemical treatment with Vitavax 200 FF (1997–48, 1998–42 infected plants). In the variants with biopreparations a significantly higher yield was recorded in 1997 as compared to the control variants and chemical treatment with Vitavax 200 FF. In 1998 the yield was insignificantly higher in variants with biopreparations.

The results show that seed treatment against harmful phytopathogens can be realized by means of using antagonisms among fungi while natural microbial life in the soil remains well-preserved.

The effect of Basagran 600 on the phytotoxicity of Fusarium equiseti and Alternaria alternata isolated from pea seeds

Vliv Basagranu 600 na fytotoxicitu Fusarium equiseti a Alternaria alternata izolovaných z osiva hrachu

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Pesticides do not affect only target organisms. There are a lot of studies concerning this problem. The influence of the herbicide Basagran 600 (bentazon)

on the growth of the mycelium of the two fungus species *Fusarium equiseti* and *Alternaria alternata*, and the effect of their metabolites on germination and growth of pea (variety Komet) were investigated. The mycelium was cultivated on a potato liquid medium, then filtered off, dried and weighed.

Six types of filtrates were tested: (1) sterile water (control), (2) filtrate with fungus metabolites, (3) filtrate with Basagran 600 (0.5 %), (4) – (6) mixture of the filtrate containing fungus metabolites and a herbicide at given concentration (0.1, 0.5 and 1 %). Pea seeds were cultivated in Petri dishes or on sandy soil both treated with filtrates. Seed germination and length of stems and roots of pea seedlings were observed.

The herbicide Basagran did not affect mycelium growth at all. Solutions including Basagran inhibited germination and seedling growth. Metabolites of *Fusarium equiseti* had a similar effect as the herbicide. Filtrates containing both components caused a stronger inhibition of germination and growth. Filtrate including metabolites of *Alternaria alternata* had no effect. Metabolites in a mixture with Basagran increased germination and length of roots compared with the effect of the herbicide only.

We conclude that metabolites of *Fusarium equiseti* are much more toxic to pea seedlings than metabolites of *Alternaria alternata*. In a mixture with Basagran 600 the toxicity of metabolites even increased.

Leaf spot diseases of wheat – Pyrenophora tritici-repentis (Died.) Drechsler

Listové skvrnitosti na pšenici – Pyrenophora tritici-repentis (Died.) Drechsler

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Leaf spot diseases of wheat are caused by various pathogenic fungi. This study deals with *Pyrenophora tritici-repentis*, the causal agent of the wheat leaf spot disease known as tan spot. This disease occurs in all major wheat growing areas of the world. *Pyrenophora tritici-repentis* was also detected in the Czech Republic and its incidence has increased very significantly the last years. The fungus has probably expanded from western Europe to our country.

Tan spot on wheat can cause significant losses under suitable epidemic conditions. It is dependent on the stage of leaf damage and the loss of assimilation leaf area. Losses are manifested in total yield, kernel weight and number of grains per head.

Four factors are important for the development of the disease: presence and amount of primary inoculum, duration of leaf wetness after inoculation, stage of crop development at the time of infection and host genotype. The primary disease

cycle on wheat is initiated by ascospores, borne on infested plant residues. The secondary infection is spread by conidia, which can be transmitted very far by wind. Only a little amount of inoculum is enough for infection.

We studied the resistance of selected winter wheat cultivars to *Pyrenophora tritici-repentis* this year. The fungus was cultivated on sterile oat grains and this inoculum was applied on experimental field plots with different cultivars of winter wheat in November and in March. The first symptoms were detected at the end of April. Periodic assessment was pursued from June (every week). Diseased leaf area, total leaf area, stage of crop development and other features were observed. For the assessment of diseased leaf area the percentage scale was used.

The analysis of variance proved the differences between affected leaf area of selected winter wheat cultivars. The most resistant cultivars were Rialto, Estica, Astella, Alana, Vlasta, Arina, the most sensitive cultivar was Samanta.

Differences in the pathogenicity of Fusarium spp. on seeds and young of pea plants (Pisum sativum L.)

Rozdíly v patogenitě izolátů druhů rodu Fusarium na semenech a mladých rostlinách hrachu setého (Pisum sativum L.)

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Species of the genus *Fusarium* have mostly been studied from the viewpoint of their ability to cause diseases of many economically important crop plants (Haglund 1984). *Fusarium oxysporum* (Schlecht.) f. sp. *pisi* (Van Hall) Snyd. et Hans. and *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. et Hans. have been reported as the most common pathogens of pea (*Pisum sativum* L.). *F. oxysporum* f. sp. *pisi* is known to cause a vascular wilt, *F. solani* f. sp. *pisi* is an important component of the complex of soil-borne pathogenic fungi causing dry root rot in pea (Tu 1994).

The pathogenicity of five monosporic isolates of *Fusarium* spp. from soil (*F. oxysporum, F. solani*) and pea roots (*F. oxysporum* f. sp. *pisi, F. oxysporum* v. *redolens, F. solani* f. sp. *pisi*) was tested on seeds and young plants of three pea cultivars (Tyrkys, Radim, SG-L-70). Two modified screening methods (inoculation of germinating seeds in Petri dishes, inoculation of seedlings grown in pots) were used. The degree of infection on the upper part of plants and on roots was evaluated according to Lebeda and Buczkowski (1986).

Significant differences in the pathogenicity in seeds and young plants were found among the isolates. The highest degree of pathogenicity of *F. solani* f. sp. *pisi* and *F. solani* was confirmed by both methods. *F. oxysporum* v. *redolens* showed to be weekly pathogenic. There is no good correlation between pathogenicity on seeds and young plants. Germinating seeds could be considered as more susceptible in comparison with young plants. No significant variations were found among isolates of the same *Fusarium* spp. obtained from soil samples and from host roots.

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Application of white rot fungi in biotechnology

Použití hub bílé hniloby v biotechnologii

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White rot fungi is a group of basidiomycetes that under natural conditions grow on wood; removing predominantly its lignin moiety they cause so-called white rot of the wood. The ability to degrade both lignocelluloses and man-made aromatic xenobiotic pollutants has brought these fungi into the focus of biotechnological application.

Cultivation of selected lignin-degrading fungi has a long history. However, during the last decades, mushroom growing has become a sophisticated and economically important food production biotechnology. It is an excellent model of closed-circle recycling biotechnology – waste of one production process (straw, saw dust, manure etc.) becomes the resource for another technology (production of edible fungi), and its possible waste – the spent substrate after mushroom collection – can serve as soil fertilizer, cattle fodder, and in some cases also as biological material for soil remediation.

Intensive research has been carried out to utilize the lignin-degrading capability of white rot fungi in pulp and paper industry. It was found that fungal pretreatment of wood chips (biopulping) results in lower energy demand and higher

quality of cellulose. White rot fungi have been introduced into the bleaching of paper pulp, and good results have been obtained in decolorization and detoxification of waste water.

White rot fungi are prospective microrganisms for the clean-up of soil polluted with different aromatic xenobiotics (chlorophenols, PCBs, PAHs, synthetic dyes, nitroaromatics, different pesticides, etc.). For the selection of fungal strains usable in soil remediation, we used a multi- step process that included: screening for the activity of ligninolytic enzymes using decolorization of model synthetic dyes, evaluation of degrading capability in liquid media, artificially contaminated soil and soil samples originating from polluted sites, and finally, testing the ability to colonize soil to be remediated.

The study was supported by grant No. 526/99/0519 of the Grant Agency of the Czech Republic.

Oyster mushroom (Pleurotus ostreatus) – its occurence, composition, active substances, and some of its physiological properties

Hliva ústricovitá (Pleurotus ostreatus) – výskyt, obsahové a účinné látky a ich niektoré fyziologické vlastnosti

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The authors investigated the occurrence of oyster mushroom (*Pleurotus ostreatus*) under natural conditions and discussed the possibilities of large-scale artificial growing of this mushroom on various lignocellulose-based substrates (straw, maize leaves, cotton, etc.). They deal with some optimal growth factors such as temperature, air humidity and proper growing technology providing maximal fructification of oyster mushrooms. We further describe the determining characteristic traits as well as the botanical classification of this mushroom species. We investigated the hypocholesterolaemic activity of the substances contained in oyster mushroom along with the specific factors capable of interfering with the metabolism of cholesterol and other fatty substances. These biologically active substances, after their application to the organism, are clearly able to decrease the level of two main risk factors of artherosclerosis, namely the concentration of cholesterol in plasma and blood pressure. Besides oyster mushroom we also deal in this study with other fungi having a similar composition and effects, such as *Lentinus edodes*, honey fungus *(Armillaria mellea)* and *Ganoderma lucidum*. We characterised and defined, as to its chemical structure, the isolate Eritadenin

and its derivatives, and also substances with strong hypocholesterolaemic activity. Besides these active substances from higher fungi we also studied some isolates of lower fungi, such as *Penicillium citrinum* and *Monascus ruber*. These substances are known as Compaktin and Monacolin K. Using chemical modifications, substances of the Lovastatin type were prepared (Mevinolin, Mevacor) that were highly effective for the investigated features and showed no serious side-effects. In the study we also discuss two of the assumed possible mechanisms of the effect of lowering the cholesterol level in blood due to the activity of biologically active substances from higher and lower fungi. It is presumed that both mechanisms work in mutual connection and in a complex way.

Degradation of polycyclic aromatic hydrocarbons by white rot fungi in soil

Degradace polycyklických aromatických uhlovodíků v půdě houbami bílé hniloby

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Polycyclic aromatic hydrocarbons (PAHs) represent a group of serious pollutants of the environment. Bioremediation is an environment friendly and cost-effective way to remove these pollutants from soil. In practice, mostly bacteria have been used for the clean-up. Over last two decades, white rot fungi have been focused on for their ability to degrade, besides lignin macromolecules, also other aromatic compounds like PCBs, PAH, chlorophenols, synthetic dyes, nitroaromatics, different pesticides etc. However, most studies have been carried out using *Phanerochaete chrysosporium*, which has become a model organism for the studies of both lignin and organopollutant degradation.

The present study was directed at the search for a fungus capable to remove PAHs efficiently from contaminated soil. The capability to produce ligninolytic enzymes was pre-screened in a set of white rot fungal cultures using a dye-decolorizing method (Šašek et al., Czech. Mycol. 50:303, 1998). Further selection was carried out on the basis of PAH degradation efficiency in liquid media. Out of six fungal species (*Phanerochaete chrysosporium, Bjerkandera adusta, Trametes versicolor, Pleurotus ostreatus, Pleurotus tuber-regium* and *Irpex lacteus*), *I. lacteus, T. versicolor* and *P. tuber-regium* significantly removed phenanthrene, anthracene, fluoranthene and pyrene. These fungal species were tested for their potential to colonize both tyndallized and untreated soil. *I. lacteus* efficiently colonized both soils. This fungus was used also for the treatment of a soil artificially contaminated with phenanthrene, anthracene, fluoranthene and pyrene (each 50

ppm). After 12 weeks of incubation, compared to the heat-killed control, the removal rates of anthracene and pyrene were close to 90 %, in the case of phenanthrene and fluoranthene they were around 50 %. In the really contaminated soil (originating from a former gas station area), *I. lacteus* removed low molecular PAHs up to 50 % during 5 weeks of incubation.

The study was supported by grant No. 526/99/0519 of the Grant Agency of the Czech Republic.

Phanerochaete magnoliae – a promising white rot fungus for decolorization of synthetic dyes

Phanerochaete magnoliae – perspektivní houba bílé hniloby pro dekolorizaci syntetických barviv

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Decolorization of model synthetic dyes has been used for an estimation of the activity of ligninolytic enzymes in white rot fungi. The low specificity of lignin-degrading enzymes suggests their possible involvement in the degradation of organopollutant xenobiotics. For this reason we screened the decolorizing ability of a set of white rot fungi. *Phanerochaete magnoliae* demonstrated quite an efficient decolorization capability. Since synthetic dyes themselves represent one of the pollutants entering the environment mostly as textile industrial effluents, we studied the ability of *Phanerochaete magnoliae* to decolorize synthetic dyes belonging to different chemical groups.

The dyes included in this study were: Methyl Orange (azo dye), Congo Red (diazo dye), Remazol Brilliant Blue R (RBBR, anthraquinone dye), Bromophenol Blue (triphenyl methane dye), Fluorescein (heterocyclic dye), and an unknown dye (extracted from industry-polluted soil). A dye mixture was prepared using all the mentioned dyes.

Agar media (malt-extract-glucose and N-limited Kirk's medium) containing all the dyes (150 ppm) were completely decolorized within 4–6 days. A further study in liquid media was made under both submerged (shaken) and static conditions at two temperatures (26 and 35 °C). Under static conditions at both temperatures a substantial decolorization (60 – 100 %) of all the dyes was observed within 10 days; at 35 °C the decolorization was more efficient and faster. In the case of the dye mixture at 26 °C, the different dyes were decolorized to a lesser extent. However, under the submerged condition no decolorization was observed. This indicates the involvement of ligninolytic enzymes in the decolorization process,

since static culture conditions have been reported to be necessary for the activity of ligninolytic enzymes.

The study was supported by grant No. 526/99/0519 of the Grant Agency of the Czech Republic.

Testing petrification solutions on selected species of micromycetes

Testovanie petrifikačných roztokov na vybraných druhoch mikromycét

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Fungal contaminants of various sculptures, canvases and frames located in the exterior or interior belong to the ubiquitous terrestrial organisms. While micromycetes are considered as universal destruents of organic matter, some of them have specific requirements, and the presence of certain chemical components in the substrate influences their growth inhibition. The physiological properties of micromycetes are utilized in practice to prevent undesirable extension, in restoration as well as protection.

In the restoration of a wooden sculpture (St. Nicolas – 18th century) after the application of three petrification solutions we registered considerable decrease in the growth of micromycetes from ten (*Aspergillus ochraceus, A. versicolor, A. ustus, Aureobasidium* sp., *Cladosporium cladosporioides, Chrysosporium* sp., *Monilia* sp., *Paecilomyces* sp., *Penicillium expansum*, *Verticillium* sp.) to five species. The micromycetes *Penicillium chrysogenum, P. glabrum, Aspergillus versicolor, A. flavus* and *Cladosporium* sp. belonged to the resistant species to the solutions used for petrification. This was also confirmed in our tests of inhibitory effects of the solutions on mixed culture of micromycetes. The species *Aspergillus versicolor* is very expansive in colonization of objects situated in depositories (polychrome wooden sculptures, wooden sculptures, canvases, frames, air and walls) and is the most resistant to the petrification solutions.

The following solutions for petrification were tested: solakryl BT-55 (used in the process of solidification, to arrange the surface and as a sticker); cloves oil (used in the process of solidification, for conservation and material impregnation); bee wax in toluene (added as a fungicide to the restoratory chalk).

This work was supported by Grant Agency, VEGA, Grant B220-G11 and 2/5048/98

The CCBAS collection of basidiomycetes – its aims and use

Sbírka basidiomycetů CCBAS – účel a využití

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Culture collections have fundamental and growing importance not only for the preservation of the endangered gene pool, but also as a basic source of material for biotechnological processes, research and teaching. CCBAS (Culture Collection of Basidiomycetes) at the Institute of Microbiology AS CR is the only integral collection of hymenomycetes in the Czech Republic, the keeper of some endangered and rare species, the protector of patented strains. It is a basis for screening of strains possessing important metabolic activities and the supplier of cultures to different institutions in this and foreign countries. CCBAS is a member of the World Center for Culture Collections of Microorganisms listed under the number 558. The collection was founded in 1959, contains about 700 fungal strains belonging to 260 species and is continually extended. Most of the cultures belong to *Agaricales, Aphyllophorales* and *Gasterales*. The main method of the culture maintenance in CCBAS is cryopreservation in liquid nitrogen. This method seems to be the most reliable, safe and perspective for a long-term maintenance of most fungal species, especially those not amenable to freeze-drying. An electronic version of the CCBAS catalogue has been published. We have also created a home page on the Internet (http://www.biomed.cas.cz/ccbas/fungi. htm). Basic research concerning culture storage is an integral part of our work. Here is one example. A principal requirement for successful maintenance of production strains is the ability to retain their important properties (e.g. production of antibiotics, enzymes and other metabolites) unchanged. Fourteen strains of white-rot basidiomycetes belonging to eight species of two genera (*Inonotus* and *Pholiota*) were tested for their ability to keep the production of laccase, peroxidase and manganese-dependent peroxidase (enzymes involved in lignin biodegradation) after a short-time preservation in liquid nitrogen with different cryoprotectives (glycerol, dimethyl sulfoxide). No negative effect of cryopreservation or the used cryoprotective on the production of ligninolytic enzymes was found in the fungi tested.

Czech mycol. 52 (2), 2000

Micromycetes as a food resource for mites (Acari: Oribatida & Acaridia) – a survey

Mikromycety jako potrava pro roztoče (Acari: Oribatida a Acaridia). Přehled problematiky

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Micromycetes belong to the major food resource for oribatid and acarid mites in the soil and are also an important food resource for storage mites. This survey recapitulates observations on mites' fungal food preferences. Mites can utilise two fungal sources of nutrition: (1) polysaccharides in the hyphal cell walls, (2) storage polysaccharides, disaccharides and lipids. Digestive enzymes splitting these compounds are necessary for utilising fungal walls or their contents. Correlations between the enzyme production, microanatomy of the digestive tract and the ability to utilise fungal food resources are studied. Chitin and β-D-glucans are the main polysaccharides in fungal cell walls. Although the enzymes splitting these fungal polysaccharides occur in some mites, neither spores nor parts of the hyphae are digested. The hyphal walls are mechanically damaged during the ingestion, therefore an enzymatic degradation of hyphal walls is not necessary for releasing the fungal cell contents. The pieces of hyphae occur in the mesenteron. There is probably no correlation between hyphae digestion and presence or absence of chitinase. Thus, classification of feeding guilds based on this enzyme is discutable. The fungal storage polysaccharides and lipids are probably utilised by more species than fungal cell polysaccharides. The enzymes splitting storage compounds have not been studied intensively. The hyphae produce a lot of secondary metabolites, which strongly influence mites. The micromycetes preferred by mites in food preference tests need not be suitable for reproduction or individual growth of mites. The energetical contribution of micromycetes as food resources for mites is usually not examined due to methodical problems. Although the mites' food preferences are studied intensively under laboratory conditions, their interpretation as for natural conditions is difficult.

Can the genus Fusarium serve as a model organism of trichothecene production?

Může sloužit rod Fusarium jako modelový organismus produkce trichothecenů?

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Species of the genus *Fusarium* are mainly considered to be facultative necrotrophs. They are mostly organisms surviving in nature as saprotrophs which can adapt to parasitic life. We studied in particular barley where *Fusarium* caused systemic infection (the mycelium of the fungus was growing through the plants, not forming conidiophores). The genus *Fusarium* is a producer of different mycotoxins, e.g. zearalenon, culmomarasmin, trichothecene mycotoxins etc. Trichothecenes (scirpenes) cause primary toxic aleukia in man and animals and are potentially carcinogenic. Therefore we were interested in trichothecene mycotoxins, which we evaluated by gas chromatography.

We used the gas chromatograph HP-5890, serie II, which was equipped with the fused silica capillary column HP-35. In the grain and in fungal cultures the following mycotoxins were evaluated: DON (deoxynivalenol), NIV (nivalenol), DAS (diacetoxyscirpenol), T-2 toxin, HT-2 toxin and FUS-X toxin. Trichothecenes were evaluated separately in grain, malt agar with the mycelium, in macroconidia, and in the mycelium. We reached the following results, presented in the table.

Table: Trichothecene mycotoxins in *Fusarium poae* and *Fusarium culmorum* (the most frequent species of the genus *Fusarium* detected in the season 1997 — 1998). Barley — var. Rubín

Toxin (mg/kg)	NIV	DON	FUS-X	DAS	T-2	HT-2
F. poae myc.	0.058	–	0.913	–	–	–
F. poae macroconidia	0.026	–	–	0.769	–	–
F. poae grain	–	–	–	–	–	0.027
F. culm. myc.	0.058	0.696	–	–	–	–
F. culm. macroconidia	0.135	–	–	1.046	–	–
F. culm. grain	0.130	0.089	–	0.161	–	–

Detection limit: 2 mg/kg

These values represented the average of 20 measurings. It is clear from the table that the values of trichothecene mycotoxins in the examined samples were under the detection limit. It seems that the different variable and low values of these mycotoxins are not suitable for long-term model experiments. The main question for phytopathology is how these mycotoxins influence the pathogenic properties of parasitic fungi, as low amounts of trichothecenes are not accumulated in macroorganisms (vertebrates and man).

The effect of Lactobacillus acidophilus CH5 on the genus Kluyveromyces

Vliv Lactobacillus acidophilus CH5 na rod Kluyveromyces

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The ability of neutralized and heated supernatant containing acidocin CH5 (SAC), a bacteriocin produced by the strain *Lactobacillus acidophilus* CH5, to inhibit the growth of yeast strains of *Kluyveromyces marxianus* var. *lactis* LCC 255 and *Kluyveromyces marxianus* var. *marxianus* LCC 269 in a complex medium was investigated. In a second experiment the effect of antimicrobials (NaCl, $NaNO_3$ and $NaH_2PO_4 \times 12\ H_2O$) alone or together with SAC on the yeast growth was tested.

The inhibition of the growth of both yeast strains (*Kluyveromyces marxianus* var. *lactis* LCC 255 and *Kluyveromyces marxianus* var. *marxianus* LCC 269) with the smallest used concentration of 0.1 % v/v SAC was detected (8 % to 9 % inhibition). Up to a concentration 2 % v/v of SAC the sensitivity of both strains was practically the same. At higher concentrations the sensitivity of *Kluyveromyces marxianus* var. *lactis* LCC 255 to SAC was higher than that of *Kluyveromyces marxianus* var. *marxianus* LCC 269. At 5 % v/v of SAC the first strain was inhibited by 52 % while the second one by 36 %.

Yeast strains were sensitive to antimicrobials in the concentration range applied (NaCl in a concentration of 0.1–5.0 % w/v, $NaNO_3$ in a concentration of 0.001–0.03 % w/v and NaH_2PO_4 . 12 H_2O in a concentration of 0.1–2 % w/v). The inhibition effect was the same or slightly higher with both antimicrobials and SAC (3 % v/v) than with antimicrobials only.

This work was supported by the Czech Grant Agency (Grant No. 104/98/P058).

Interactions between lactic acid bacteria and moulds in a milk environment

Interakce bakterií mléčného kvašení a plísní v mléce

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The contamination of fermented dairy products such as fermented milk and cheeses with undesirable yeasts and moulds is a serious problem. Mould contamination is responsible for causing technological problems during production

and therewith causes substantial economical losses. Additionally, it may even constitute a health risk as the result of mycotoxin production. Many fungal genera including *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Geotrichum*, *Mucor*, *Rhizopus*, and *Fusarium* were found to be toxic to biological systems[1,2,3].

The antibacterial effects of lactic acid bacteria (LAB) have been well documented[4,5]. However, less has been published about their antifungal characteristics. The relative low number of studies on the antifungal properties of LAB thus emphasises the need for further research. Therefore, the main goal of this study was to screen LAB of different origins for their antifungal activity and to observe their interactions with moulds in a milk environment in view of their potential application in the dairy industry.

Regarding the experimental work done in the Department of Dairy and Fat Technology, 77 LAB strains of different origins were screened for their antifungal properties. The milk agar plate method appeared to be a suitable method for testing the interactions between LAB strains and moulds in a milk environment, simulating the real surfaces of fermented dairy products.

The strongest inhibition of fungal growth and spore production was exhibited by the following strains: *Lactobacillus reuteri* CCM 3625, *Lactobacillus rhamnosus* VT1, and *Lactobacillus rhamnosus* LBK7.

Both tested *Fusarium* sp. strains were more sensitive than *Penicillium* sp. and *Aspergillus* sp. to the inhibition effects of lactobacilli. The growth of *Fusarium* sp. DMF 0101 was totally suppressed by the most active lactobacilli, while only the inhibition of spore production was observed for *Penicillium* sp. and *Aspergillus* sp. strains.

The highest antifungal effect was found for lactobacilli actively growing in milk with a 0.5 % w/w yeast extract, but some effect (mainly for *Lactobacillus reuteri* CCM 3625) was demonstrated even after a 20 % v/v addition of cell-free MRS broth supernatants into the milk.

The addition of 0.5 % w/w N-sources (especially yeast and beef extracts) stimulated the antifungal effect of lactobacilli in milk.

This work was supported by the Czech Grant Agency (Grant No. 525/98/0652).

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Soil micromycetes – biosorbents of heavy metals

Pôdne mikromycéty – biosorbenty ťažkých kovov

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The results of a model experiment are presented the objective of which was to evaluate the ability of soil micromycetes to absorb heavy metals (Pb, Hg, Cd) from humic acid as natural substrate. Biosorption of the mentioned metals by micromycetes and their influence on micro- and macro-morphological mycelium changes was studied. The cosmopolitan species *Trichoderma viride* isolated from soil was selected as a model micromycete. Microscopic fungi as universal destruents of organic matter are considered part of terrestric and aquatic ecosystems as well as other natural systems where they participate in chemical elements mobility. The humic acids as the essential component of humic substances were obtained from turf using alcaline extraction. Under experimental conditions the metals under study – Pb, Hg, Cd – remained bound to the humic substances during purification and sterilization.

A certain part of the metals was transferred into various parts of the culture where biosorption took place by the species *Trichoderma viride*. The presence of the observed metal had a negative effect in the order Hg < Cd < Pb, which corresponds with the content of individual metals analytically determined in the mycelium of *Trichoderma viride* (23.97 ppm Hg; 0.49 ppm Cd and 0.28 ppm Pb). Due to the presence of Hg a very weak air mycelium was formed, the conidiophores were branching and sporulation considerably suppressed compared to the control. The weak air mycelium and sporulation was the result of a negative Cd effect compared to the control. Addition of Pb had a weak negative effect. The cover of airy mycelium was dense and sporulation at the same level compared to the control.

This work was supported by Grant Agency VEGA, Grant Nos. 1/6167/99 and GJG 1/4236/97.

Biochemical and physiological changes during photo-induced conidiation of Trichoderma viride

Biochemické a fyziologické zmeny počas fotoindukovanej konidiácie Trichoderma viride

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Fungi of the genus *Trichoderma* are important industrial organisms mostly used for the production of polysaccharide-degrading enzymes and antibiotics. Furthermore, they are increasingly being applied as biocontrol agents against various fungal plant diseases. An interesting property of *Trichoderma* spp. is that their sporulation can be induced by light and/or starvation. Among the primary biochemical and physiological changes following illumination of the mycelia of *Trichoderma* grown in the dark are the increase in the intracellular levels of ATP and cAMP, the increased rate of respiration, phosphorylation of sporulation-specific proteins, hyperpolarization and depolarization of plasma membrane and degradation of glycogen reserves. Exogenously added cAMP as well as compounds inhibiting cAMP-phosphodiesterase or drugs stimulating adenylyl cyclase can mimick the light effect to a certain extent and induce conidiation of the mycelia also in the dark.

Comparison of secreted aspartic proteinases of four pathogenic Candida spp.

Srovnání extracelulárních aspartylových proteas čtyř patogenních druhů rodu Candida

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Candida infections cause diseases ranging from superficial disorders to invasive, mostly fatal infections in a host with reduced immunity. The major human pathogen classified in the genus *Candida* is *Candida albicans*. However, other species of this genus have been recently shown to cause an increasing incidence of mycoses. Secreted aspartic proteinases (SAPs) produced by *Candida* are believed to act as one of the virulence factors. Most of the efforts of characterization of SAPs were focused on aspartic proteinases of *C. albicans*. Studies of proteinases secreted by other *Candida* spp. are less frequent.

To examine and compare SAPs of different *Candida* spp., we collected a series of isolates of *Candida* spp. from Czech patients. The strains identified as *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. famata* were cultivated and proteinases secreted into the medium were tested for proteolytic activity with hemoglobin or peptide substrates. Cultivation conditions of *Candida* spp. were optimized and proteinases isolated using ion exchange chromatography. The stability and pH optima of the SAPs were tested. In order to design subnanomolar inhibitors of secreted aspartic proteinases of different pathogenic *Candida* spp., we designed, synthetized and tested in vitro a series of inhibitors varying in the type of scissile bond replacement as well as N- and C-terminal modification. We found inhibitors with K_i in the range of 10^{-9}–10^{-10} M for all the proteinases tested here. We also prepared an inhibitor containing a cyclohexylstatine residue with K_i differing by several orders of magnitude for individual SAPs. We further tested HIV proteinase inhibitors used clinically for inhibitory activity of the proteinases tested here.

Submerged preparation of sodium gluconate by a strain of the fungus Aspergillus foetidus var. pallidus

Submerzní příprava glukonátu sodného pomocí plísně Aspergillus foetidus var. pallidus

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An industrial strain of the filamentous fungus *Aspergillus foetidus* var. *pallidus*, which has a 6 to 8 -fold higher glucose oxidase activity as compared with the original strain, was improved and patented.

By submerged cultivation on synthetic glucose media, with a continuous regulation of pH by means of sodium hydroxide, glucose was nearly completely oxidatively converted into sodium gluconate. The improved strain was capable of growing also on highly concentrated substrates.

Further experiments showed, that this strain contains, in addition to the high glucose oxidase level, also a highly active invertase (β-fructofuranosidase) in spite of not being able to ultilize fructose. In fermentations on sucrose substrates, sucrose was first inverted and the resulting glucose was almost immediately converted to sodium gluconate. After depletion of glucose from the medium, the supernatant contained practically only fructose and sodium gluconate, the latter very simply isolated from the supernatant by using polar solvents.

Fermentations on highly concentrated sucrose substrates were monitored with liquid chromatography. The results showed that in certain stages of the fermentation the medium contains besides sucrose, glucose, sodium gluconate and fructose also trisaccharides and tetrasaccharides, whose level then gradually decreases so that only trace amounts remain in the medium by the end of the fermentation.

Induction of Candida albicans extracellular proteinase production by oligopeptides

Indukce tvorby extracelulární proteinasy Candida albicans oligopeptidy

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Secreted aspartic proteinases (SAPs) produced by *Candida albicans* are believed to act as one of the virulence factors playing an important role in *Candida* infection. In vitro, the SAPs are produced in presence of an exogenous protein as the sole nitrogen source, during cultivation in liquid media. The isoenzyme called SAP 2 is the major extracellular proteolytic enzyme present under these conditions. Although proteinase secretion is induced by a variety of protein substrates, the precise molecular mechanisms controlling this phenomenon are unknown.

We used a panel of peptides as putative inducers of *C. albicans* SAPs. We synthesized a series of 6-mer to 15-mer peptides derived from a fragment of epidermal cystatin A, which is a substrate of SAP 2. The extracellular proteolytic activity was examined also in the presence of random tetra-, octa- and nonapeptides. The aim of this study was to investigate if (and how) SAP production is affected by the length of peptide backbone and particular amino acid residues.

We found that the best SAP inducers were 9-, 13- and 15-mer peptides of the series tested. Simultaneously, all of these peptides were cleaved by SAP at the expected sites. However, extracellular proteinase production was induced even by peptides that are not substrates of SAP. Nevertheless, our observations indicate that SAP induction does not depend only on the presence of certain peptide or protein molecules, but rather on the level of exogenous ammonium ions.

Antifungal activity of new Cu(II) compounds with some bio-active ligands

Antifungálna aktivita nových meďnatých komplexov s bioaktívnymi ligandami

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Many metal cations play an active role in a great number of various biological processes, especially the six-membered ring system, being a component of several vitamins and drugs. The CuX_2L_2 – X = clofibrinate (clof), 2,6-pyridinecarboxylate (2,6-pyr), salicylate (sal) or nicotinate (nic) and its derivates (isonicotinate – inic, methylthionicotinate – mnic, bromnicotinate – Brnic); L = nicotinamide (nia), ronicol (ron), caffeine (caf) – have been prepared and characterized mainly by elemental analysis, infrared, electronic and EPR spectra. Their antifungal effects have been tested on various strains of yeast and filamentous fungi. Inhibitory concentration IC_{50} and MIC were determined by microdilution methods with vigorous shaking (yeast) and by macrodilution methods during static culturing (filamentous fungi). The mutagenicity assay with *Salmonella typhimurium* TA 97 and *S. typhimurium* TA 100 was performed with $Cu(clof)_2(caf)_2$. Also its effect on energy requiring processes (biosynthesis of nucleic acids and proteins) in *Pseudomonas aeruginosa* was studied. Some of the newly synthesized Cu(II) compounds inhibited the growth of tested yeast and filamentous fungi. The strongest effects on *Candida albicans, C. parapsilosis, Rhizopus oryzae* were magnifested by $Cu(clof)_2(caf)_2$, $Cu(mnic)_2 . H_2O$, $Cu(5Brnic)_2 . H_2O$ (IC_{50} = 1.5–2.1 mmol.l^{-1}). The same compounds also induced morphological changes in growing colonies of *Alternaria alternata* at concentrations, which partially inhibited growth. The most effective compounds against *Microsporum gypseum* were copper clofibrinates and $Cu(5Clsal)_2$, IC_{50} = 1.7 mmol.l^{-1}.

$Cu(clof)_2(caf)_2$ influenced the incorporation rate of [^{14}C] adenine and [^{14}C] leucine into the biomolecules. Biosynthesis of nucleic acids indicated by the incorporation of [^{14}C] adenine was approximately twice as "sensitive" (IC_{50} = 0.380 mmol.l^{-1}) than proteosynthesis indicated by the incorporation of [^{14}C] leucine (IC_{50} = 0.642 mmol.l^{-1}). $Cu(clof)_2(caf)_2$ demonstrated no mutagenic activity.

This work was supported by the Slovak Committee for Scientific Research VEGA, Grant No. 1/4203/97, 95/5195/199 and 95/909.

Occurrence and induction of β-*N*-acetylhexosaminidase in filamentous fungi

Výskyt a indukce β-*N*-acetylhexosaminidasy u vláknitých hub

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β-*N*-Acetylhexosaminidases can be used for the preparation of many glycosidic structures, e.g. Lewis antigen, selectines, glycoconjugates of aminoacids and modified chitooligomers having immunostimulatory activity.

As glycosidases of fungal origin have better transglycosidation capabilities than bacterial enzymes, we have started an extensive screening to obtain β-*N*-acetylhexosaminidases from different fungi. In the course of the screening we found slight extracellular production of this enzyme in many fungi. This production can be frequently improved by induction. An excellent inductor for β-*N*-acetylhexosaminidase is crude chitin hydrolysate (chitooligomers containing 2–10 glycosyl units). In some strains β-*N*-acetylhexosaminidase is specifically induced even by free *N*-acetylglucosamine. Further improvement of induction (almost 2×) was reached by alkali catalyzed ($Ca(OH)_2$) epimerization of chitooligomers (Lobry de Buyn-Alberda van Ekestein reaction) giving derivatives of *N*-acetylmannosamine. It was proved that *N*-acetylmannosamine and its derivatives serve as non-metabolizable inductors.

Differences in the production and inducibility of β-*N*-acetylhexosaminidase and other glycosidases led, in cooperation with the Culture Collection of Fungi at Charles University, Prague, to the re-determination of six fungal strains.

Acknowledgement: This work was supported by the grant No. 309/99/1382 from the Grant Agency of the Czech Republic.

Structural characterization and biological activity of mannans of pathogenic Candida species

Štruktúra a biologická aktivita manánov patogénnych druhov rodu Candida

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On the basis of structural analysis of cell wall polysaccharides of pathogenic *Candida* species it was found that they are mannans with highly branched structures containing α-(16)-linked D-mannosyl units in the main chain, and α-(12) and α-(13)-linked mannosyl units in the side chains. Highly pathogenic *Candida* species such as *C. albicans, C. tropicalis, C. glabrata* have a high degree of branching and

possess long side chains (penta- to hepta-mannooligosaccharides), while species showing low pathogenicity such as *C. krusei* or *C. pseudotropicalis* have low branched mannans with short side chains (tri- and tetra-mannooligosaccharides). Immunochemical cross-reactivity of the yeast strains corroborated the established structures of the surface mannan antigens.

Surface mannans and mannoproteins of pathogenic yeasts are important constituents of the adhesins that mediate specific attachment to the host cells. A correlation was found between the adherence and pathogenicity of the pathogenic yeast strains and the structure of their surface polysaccharides. Adherence of the yeast cells to host tissue is an important initial step in the pathogenesis of candidal infections. We have found that several mannans show a strong inhibition of adherence of *C. albicans* to human epithelial cells. Some mannans were investigated for inhibition of plant viruses and revealed high protective activity.

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Changes in glucan- and chitin synthase activity in Trichoderma viride caused by illumination

Zmeny v aktivite glukán- a chitinsyntázy Trichoderma viride spôsobené osvetlením

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In *Trichoderma viride* a number of biochemical and developmental responses are controlled by blue light. We have investigated the response of the cell-wall biosynthesizing enzymes glucan synthase and chitin synthase to illumination.

We found that a 10 min. illumination of mycelia with white fluorescent light stimulated glucan synthase activity by about 230 % in relation to the dark control and caused a decrease of chitin synthase activity by about 50 % in relation to the dark control. These changes were probably caused by *de novo* synthesis of

corresponding synthases and their regulatory components since they were not observed in the presence of 50 μg/ml cycloheximide.

Preparation, characterization and antimutagenic properties of chitin-glucan from Aspergillus niger

Príprava, charakterizácia a antimutagénne vlastnosti chitín-glukánu z Aspergillus niger

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In recent years increased attention has been paid to β-glucans isolated from the cell walls of the yeasts and moulds that act as non-specific modulators of the immune system and are applied as immunoadjuvants, antitumor agents, etc. The cell wall of *Aspergillus niger* contains predominantly glucan, chitin and galactomannan. The alkali-soluble fraction obtained from the cell wall is composed of α-(13)-D-glucan, galactomannan and glycoprotein. The alkali-insoluble fraction contains a covalently linked complex of β-(13)-D-glucan and chitin which probably plays a critical role in fungal morphogenesis.

The crude, water-insoluble chitin-glucan complex was isolated from cell walls of the industrial strain *Aspergillus niger* by hot alkaline (1 m NaOH) extraction and after derivatization with monochloroacetic acid, carboxymethylated chitin-glucan (CM-CG) with M_w 220 kDa was obtained. A protective effect of the high molecular CM-CG complex (220 kDa) against cyclophosphamide (CP)-induced incidence of micronuclei in reticulocytes of mice was demonstrated after intravenous and intraperitoneal administration. However, this effect was not observed when CM-CG was administered orally. Oral pre-treatment of mice with original high-molecular CM-CG did not decrease the clastogenic effect of CP. The reason for such ineffectiveness of the orally administered CM-CG probably lies in its inability to pass to the bloodstream from the gastro-intestinal tract. In order to overcome this hindrance, a CM-CG$_I$ fragment with lower molecular weight (19 kDa) was prepared. The anticlastogenic effect of CM-CG$_I$ with ultrasonically lowered molecular weight against CP also by oral administration was confirmed.

Acknowledgements. This work was supported by grants from VEGA: No. 2/6024/99, 2/5059/98 and 2/5062/98.

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DNA vaccines – new strategic possibilities in the prevention and prophylaxis of mycotic infections

DNA vakcíny – nové strategické možnosti v prevenci a profylaxi mykotických infekcí

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The therapy of many mycotic diseases is time-consuming and often imperfect. Although fungi are not frequent infectious agents, in consequence of the advance in medicine their importance increases together with the number of long-term immuno-suppressed patients. Also rising migration of people increase the frequency of patients infected with endemic infectious agents (e.g. *Histoplasma capsulatum*) normally eliminated through natural resistance in local populations.

At present the treatment of mycoses is based on the application of some antimycotics (either locally or systematically) according to the type of mycose. Another method is based on the construction of vaccines derived from proteins which play an important role in the pathway of infection. Promising results are reached for example in the application of recombinant Heat Shock Proteins HSP60 or HSP70 or their fragments derived from *H. capsulatum* in mice. Some efforts to prepare a multispectral vaccine based on surface glycoproteins have not been successful, but this option is presently discussed as a promising immunomodulation. Modern molecular biology offers an alternative in the prevention of particular infections – DNA vaccination. Because resistance to mycoses is supposed to be based on the activity of cytotoxic lymphocytes, DNA vaccination seems an optimal option.

A lot of tests with DNA vaccination confirmed the hypothesis of stimulation of specific cytotoxic T-lymphocytes after vaccination in mice, rabbits, but also some non-primate monkeys. DNA vaccine is a plasmid carrying target protein coding gene suspected to be an important factor of pathogenity. For the vaccine function of this plasmid it is important that after administration into a vaccinated organism the gene inserted into the plasmid is expressed by the expression apparatus of the host cells. The particular protein product is presented on the cell surface to the immune system as main histocompatibility complex I – restricted and stimulates immune response by cytotoxic T lymphocytes.

Our target organism was the filamentous fungus *Trichophyton mentagrophytes*, a causing agent of dermatophytoses. We were concerned with the possible role of the HSP60 based cDNA vaccine and its protective effect in mice. According to the above mentioned presumptions a 800 bp long DNA fragment suspected to be HSP60 gene part was prepared and inserted into a cloning vector. We then tested the optimal vaccination vector for an immunological response test in mice.

This work was supported by Grants (No. 11201102 and 14501104) of the Medical Faculty of Palacký University, and by research programme CEZ: J14/98: 15510002.

Molecular epidemiology of nosocomial candidiasis – the use of PFGE and RAPD

Molekulární epidemiologie nozokomiální kandidózy – využití PFGE a RAPD

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Candida yeasts cause life threatening mycoses in immuno-deficient patients. Hospital units where these patients are treated may be in danger of mini-epidemics. In such cases powerful and fast typing techniques are essential for the discrimination between true epidemics and coincidental clusters of infection. Further surveillance of the health care personnel and environment makes eradication of potential causes of infection possible. DNA-fingerprinting is evaluated as the most powerful typing method, whereas Pulsed Field Gel Electrophoresis (PFGE-typing, karyotyping) and Random Amplification of Polymorphic DNA (RAPD) are classified as more cost-effective and less time consuming alternatives. It is recommended to use at least two typing techniques to increase the reliability of results.

We typed 51 isolates from 30 patients using PFGE, 25 of which were *C. albicans*, 9 *C. glabrata*, and 17 *C. lusitaniae*. For comparison, 18 of these isolates were typed using RAPD. Optimization of PFGE parameters allowed us to obtain very well-defined differences between strains of *Candida* yeasts, even in the case of *C. albicans* isolates, for which some authors question the discriminative power of PFGE. There were no discrepancies between the results of PFGE-typing and RAPD-typing. Colonization by different strains was detected in some cases where isolates from different sites of the same patient were typed. Our results demonstrate very well the discriminative power of PFGE-typing.

This research was supported by grants CEZ J14/98: 15110004 and 15110002 and by the internal grant of the Faculty of Medicine, Palacký University No. 11701102.

The effect of the optical brightener Rylux BSU on spore germination rate and spore survival after UV irradiation in Trichophyton mentagrophytes.

Vliv optického zjasňovače ryluxu BSU na klíčení a na přežívání spor po působení UV záření u Trichophyton mentagrophytes.

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We tested the effect of the optical brightener Rylux BSU on the germination rate of spores of *Trichophyton mentagrophytes*. Spore suspensions were exposed to various concentrations of brightener in distilled water and incubated on a reciprocating shaker in darkness at laboratory temperature for 24 hours. The suspensions were then diluted and spread on the surface of SGA. The stimulation effect of Rylux was confirmed. The spore germination rate was increased to 105 % of the control at 0.1 % (w/v) and to 129 % of the control at 0.2 % (w/v) Rylux BSU concentration. Higher Rylux BSU concentrations decreased spore germination rate. We further tested the effect of Rylux BSU on the killing effect of UV radiation. A UV dose which decreased the germination rate of the spore suspension to 18 % of the control was applied. If the spore suspensions were treated with Rylux BSU prior to UV irradiation, considerably more colonies were formed. The decrease in killing effect and/or the increase in spore germination rate if compared with the control was as follows: 1.6 at 0.1 % (w/v) Rylux BSU, 2.2 at 0.2 % (w/v) Rylux BSU, 1.0 at 1 % (w/v) Rylux BSU, 3.1 at 5 % (w/v) Rylux BSU, 3.9 at 10 % (w/v) Rylux BSU, and 4.0 at 15 % (w/v) Rylux BSU. If spore suspensions were treated with Rylux BSU, the UV mutagenization resulted in a significantly higher variability in colony morphology, growth rate, sporulation, pigmentation, surface texture and colonial form.

The observed stimulatory effect of Rylux BSU on germination rate could be useful to improve the sensitivity of detection of pathogenic fungi in clinical specimens and to increase the recovery of mutants in UV induced mutagenesis.

Stress proteins in fungi

Stresové bílkoviny u hub

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Cell and molecular biology studies of the stress response have become a self-contained field with many connections to the basic questions of living systems. On

the other hand, knowledge about stress proteins has opened a new era of their practical exploitation. In this context, fungi play two important roles: 1) as model organisms and 2) as important pathogens for humans, animals and plants.

In this review the roles of stress proteins in individual fungi and their families are discussed. Special interest is focused on the stress proteins of pathogenic fungi, which could be immunodominant antigens, and their potential use in DNA vaccine development.

This work was supported by Grant No. 11201102 of the Medical Faculty of Palacký University, and by research programme CEZ:J14/98:15510002.

Effect of peroxynitrite on resting spores and germlings of Aspergillus fumigatus in vitro

Účinek peroxynitritu na dormantní a klíčící spory Aspergillus fumigatus

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Our previous investigations (Kunert 1995) led to the conclusion that nitric oxide (NO^-) is not a major effector molecule in the killing of *Aspergillus fumigatus* conidia by phagocytes. Recently, peroxynitrite ($ONOO^-$), a strong oxidant arising in the reaction of nitric oxide with superoxide (O_2^-), was shown to be the principal candidacidal molecule of activated macrophages (Vázques-Torres et al. 1996). We tested the killing of resting and swollen conidia of *A. fumigatus* by authentic peroxynitrite and a peroxynitrite donor, SIN-1 (3-morpholinosydnonimine). The decrease in colony-forming units after treating the conidia was evaluated. Inhibition of germination and growth by both effectors was also tested on conidia germinating in nutritive media in microtitration plate wells. Authentic peroxynitrite did not kill any resting or medium-swollen (4 h) conidia in concentrations up to 6.25 mM. Germlings (after 6 and 9 h in the glucose-peptone medium) were only slightly inhibited in further growth when peroxynitrite (5 mM final concentration) had been added. Likewise, SIN-1 (up to 10 mM, 24 h in pH 7 buffer) did not kill any conidia. However, it had an inhibitory effect on germination and growth, depending on the medium. In the glucose – sodium nitrate medium 5 mM SIN-1 slowed down the germination and 10 mM SIN-1 stopped it completely for 20 hours. This effect was, however, only fungistatic and all spores germinated in the fresh medium. In a rich (glucose-peptone) medium the effects were much weaker. In ten other strains of *A. fumigatus* from the Czech Republic, Slovakia, Germany and France the effects of peroxynitrite and SIN-1 were very similar to those in the model strain.

One *Candida albicans* strain used for comparison was much more susceptible to peroxynitrite and its donor than *A. fumigatus*. E.g., 2.5 mM peroxynitrite killed all yeast cells in 2 hours.

The results indicate that peroxynitrite is even less effective against conidia and germlings of *A. fumigatus* than its precursor molecule – nitric oxide. Therefore it does most probably not contribute substantially to the fungicidal activity of phagocytes against the above fungus.

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Conserving of microscopical fungi for collection and demonstration

Uchovávání kultur mikromycetů pro sbírky a demonstrace

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The author describes methods used for establishing collections of microscopical fungi and conserving their typical macroscopical and microscopical traits. This is important for comparison with further isolates as well as for purposes of teaching.

Sabourauds glucose agar enriched with thiamine and histidine and cultivation at 27 °C are recommended for reference cultures. The "macroculture method" includes inoculation of the fungi onto 3 mm thick layer of the medium in plastic Petri dishes. Mature, typical colonies are fixed in formaldehyde vapours and the medium dried to one half of its original thickness. Petri dishes are sealed airtight with acrylate glue and kept at room temperature in dark. In the authors laboratory, prepared colonies are stable for at least ten months. Only about 15% of the cultures had to be discarded because of contamination or insufficient fixation.

For microcultures the fungi are cultivated on thin agar blocks covered with microscopical cover glasses, fixed with methanol and mounted in a gelatin medium. Such cultures can be kept for a long time; in the authors laboratory 15 years old preparations are still being used.

The chitin synthase inhibitor Nikkomycin Z causes disorganized cell wall synthesis in hyphal apices of Basidiobolus ranarum

Inhibitor chitin synthas Nikkomycin Z způsobuje dezorganizaci polarizované syntézy buněčné stěny u hyfových vrcholů Basidiobolus ranarum

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Chitin plays an important role in fungal cell wall rigidity. Stimulation of chitin synthesis was detected in cases of cell wall damage, in mutants with cell wall defects and in response to hypotonic shock. Nikkomycin Z is a tripeptide which inhibits chitin synthases competitively. In the yeast *Saccharomyces cerevisiae* K_i varies for different chitin synthase isozymes between 0.21 and 890 μM. In the filamentous fungi *Mucor plumbeus* and *Geotrichum candidum* 1 μM Nikkomycin Z was described to induce swelling of hyphal apices.

We tested the effect of Nikkomycin Z in the filamentous fungus *Basidiobolus ranarum*. The effect of Nikkomycin Z was concentration-dependent. The highest concentrations of Nikkomycin Z caused immediate swelling of hyphal apices and lysis. Intermediate concentrations of Nikkomycin Z caused formation of large spherical cells with irregular cell wall deposits formed at the inner side of the cell wall after prolonged exposition (24 hours). The lowest effective Nikkomycin Z concentrations caused only minor changes in growth pattern – the hyphae were not as straight as in the controls and dumbbell-like cells were observed. The lowest effective concentration of Nikkomycin Z was determined to be 0.5–1 μM.

Formation of irregular cell wall deposits after prolonged exposition to Nikkomycin Z indicates that the inhibition of chitin synthases causes spatial disorganization of cell wall synthesis and induces cell wall synthesis. As the material of these deposits was stained with Rylux BSU very intensively, it might consist of chitin. However, this has to be proved by chemical analysis.

Position and prospect of Slovak mycology at the turn of the second millennium

Postavenie a výhľady slovenskej mykológie na prelome tisícročia

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Slovak mycology was immediately connected to Czech mycology until the last decade of this century. In the future this historical fact must always fully be respected and correctly interpreted. Today mycology in Slovakia already has

a comparatively rich tradition. Its roots reach down to the past centuries from which the first information on the occurrence of fungi on this territory originate. History shows that the territory of Slovakia, already in the past time, was very interesting in respect of mycology.

The results attained during the last 40 to 50 years document the good level of Slovak mycology which gain respect and appreciation among biological disciplines. In the last years a big advance was made in the field of promotion of macromycetes and prevention against poisoning by poisonous fungi. To the progress of this field largely contributed not only professional mycologists but also amateurs.

At present scientific mycology is developed at the institutes of Slovak Academy of Sciences, universities and some departments of institutes focused on phytopathological mycology in agriculture and forestry. Many research tasks are investigated through scientific projects. Remarkable results were attained by studies of physiology, biochemistry and genetics of fungal pathogens of cereals, sugar beet, grapevine, tobacco, introduced and native trees as well as medicinal plants. It is necessary to inform on the significant results of research in the field of dermatomycoses and mycotoxins. Slovakia attained a prominent international position by significant results of the study in the field of yeasts. Rightly can be spoken of a Slovak school in basic and applied research. Similar results were attained in the study of wood-destroying fungi. In the future attention need to be payed above all to the study of fungal systematics with regard to elaborated mycoflora of Slovakia. The systematics of fungi must be developed on modern basis, i.e. to bring the morphology and anatomy of fungi, close to general mycology, to pay more attention to fungal physiology and ecology.

New possibilities and demands of the research are unceasingly opened in the field mycogeography. It appears that bigger space in research must be provided for micro- and macromycetes because their mycelium forms mycorrhiza with forest trees, shrubs and plants.

For the future an urgent need appears to be the study of theoretical and practical questions of fungal physiology and genetics.

Attention must be paid to the collection of vital fungal cultures and mycosociology.

To fulfil the given theoretical and practical questions it will be necessary to expand special staffs of mycologists, support their further specialisation and coordinate the mycological research according to the demands of the newest scientific knowledge.