Colloquium "Fungi as Model Organisms in Research and Biotechnology – II" Olomouc, Czech Republic, September 5th-6th, 2002

The colloquium was a continuation of a previous scientific meeting that took place in Olomouc in 1999 (Czech Mycology 52: 139-178, 2000). It was organised by the joint Commission for Experimental Mycology of the Czechoslovak Microbiological Society and the Czech Scientific Society for Mycology together with the Institute of Biology, Faculty of Medicine of Palacký University, Olomouc. 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: biochemistry, biotechnology and genetics of fungi; phytopathogenic fungi; fungi pathogenic to humans and animals; and mycology of food and mycotoxins. Each topic was opened with a plenary lecture (30 min.), followed by short communications (10 min.) and accompanied by poster presentations. Besides five plenary lectures, 20 short communications and 24 posters were presented. In total 42 researchers took part in the colloquium and discussed various topics important for the further direction of experimental mycology. Abstracts of the contributions are given below.

Jiří Kunert and Václav Šašek

Biochemistry, biotechnology, and genetics

Interactions of wood-rotting fungi and microorganisms

Interakce dřevokazných hub s mikroorganismy

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White-rot fungi are able to degrade lignin and related compounds. Under natural conditions, the process occurs in the presence of other microorganisms. Introduction of microorganisms to liquid cultures of Trametes versicolor led to an increase of activity of the ligninolytic enzyme laccase. A high increase was achieved with soil fungi, e.g. Trichoderma harzianum (2810 % of control), Penicillium rugulosum (1940 %) and Fusarium reticulatum (1690 %), with non-sterile soil or soil extracts. The increase was lower after addition of bacteria or yeasts. After one-week cultivation with Trichoderma harzianum, the mycelium of Trametes versicolor was killed, which was accompanied by a decrease of Mn-peroxidase activity. Increase of laccase activity is a common response – it was found also in other white-rot fungi, e.g. Abortiporus biennis, Coriolopsis occidentalis, Pleurotus ostreatus, Pycnoporus cinnabarinus and Trametes hirsuta. It might be involved in active defense, since some products of laccase exhibit antimicrobial activity (Eggert 1997). Interestingly, laccase is also increased in heavy metals-stressed cultures (Baldrian et al. 2000, Baldrian and Gabriel 2002). Increase of laccase activity correlated with an increase of decolorisation of the synthetic dye Remazol brilliant blue R. It seems that interspecific interactions can affect the biodegradative activity of white-rot fungi in situ.

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Aspartic proteinases of Candida spp.

Aspartátové proteasy u kvasinek rodu Candida

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The incidence of life-threatening mycoses caused by *Candida* species has increased dramatically in recent years. Candidiasis is a common infection of the skin, oral cavity, vagina and vascular system of humans. Virulence of the *Candida* pathogens is enhanced by the ability to adhere to the host surface, by a phenotypic switch from yeast to hyphal form and by production of extracellular proteolytic enzymes. The role of extracellular aspartic proteinases (Saps) is to degrade a number of cellular substrates, including proteins related to immunological and structural defenses. Saps are therefore studied as a possible target for chemotherapy.

We have developed a screening system based on a solid medium containing hemoglobin as the sole nitrogen source. We have collected *Candida* samples (696) from patients treated in the hospital of the Faculty of Medicine in Olomouc, Czech Republic. We have monitored Saps production in these strains using the novel screening system. Furthermore we have designed, synthesised and tested a set of Sap nanomolar inhibitors derived from pepstatin A structure. We have tested the growth of different *Candida* strains in the presence of these inhibitors. The growth inhibition was found to correlate with Ki values obtained for the individual inhibitors with purified Saps. We also tested HIV proteinase inhibitors used clinically for inhibitory activity of the proteinases studied here. The results can provide new information for the methodological progress in *Candida* diagnosis in clinical work.

This work was supported by IGA MZ, grant no. NI/6485–3 and GA CR, grant no. 303/01/0831.

Effects of N-heterocyclic copper carboxylates on the growth and morphology of filamentous fungi

Účinky N-heterocyklických karboxylátov meďnatých na rast a morfológiu vláknitých húb

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Antifungal activity of 11 newly synthesised copper(II) complexes of isonicotinate (isonicH), 2-methylthionicotinate (2-MeSnicH), 2,6-pyridinecarboxylate ($pdcaH_2$) and their adducts with the bioactive ligands bipyridine (bipy), ethylendiamine (en) and diethylentriamine (dien) were tested on various strains of filamentous fungi with the macrodilution method. TLC was used to determine changes in pigmentation of the model representative.

The majority of the tested compounds influenced growth of the model fungi weakly. Only the antifungal effects of bipy $(IC_{50} \ge 0.12 \text{ mmol.}l^{-1})$, $[Cu(isonic)_2 (bipy)(H_2O)].(H_2O(IC_{50} \ge 0.60 \text{ mmol.}l^{-1}), [Cu(H_2O)_2(bipy)_2] (2-MeSnic)_2 (IC_{50} \ge 1.12 \text{ mmol.}l^{-1}) and [Cu_2(2-MeSnic)_4(DMSO)_2] (IC_{50} \ge 1.15 \text{ mmol.}l^{-1}) could be noticed. The lowest inhibition effect was observed against$ *Rhizopus*oryzae; growth of Alternaria alternata and Botrytis cinerea was influenced at approximately the same level; Microsporum gypseum was the fungus most sensitive to the tested compounds.

Inhibition of sporulation (>80 %) of Alternaria alternata with 1.5 mmol.l⁻¹ $[Cu_2(2-MeSnic)_4(DMSO)_2]$ and 1.5 mmol.l⁻¹ $[Cu_2(2-MeSnic)_4(DMF)_2]$ was observed as a change in the colour of the colonies caused by a decrease in spore concentration. Cultivation of A. alternata in the presence of 1.5 mmol.l⁻¹ $[Cu_2(pdcaH)_2(bipy)_2(NO_3)_2].4H_2O$ in the growth medium caused a defect in melanin synthesis. At the same time the fungus was more sensitive to UV-light than the control without the complex. Both morphological changes of A. alternata were reversible. $[Cu(H_2O)_2(bipy)_2](2-MeSnic)_2$ at 3 mmol.l⁻¹ induced intensive branching in growing hyphae of Botrytis cinerea.

This work was supported by the Slovak Grant Agency VEGA, grant nos. 1/7342/20 and 1/6106/99.

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Determination of the effect of several chemical compounds on wood-destroying fungi

Zisťovanie účinnosti niektorých chemických látok na rast drevokazných húb

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Several chemical compounds were investigated for their influence on the growth of the following wood-destroying fungi: *Coniophora puteana*, *Serpula lacrymans* and *Trametes versicolor*.

For this purpose the "filter paper method" was used. The results show that ammonium isothiocyanato-(N-salicylidene-glycinato)copper II monohydrate acts as an inhibitor of growth in all three tested fungi. The tested fungi showed different sensitivity to ammonium isothiocyanato-(N-salicylidene- β alaninato)copper II.

Other tested compounds, zinc salicylate and copper salicylate dihydrate, had no antifungal activity.

These results were compared to the results of experiments with the commercial fungicides Tebuconazol and TCMTB.

Biocorrosion of stone substrates by soil micromycetes

Biokorózia kamenných substrátov činnosťou pôdnych mikroskopických húb

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The occurrence of microscopic soil fungi on different substrates (limestone, sandstone, objects of art and walls of buildings) with the aim of identifying species of microscopic fungi capable of growing on this extreme type of substrate and also their interactions were monitored. Samples of microscopic fungi were collected from walls (8 samples), from objects of primitive African art made from green serpentine (3 samples) and from tombstones in the crypt of Chatam Sófer in Bratislava (10 samples). Microscopic fungi were isolated from the surface of all substrates by wipping fragments off with sterile cotton plugs and then inoculated on media in Petri dishes (CD, SAB, PDA, MEA, DG-18) and cultivated for 10 days. Altogether, 53 species of microscopic fungi belonging to 23 genera were isolated from the walls, green serpentine and tombstones. Most microscopic fungi were recorded on tombstones (36 species). Dominating species belonged to the genera Alternaria, Cladosporium, Fusarium, Mucor, Penicillium and

Trichoderma. The mycoflora of the walls was characterised by the smallest amount of identified micromycetes with species of the genera Aspergillus (7 species) and Cladosporium (3 species) dominating. From the walls, we also identified a new species for Slovakia, Engyodontium album. From the green serpentines 22 species of microscopic fungi were isolated. The species Aspergillus versicolor, Cladosporium sp., C. cladosporioides, C. herbarum and Penicillium sp. were common on all analysed substrates.

Microscopic fungi isolated from the walls modified pH/H_2O of these walls from 10.37–11.82 to 9.32–9.41, whereby the plaster contained mainly Ca, Si, Al, Mg, Fe, Ba and Na. From the mineralogical aspect, green serpentine is $3MgO.2SiO_2.2H_2O$, tombstones are made from limestone (calcite is dominant) and from sandstone (silica and calcite are dominant). They thus represent an extremely difficult type of biotope for microscopic fungi, but they are able to grow on it and penetrate it. The activities of microscopic fungi (production of metabolites) cause an irreversible process of slaking by chemical or physical corrosion. The effects of these activities are transformation of mineral components and accumulation of biogenic and also toxic elements in organisms.

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Effects of cadmium on the metabolism of wood-rotting fungi: induction of -SH groups and formation of sulphide

Vliv kadmia na metabolismus dřevokazných hub: indukce –SH skupin a tvorba\$ sulfidu

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Stress response of wood-rotting fungi to heavy metals comprises among others reduction of growth rate, changes in morphology including colour changes of mycelium and induction or inhibition of enzymes of both primary and secondary metabolism (Baldrian and Gabriel 1997). Unlike in yeasts and some other fungi, induction of metal-binding compounds containing sulphhydryl groups (metallothioneins or phytochelatins) or phosphates (mycophosphatins) has not been reported so far (Vaccina et al. 2002). This study was focused on changes in concentrations of –SH groups in *Phanerochaete chrysosporium* and *Trametes versicolor* caused by cadmium. Both fungi were cultivated submersed in glucosecorn-steep medium. Addition of Cd increased concentrations of intracellular sulphhydryl groups. Formation of inorganic sulphide was also found. The response of fungi was higher when Cd was added to the culture in the exponential

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phase of the growth. In 1 mM Cd-treated Phanerochaete chrysosporium, the amount of inorganic sulphide reached 1.13 μ mol/mg proteins; no compound was detected in control mycelium. Addition of the metal affected also protein spectra. Analyses of FPLC fractions showed induction of proteins of MW higher than 20 kDa with an increased content of -SH groups. However, cadmium in both Phanerochaete chrysosporium and Trametes versicolor was found in low-molecular weight fractions and no metal-binding protein was detected under the conditions of the experiment. The results confirmed that formation of inorganic insoluble compounds plays an important role in detoxification processes.

This work was supported by GA CR (204/99/1528).

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Some biological properties of new copper(II) halogenosalicylates

Niektoré biologické vlastnosti nových halogénosalicylátomeďnatých komplexov

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Copper ions play a vital role in a number of widely differing biological processes and their interaction with drugs administered for therapeutic reasons is of considerable interest. Copper compounds are important in chemotherapy of some rheumatic diseases as non-steroid antiphlogistic drugs (e.g., the well-known aspirin – acetosalicylic acid, or brufen – a derivative of propionate). Copper complexes were found to have anti-inflamatory, anti-ulcer, antidiabetic, antimutagenic, radioprotective and antimicrobial activity. The new halogenosalicylatocopper(II) complexes of CuX_2 and CuX_2L composition [where X = halogenosalicylato anion (Clsal, Brsal, Isal), L = nicotinamide (nia)], containing in some cases also H₂O, have been prepared and characterised mainly by elemental analysis, infrared, electronic and EPR spectra. The assessment of bioactivity of the tested compounds was concentrated primarily on determination of antimicrobial activity against

bacteria, yeasts and filamentous fungi. Inhibitory concentration IC₅₀ ad MIC were determined by the macrodilution technique in shaken (bacteria, yeasts) or stationary cultures (filamentous fungi). The results of antimicrobial study show greatly increased activity of the substances which were already biologically active, with addition of the copper ion. Presence of halogenosalicylates influence the antimicrobial activities of the complexes under investigation and their activities increase in the sequence Clsal < Brsal < Isal. The highest antifungal effects against Candida parapsilosis, Rhizopus oryzae, Alternaria alternata, Trichoderma viride, Botrytis cinerea, and Microsporum gypseum were obtained with Cu(3,5- I_2 sal)₂(H_2O)₂. The same compound demonstrated no mutagenic activity in Ames assay. The effect of Cu(3,5-I₂sal)₂(H₂O)₂ on energy yielding and energy requiring processes in Salmonella typhimurium was also studied. This compound influenced the incorporation rate of $({}^{14}C)$ adenine and $({}^{14}C)$ leucine into the biomolecules and also markedly inhibited oxygen consumption. All tested halogenosalicylates inhibited sporulation of Alternaria alternata, elicited changes in the morphology of hyphal tips of *Botrytis cinerea*, and increased permeability of the plasmalemma of plant cells.

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Characterisation of chlorpromazine-resistant Trichoderma viride mutants

Charakterizácia chlórpromazínrezistentných mutantov Trichoderma viride

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We found previously that phenothiazine drugs known as calmodulin antagonists strongly inhibit growth and conidiation in several filamentous fungi. Eleven chlorpromazine-resistant UV mutants were prepared from a *Trichoderma viride* M-108 brown conidia mutant strain. The growth and conidiation of *T. viride* M-108 and its chlorpromazine-resistant (CPR) mutants and response of these strains to light were investigated. The growth kinetics of these fungal strains cultivated both in the dark and in circadian light was equal and the number of conidia was lower in the dark compared to illuminated cultures. The number of conidia under identical conditions was higher in CPR mutants than in the parental *T. viride* M-108 strain. Attempts were made to isolate revertant strains by co-cultivation of pairs of mutants supposing that wild-type conidia will be created by anastomosis. Surprisingly, pairs of CPR mutants created variable boundaries which could be Colloquium Fungi as Model Organisms: Biochemistry, Biotechnology, and genetics

divided into seven types (tentatively named A-G). Type A represents a boundary with total coalescence of mycelia of fungal colonies. These mutants were found to exhibit functional anastomosis. On the opposite side, type G represents pairs of mutants which created a boundary with clearly separated mycelia even upon prolonged cultivation. This mycelial "incompatibility" was demonstrated in two mutants. We did not find evidence that the "incompatibility" of these mutants was due to the production of secondary metabolites. On the other hand, the cocultivation of these strains in a stationary liquid culture led to appearance of proteolytic activity in the strains yielding G-type boundaries but not in those with A-type boundaries. These results show that the mutation conferring resistance to chlorpromazine affects also the processes of mutual "recognition" of individual fungal strains.

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Induction of proteolytic enzymes by several inducers in a submerged culture of Trichoderma viride

Indukcia proteolytických enzýmov rôznymi induktormi v submerznej kultúre Trichoderma viride

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The activity of secreted proteinases was measured by means of the chromogenic substrate N- α -Benzoyl-DL-Arg-p-nitranilide (BAPA) during submerged cultivation of Trichoderma viride in the Czapek-Dox medium (CzDM) supplemented with yeast autolysate, the bovine serum albumin or casein (as inducers). After 72 h cultivation with inducers, the secreted proteolytic activity was higher in media supplemented with yeast autolysate (3.2 μ kat) or casein (4 μ kat) than in media with albumin (2.6 μ kat). Cultivation of mycelia in CzDM without any inducer did not lead to the induction of proteolytic activities. After partial purification of proteinase activities with ammonium sulphate precipitation, the effects of proteinase inhibitors were studied. Proteolytic activity isolated from CzDM with yeast autolysate was inhibited with EDTA and TLCK, whereas the activity isolated from CzDM with albumin was inhibited with EDTA and TPCK. The fraction precipitated with 60 % $(NH_4)_2SO_4$ was analysed for the presence of proteinase activities using native PAGE with incorporated gelatin, which displayed the activities of acidic proteinases. Proteinase inhibitors such as PMSF and EDTA inhibited proteinase activities induced by yeast autolysate or casein. However, pepstatin had a more pronounced inhibitory effect when yeast

autolysate was used as inducer, whereas leupeptin inhibited proteinase activity induced by casein better. Proteinases were isolated from the cultivation broth using bacitracin-Sepharose 4B. It was found that protein patterns from the broths containing yeast autolysate or casein as inducers were different in SDS-PAGE and in native PAGE with gelatin. Thus, results indicate that the properties of induced proteinase are dependent on the properties of proteins used as inducers.

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Study of inducibility of citrate uptake into the fungus Penicillium simplicissimum

Stúdium indukovateľnosti vtoku citrátu do vláknitej huby Penicillium simplicissimum

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When citrate was used as a sole source of carbon, citrate uptake by *Penicillium* simplicissimum increased 267-fold (if glucose-grown mycelium was adapted to citrate) or 1400-fold (if the fungus was grown on citrate) compared to glucosegrown mycelium. Inhibition of macromolecular synthesis prevented this stimulation of citrate uptake. Citrate uptake by glucose-grown mycelium was low (0.0015 nmol.min⁻¹ (mg DW)⁻¹) and most probably due to diffusion of undissociated citric acid. Citrate-adapted mycelium had a K_M of 65 μ mol.l⁻¹ and a V_{max} of 0.34 nmol.min⁻¹ (mg DW)⁻¹. In citrate-grown mycelium K_M was 318 μ mol.l⁻¹ and V_{max} was 8.5 nmol.min⁻¹ (mg DW)⁻¹. Citrate uptake was inhibited by sodium azide and uncouplers (TCS, 3,3',4',5-tetrachlorosalicylanilide; FCCP, carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone). Because of this we postulate that the induced citrate uptake must be an active transport process. The pH optimum of citrate uptake was between pH 6 and 7. EDTA, Mg²⁺, Mn²⁺, Cu²⁺, Zn²⁺, Fe²⁺, and Ca²⁺ only weakly influenced the induced citrate uptake. The properties of citrate uptake by Aspergillus niger and Penicillium simplicissimum are compared.

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Discrimination of Armillaria species in the Czech Republic with molecular-biological methods

Identifikace václavek v České republice molekulárně-biologickými metodami

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The genus Armillaria belongs to the basidiomycetes and is known to induce root rot disease and to cause extensive economic losses to forest crop in the Czech Republic. The main function of these fungi in the ecological system is the decomposition of wood waste, but it can very often turn to necrotrophic parasitism and attack a wide range of tree species. Seven species of Armillaria have been identified in Europe up to now: A. borealis, A. cepistipes, A. ectypa, A. gallica, A. mellea, A. ostoyae and A. tabescens. These species have a different pathogenic behaviour and thus forest management necessitates an identification of individual Armillaria species present in the forest. The molecular biological technique was used for the identification. This technique provides very good reproducibility and the analysis is very rapid. The aim of our study was to introduce the molecular-biological technique of Armillaria identification in laboratory practice. We analysed about 40 isolates from the surroundings of Brno.

The restriction analysis of internal transcribed region (ITS), which lies between small nuclear rDNA and large nuclear rDNA sequences, using restriction endonucleases Alu I, Mbo I and Hinf I was applied in the identification. The restriction fragments were analysed both on 3 % agarose gels and by ion-exchange HPLC. Only restriction endonuclease Hinf I was able to discriminate all six investigated species. ITS of some isolates were sequenced. HPLC enabled to discriminate between hetero- and homozygotes. About 20 % of isolates were identified as heterozygous. Homology of the ITS region between individual species was compared on the basis of the sequences. The homology between A. borealis, A. cepistipes, A. gallica and A. ostoyae was about 98 %. On the other hand, the homology between A. mellea and other species was only about 80 %. The resolution and sensitivity achieved with HPLC was comparable or better than on 3 % agarose gel.

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Oxidation of glyoxylate by enzymes of the brown-rot fungus Fomitopsis pinicola

Oxidace glyoxylátu enzymy houby hnědé hniloby Fomitopsis pinicola

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Most hitherto published studies indicate that brown-rot fungi in contrast to white-rot fungi accumulate oxalic acid in cultivation media under high nutrient conditions. Oxalate is supposed to have an important function (among others) in lignin biodegradation by wood-destroying fungi. One of the ways in which oxalate can be formed within the metabolism is oxidation of glyoxylate. Two types of enzymes oxidising glyoxylate were purified from basidiomycetes as yet. One of them was identified as glyoxylate dehydrogenase, the other as glyoxylate oxidase, both from Tyromyces palustris. We partially purified an activity responsible for enzymatic glyoxylate oxidation from the brown-rot fungus Fomitopsis pinicola. The purification procedure consisted of $(NH_4)_2SO_4$ precipitation of a cell-free extract from F. pinicola, ion-exchange chromatography on Sepharose Q and chromatofocusing on MonoP column. Results of ion-exchange chromatography indicated that two proteins contributed to the activity. One of the enzymes has a very low stability, so only one of them was characterised in more detail. We determined the isoelectric point of this enzyme by means of chromatofocusing to be about 4.8. Among the compounds tested, the best substrate was glyoxylate. Glycollate and glyoxal were little utilised, but none of the other used substrates, such as ethylene glycol, oxalate, formaldehyde, formate, were effective. 2,6-dichloroindophenol, potassium ferricyanide and very little even cytochrome c served as electron acceptors but neither NAD⁺, NADP⁺ nor FMN was effective. M_r of native enzyme was estimated to be about 200,000 on a Superose 6 gel filtration column.

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Cloning and bacterial expression of proteinase Sapp2p from Candida parapsilosis

Klonování a bakteriální exprese proteasy Sapp2p z Candida parapsilosis

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Yeasts belonging to the genus *Candida* are the major cause of fungal infection in immunocompromised patients. These opportunistic pathogens produce secreted aspartic proteinases (Saps) that are considered as one of the virulence factors. While Saps of *C. albicans* have been studied extensively, information concerning proteinases secreted by other pathogenic *Candida* species is scarce.

Two different DNA sequences coding for putative Saps in *C. parapsilosis* were detected. One of the genes was identified as a Sap using amino terminal sequencing of extracellular protein isolated from the culture of *C. parapsilosis*. However, information about the second gene and its protein product (Sapp2p) is contradictory.

Our experimental data show that the gene of Sapp2p is transcripted to mRNA but its product is not expressed or secreted. Therefore we cloned a cDNA fragment which encodes Sapp2p from the yeast *C. parapsilosis* into the bacterial expression vector pET-24d(+). Recombinant Sapp2p that was expressed into inclusion bodies in the cytosol of *Escherichia coli* was purified using chromatography on a QAE-Sephadex column. This protein will be used for a study of its folding and activation. The results will be compared to that obtained for Sapp1p.

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Capability of white-rot fungus Dichomitus squalens to degrade azo-, anthraquinone and thiazine dyes

Degradační schopnost ligninolytické houby Dichomitus squalens odbourávat syntetická barviva

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Dye degradation by *Dichomitus squalens* and *Phanerochaete chrysosporium* was compared in solid and liquid media. Agar cultures of D. squalens were able to completely decolorise Reactive Orange 16 (RO16; azo), Disperse Blue

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3 (DB3; anthraquinone) and Methylene Blue (MB; thiazine) within 6–8 days. Decolorisation by *P. chrysosporium* was more rapid and was accomplished within 5 days. Contrary to *D. squalens*, *P. chrysosporium* was not able to decolorise MB in N-limited, mineral medium (NMM).

Liquid stationary NMM cultures of D. squalens reduced the colour of RO16, DB3 and MB (each 100 mg/l) by 81, 92 and 48 %, respectively. The respective values obtained with P. chrysosporium were 98, 93 and 8 %. Manganese-dependent peroxidase (MnP) and laccase were major enzymes present in stationary cultures of D. squalens containing the dyes whereas only MnP was present in significant amounts in stationary cultures of P. chrysosporium. The efficiency of color removal in submerged cultures of D. squalens was similar to the stationary ones but the decolorisation process was slower. The difference between decolorisation ability of submerged and stationary cultures was greater in P. chrysosporium, where the respective removal rates in the former cultures with RO16, DB3 and MB were only 10, 50 and 20 %. The much lower MnP activity in submerged cultures of P. chrysosporium, compared to the stationary ones, could explain this difference.

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Degradative activity of white-rot fungi

Degradační aktivita lignivorních hub

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White-rot fungi are principal degraders of the most recalcitrant natural product – lignocellulose, the predominant form of terrestrial biomass. The mechanisms they employ are not only fundamental to the global carbon cycle, but also potentially useful in environmental applications. A review of information related to the biochemistry of lignin and other recalcitrant compound degradation is presented. White-rot fungi produce one or more of three major classes of extracellular enzymes (laccase, lignin-peroxidase and Mn-peroxidase) that are believed to be involved in lignin degradation. However, it was proved that neither these enzymes nor their mixture can either directly initiate or completely degrade lignin because the enzymes are too large to penetrate native wood. This implies that diffusible low molecular weight oxidants are involved in the degradation. Reactive oxygen species like hydroxyl radicals have been implicated in this connection. In this contribution we specifically focused on non-enzymatic systems similar to the Fenton reagent. Our systems, consisting of transition metal-ligand COLLOQUIUM FUNGI AS MODEL ORGANISMS: BIOCHEMISTRY, BIOTECHNOLOGY, AND GENETICS

plus hydrogen peroxide, produce hydroxyl radicals that were proved by EPR, and are able to degrade different recalcitrant compounds efficiently. Although the involvement of these systems in fungal degradation processes is still hypothetical, they may find potential application in environmental biotechnology.

The Czech Collection of Microorganisms (CCM) and Federation of Czechoslovak Collections of Microorganisms (FCCM) – biological resource centres

Česká sbírka mikroorganismů (CCM) a Federace československých sbírek mikroorganismů (FCCM) – centra biologických zdrojů

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The Czech Collection of Microorganisms (CCM) belongs to the most important and greatest culture collections of the Czech Republic. It maintains at present about 2500 strains of more than 810 species of bacteria, about 700 strains of more than 570 species of fungi and approximately 500 strains of more than 130 species of aquatic hyphomycetes. Most of the fungal cultures belong to Hyphomycetes, Coelomycetes, Ascomycetes and Zygomycetes. The CCM keeps many strains used in industry, medicine, research and teaching. The specialised collection of aquatic hyphomycetes is very valuable for fungal taxonomy, systematics and ecology. Many strains are derived from type specimens. The principal method of preservation of microorganisms is freeze-drying. Non-sporulating strains of fungi are kept under mineral oil. Preservation of all strains of bacteria and fungi in liquid nitrogen is in preparation. CCM is a member of the World Federation of Culture Collections (WFCC), European Culture Collections Organization (ECCO) and Federation of Czechoslovak Collections of Microorganisms (FCCM). The collection is an International Depositary Authority for deposits of bacteria and fungi for patent purposes under the Budapest Treaty. These organisms are accepted on national level, too. A list of strains is published in the catalogue. Our web site provides a lot of useful information (http://www.sci.muni.cz/ccm).

The Federation of Czechoslovak Collections of Microorganisms (FCCM) associates culture collections of algae, bacteria, fungi (including yeasts) and viruses from the Czech Republic and the Slovak Republic. At present, the members (16 collections from the Czech Republic and 5 collections from the Slovak Republic) keep more than 21 700 strains of microorganisms. A home page on the Internet was created (http://prfdec.natur.cuni.cz/fccm/). A list of all species kept by members of the FCCM is in preparation.

Testing the fungicidal effect of chemicals on microscopic filamentous fungi – moulds

Testování fungicidního účinku chemických látek na mikroskopické vláknité houby – plísně

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Contamination of the indoor environment in homes and at workplaces with filamentous fungi – moulds – has a negative effect on human health. Even if previously considered harmless saprophytes, some species of these fungi may cause serious infections. That is why targeted disinfection is needed. Fungicides are used for indoor disinfection not only in the presence of visible mycelial growth but also if high counts of spores are found in indoor air. Fungicidal activity of disinfectants is identified with different methods. Fungicidal activity of eight disinfectants, containing aldehydes, quarternary ammonium compounds, peroxo- and chlorinebased compounds as active ingredients, was compared. The activity was tested on spores of the following species of filamentous microfungi resistant to chemicals: Aspergillus niger, Penicillium aurantiogriseum and Mucor racemosus, obtained from CCF Collection, Department of Mycology, Faculty of Natural Sciences, Charles University, Prague. The diffusion method and suspension method were used for fungicidal activity testing. The principle of the former consists in pipetting samples of the test products into wells in agar medium on a Petri dish inoculated with fungal spores. The size of inhibition zones is assessed. The suspension method is based on pipetting a suspension of fungal spores into a test solution and subsequent inoculation into liquid culture medium after given exposure intervals. Growth on the surface of liquid medium is recorded until sporulation appears. The diffusion method did not prove suitable for determining fungicidal activity of disinfectants. It is only indicative of the physical nature of test products. The standard method for fungicidal activity testing is therefore the suspension method, which allows differentiation between fungicidal and fungistatic activity and determination of the effective concentration and exposure time of disinfectants.

COLLOQUIUM FUNGI AS MODEL ORGANISMS: BIOCHEMISTRY, BIOTECHNOLOGY, AND GENETICS

Use of the library of fungal β -N-acetylhexosaminidases for the synthesis of glycosaminoglycosides

Použití knihovny houbových β -N-acetyl
hexosaminidas k syntéze glykosaminoglykosidů

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Fungal glycosidases are very useful in the preparation of many glycosides by transglycosylation or reversed glycosylation. Glycosidases are obtained in sufficient quality and quantity by induction. Oligosaccharides containing Nacetylhexosamines (GlcNAc; GalNAc; ManNAc) are important because of their biological activities. Oligosaccharides comprising e.g. ManNAc are important immunodeterminants of some pathogenic bacteria, derivatives of chitooligomers have a high affinity to NKR-P1 protein, the major activating receptor at the surface of natural killer cells of rats. We have performed extensive screening for new glycosidases, namely β -N-acetylhexosaminidases. The library of enzymes with different biochemical parameters comprises more than 100 different types.

By transglycosylation or reverse glycosylation using β -N-acetylhexosaminidases from this library the following oligosaccharides were prepared: GalNAc $\beta(1\rightarrow 6)$ GlcNAc, GlcNAc $\beta(1\rightarrow 6)$ GlcNAc, GlcNAc $\beta(1\rightarrow 6)$ GalNAc, p-nitrophenyl β -chitobioside, GalNAc $\beta(1\rightarrow 4)$ GlcNAc $\beta(1\rightarrow 4)$ GlcNAc,



GalNAc $\beta(1\rightarrow 4)$ GlcNAc $\beta(1\rightarrow 4)$ ManNAc. Enzymatic transfer of β -GlcNAc to the anomeric position of D-Man or D-Gal forming GlcNAc $\beta(1\leftrightarrow 1)\beta$ Gal and GlcNAc $\beta(1\leftrightarrow 1)\beta$ Man represents the first example of non-reducing disaccharides prepared with glycosidases.

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Decolorisation of anthraquinone dyes Remazol Brilliant Blue R and Disperse Blue 3 by white-rot fungus Irpex lacteus

Dekolorizace barviv Remazol Brilliant Blue R a Disperse Blue 3 ligninolytickou houbou Irpex lacteus

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This work was focused on decolorisation of anthraquinone dyes, Remazol Brilliant Blue R (RBBR) and Disperse Blue 3 (DB3), by various isolates of whiterot fungi. Decolorisation rates with the two dyes on agar media were compared. *Irpex lacteus*, capable of rapid and efficient decolorisation, was chosen for further study using a nitrogen-limited, liquid mineral medium (NMM) in stationary and submerged cultures. Stationary cultures of *Irpex lacteus* removed 100 % of RBBR (150 mg/l) in 9 days and submerged cultures 95 % in 10 days. The former cultures exhibited higher levels of lignin peroxidase (LiP), manganese dependent peroxidase (MnP), manganese independent peroxidase (MIP) and laccase than the latter, and selective inhibition by NaN₃ and n-propylgallate showed that MnP played a major role in the decolorisation of the dye.

Irpex lacteus was also immobilised on polyuretane foam (PUF) or pinewood cubes and the degradation capacity of these cultures were compared. Both immobilised cultures were able to rapidly decolorise RBBR and could be reused in up to 8 decolorisation cycles. Five-fold MnP levels were detected in PUF cultures, whereas the laccase activities were similar. No LiP was detected in either immobilised culture. The immobilised cultures of *Irpex lacteus* were also capable of efficient decolorisation of textile colour bath effluents.

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COLLOQUIUM FUNGI AS MODEL ORGANISMS: BIOCHEMISTRY, BIOTECHNOLOGY, AND GENETICS

Growth of selected microscopic fungi isolated from malt barley in presence of Bacillus subtilis, Geotrichum candidum or their free-cell filtrates

Růst vybraných mikroskopických hub izolovaných ze sladovnického ječmene v přítomnosti Bacillus subtilis, Geotrichum candidum nebo jejich bezbuněčných filtrátů

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Microscopic fungi are an important part of the microflora of malt barley. Their occurrence in barley grain or malt can present various hazards (mycotoxins, gushing, undesirable off-flavour or odours of beer, decreased germination of grains, etc.). At present some microorganisms (e.g. *Geotrichum candidum* and lactic acid bacteria) with antifungal or antibacterial qualities are utilised to reduce undesirable microflora in different branches of food industry or in biological protection of plants.

Interrelationships between strain G3 of Geotrichum candidum, of Bacillus subtilis strain S1 or their free-cell filtrates and twelve selected strains of microscopic fungi isolated from barley and malt were studied. Interactions among these microorganisms were tested on solid medium malt extract broth agar at first. The forming of an inhibition zone was noticed. The influence of cell-free filtrates prepared from a forty-eight hour old culture of Geotrichum candidum G3 and Bacillus subtilis S1 was tested in the liquid medium malt extract broth using the method of dry weight. These filtrates were added to the cultures of microscopic fungi at the beginning of the cultivation and then after six, twelve and twenty-four hours of cultivation. Antagonistic interactions were found between Bacillus subtilis S1 and the strains F1 and F2 of *Fusarium poae* on solid medium. Antagonistic interactions between Geotrichum candidum G3 and Fusarium poae F1 and F2, Penicillium sp. and P. brevicompactum P2 were recorded, too. Cell-free filtrates of Bacillus subtilis S1 and Geotrichum candidum G3 reduced the production of biomass of all strains of the tested fungi (Aspergillus clavatus A, Fusarium poae F3, F. poae F4, F. sporotrichioides F5, Mucor circinelloides M, Penicillium brevicompactum P2, P. crustosum P3, P. chrysogenum P4, Rhizopus oryzae R). The greatest influence of filtrates on the production of micromycete biomass was recorded at the application of the filtrates at the beginning of the cultivation.

Accumulation of toxic elements by the biomass of microscopic fungi

Akumulácia toxických kovov biomasou mikroskopických húb

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Since 1998, we have been observing under laboratory conditions the relations of selected species of microscopic fungi (Aspergillus niger, A. clavatus and Trichoderma viride) and some chemical elements (As, Cd, Hg and Pb). We are trying to measure quantitatively the properties of the accumulation of these elements from a fluid medium by selected species of microscopic fungi under different pH values, metal concentration and periods of time.

Microbial uptake and fixing of ions is mostly limited to the structures of the cell walls and minor amounts are transported to the cytoplasm. The uptake and fixing of excessive metal amounts, including toxic ones, by microscopic fungi are realised without their metabolic utilisation. These properties are reflections of their large adaptability, structural and functional composition, which is mostly related to the cell walls.

The fixing of metal ions in the cell walls is in principle based on two mechanisms: interaction with active functional groups of their polymeric components and physico-chemical fixing by adsorption or inorganic precipitation.

The above mechanisms of fixing of ions may take place within the metabolismdepending processes of living cells and within the processes independent of metabolism. Active metabolic metal accumulation is connected with energy consumption and takes place in the intracellular space, organelles and subsurface structures of the cell walls. It is also connected with passive adsorption of metal ions on the cell wall surface.

Non-metabolic passive adsorption of metals onto cell wall structures as a whole is the only mechanism found in the dead microbial biomass.

Although a certain degree of generalisation of the bioaccumulation and biosorption processes of metal ions from the environment by microscopic fungi and microbial biomass is possible, a unified theory of the processes listed above is not available.

Within this seminar, the results of the uptake and fixing of Cd by Aspergillus niger and Trichoderma viride after 10 and 30 days, at concentrations of 50 and 100 ppm Cd in the medium and at initial pH values of 6.1, are presented. The Cd content in Aspergillus niger mycelium after 10 and 30 days of cultivation was nearly identical, while in Trichoderma viride it was higher after 30 days. The mycelium dry weight of Aspergillus niger after 10 and 30 days of cultivation was very similar, the weight of Trichoderma viride was different, higher after 10 days than 30 days. There was no direct relation between the Cd content in mycelium Colloquium Fungi as Model Organisms: Biochemistry, Biotechnology, and genetics

and its weight. The Cd content in mycelium of *Aspergillus niger* was higher on average than that of *Trichoderma viride*.

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Microscopic fungi new for Slovakia

Mikroskopické huby nové pre Slovensko

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During the monitoring of occurrence and identification of micromycetes in various environments, from water to growths on visibly contaminated inner walls of buildings, further textile material, wooden and serpentine sculptures from objects of primitive African art and from soil, the following relatively rare microscopic fungi were found.

Nigrospora sphaerica (Sacc.) Mason was isolated from water-supply reservoir Nová Bystrica and from water mains in Žilina. Myxotrichum deflexum Berk. was isolated from water mains and the wall of a kitchen in a house and from African textile material. Engyodontium album (Limber) de Hoog was isolated from a wet, damaged wall of the Museum of Primitive African Art. Syncephalastrum racemosum Cohn ex J. Schröt. was isolated from a wooden sculpture in the Slovak National Museum in Bratislava. Idriella lunata P. E. Nelson et S. Wilh. was isolated from cambic podzols contaminated with As and Hg. The species Penicillium arenicola Chalab. and Fusarium sporotrichoides Sherb. were isolated from stone monuments from the crypt of Chatam Sófer in Bratislava. Melanopsama pomiformis (Pers.: Fr.) Sacc. was isolated from the floor of a water storage and Polyscytalum fecundissimum Riess was isolated from the wall of a wine vault.

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Ca²⁺ fluxes in developing Trichoderma viride mycelium

Toky Ca^{2+} v rastúcom mycéliu Trichoderma viride

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The properties of both Ca²⁺ influx and efflux in mycelium were studied during the life cycle of Trichoderma viride by means of ${}^{45}Ca^{2+}$ and by Xray fluorescence spectroscopy measuring. Evidence was obtained (temperaturedependence, and saturability with Ca^{2+}) that the Ca^{2+} influx is mediated by a carrier. The possibility of endocytosis-mediated Ca²⁺-accumulation was excluded by a parallel measurement of ⁴⁵Ca²⁺ and ³H-inulin uptake. The properties (pHand temperature- dependencies) of the Ca²⁺ efflux were different from those of the Ca²⁺ influx. The rate of ⁴⁵Ca²⁺ influx (in nmol.mg dry weight⁻¹.h⁻¹) dramatically changed during the development of the vegetative mycelium. It was at maximum after about 30 h of submerged cultivation and then decreased. This decrease was not accompanied by a corresponding increase of the Ca^{2+} efflux. These results were corroborated by measurements of the Ca^{2+} content of both submerged and aerial mycelium by means of X-ray induced fluorescence spectrometry, and showed that mycelial Ca^{2+} content (in nmol.mg dry weight⁻¹) continuously decreased during vegetative growth. The appearance of conidia in the aerial mycelium was accompanied by an increase of Ca²⁺ content. The results show that loading of internal Ca²⁺ stores occurs in the early stages of development of the mycelium only, and the Ca^{2+} influx mechanism is developmentally down-regulated, being almost silent during its later stages. Thus, in older mycelia, the growth of the mycelial mass seems to be independent of extracellular Ca^{2+} . The identity of the Ca²⁺ store remains uncertain and probably consists of more than one organelle.

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Phytopathogenic fungi

Microbial seeds contamination, one of the causes of low germination rate in Karwinskia humboldtiana (Rhamnaceae)

Mikrobiálna kontaminácia semien, jedna z príčin nízkej klíčivosti Karwinskia humboldtiana (Rhamnaceae)

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Species of the genus Karwinskia (Rhamnaceae) occurring in Mexico and Central America are potentially applicable in medicine; they produce secondary metabolites which exhibit selective antitumor effects. Isolation of the most important metabolite from the anthracenone group, peroxisomicine A_1 , requires a sufficient amount of biological material whose production depends on an effective mode of plant multiplication. Karwinskia parvifolia and K. humboldtiana with the highest content of the metabolite can be obtained by cultivation in vitro and also vegetatively. These species may also be grown from seeds but bad seed germination and sprouting problems were observed in K. humboldtiana. Inadequately developed or undeveloped embryos and the presence of inhibitory substances in the seed decrease plant production. The cause of a low germination rate in seeds of various plant species is a hard lignified pericarp and/or seed contamination with microflora. The negative effect of seed contamination by microorganisms is eliminated by disinfecting the seeds, eventually by sterilising seed surfaces. Seeds of K. humboldtiana (Villa de García Nuevo, León, Mexico, 1997) were contaminated with bacteria, yeasts and filamentous fungi. The concentration of microorganisms in unscarified seeds ranged from 3.0×10^3 to 7.5×10^3 CFU/g. Bacterial isolates were predominant. Of filamentous fungi, Alternaria sp., Aspergillus niger, Cladosporium sp., Fusarium sp., Mucor sp., Penicillium commune, and Trichothecium sp. were identified, yeasts included Rhodotorula sp. and Saccharomyces cerevisiae. Seed scarification reduced their microbial contamination by approximately 80 %. Treatment of seeds with disinfectans significantly increased their germination. The effect of disinfectans decreased in the order Supresivit (Trichoderma harzianum), Vitavax 200 WP (carboxin + thiram) and Pomarsol Forte 80 WP (thiram).

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Fusarioses on barley – a model for experiments with mycotoxins

Fuzariózy ječmene – model pro pokusy s mykotoxiny

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In our previous study (Hýsek et al. 2000) we posed the question whether fusarioses could serve as a model for experiments with mycotoxins. This time we report on some experiments with trichothecene mycotoxins, which can be summarised as follows:

1) After artificial infection with *Fusarium culmorum* (producer of the trichothecenes nivalenol and deoxynivalenol) from ears of barley other species were isolated after harvest: *Fusarium tricinctum*, *Fusarium poae* (after pre-crops of sugar beet, maize and cereals) which produced no mycotoxins.

2) Some strains of *Fusarium culmorum* produced more deoxynivalenol in young plants than in the mycelium.

3) The lowest occurrence of grain contaminated with *Fusarium* was after rape (13.31 %) and sugar beet (20.47 %) as the pre-crop. The highest occurrence was after cereals (33.91 %) and after maize (42.42 %).

4) The most effective fungicide against fusarioses and mycotoxins was the commercial product Charisma.

5) After pre-crop of maize the trichothecene level was about one order higher in all varieties of barley in comparison with cereals and sugar beet as pre-crops.

6) The content of deoxynivalenol (DON) after artificial infection with *Fusarium culmorum* varied from 0.5 ppm (the cultivar Chevron) to 9.0 ppm (new selection SG-S 2626). The mean value was 4.8 ppm of DON.

7) The highest values of gushing (overfoaming of beer) were found in the variety "Akcent" and the lowest ones in the varieties "Forum" and "Jersey".

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COLLOQUIUM FUNGI AS MODEL ORGANISMS: PHYTOPATHOGENIC FUNGI

Aggressiveness of Erysiphe cichoracearum isolates pathotype AB1B2CCm on cucumber and watermelon

Agresivita izolátů Erysiphe cichoracearum patotypu AB1B2CCm na okurce a vodním melounu

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Pathogenicity of obligate biotrophic fungi is basically characterised by virulence of isolates. However, quantitative differences in infection development among isolates were noticed. The purpose of this study was to describe the variation in aggressiveness within isolates of cucurbit powdery mildew Erysiphe cichoracearum of identical virulence phenotype (pathotype). Eight isolates of E. cichoracearum were collected in the years 1997–1998 on Cucurbita pepo, C. maxima and Cucumis sativus in five eco-geographically distinct regions of the Czech Republic. They were virulent by in-vitro tests to pathotype differential genotypes A (C. sativus cv. Marketer), B1 (C. melo cv. Védrantais), B2 (C. melo PMR 45), C (C. pepo cv. Diamant F1), Cm (C. maxima cv. Goliáš) and avirulent to genotype D (C. lanatus cv. Sugar Baby). The isolate aggressiveness derived from their infection development in vitro, and expressed as total infection degree (TID-%) on differential genotypes A, C, Cm, B2, B1, and D were: 55.2a, 46.6bc, 44.9bc, 40.2bc, 31.3b, and 5.6a. These differences in infection development in differential genotypes correspond to data on common response of cucurbit species to the powdery mildew. Moreover, differences in aggressiveness among isolates in each host genotype were observed. They were not related to the original host plant species of isolates and/or region of their collecting. Isolates AB1B2CCm of higher virulence potential, i.e. with the capacity to sporulate also on watermelon (D), expressed a lower aggressiveness level on cucumber (A) and vice versa.

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Natural isolates of Trichoderma species for purposes of biological plant protection

Prírodné izoláty húb z rodu Trichoderma pre účely biologickej ochrany rastlín

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The genus Trichoderma is typical of symbiosis with roots of higher plants where it can serve as a protection against pathogenic fungi. Therefore, isolation and characterisation of species of Trichoderma can contribute to increasing knowledge about biocontrol. In a specific environment, Trichoderma strains of home origin are very important. In the present project, we collected samples of soil from at least 80 sites in Slovakia. The majority of collected soil (63 %) was of neutral pH, 28 % of soil samples were slightly acidic. This sort of differentiation of strains will be relevant for their application in different soil conditions. From the 80 collected soil samples, 65 Trichoderma strains were isolated. From locations with sugar beet, roots of sugar beets were analysed by the serologic ELISA test using monoclonal and polyclonal antibodies for the presence of two viruses (BNYVV- Beet necrotic yellow vein virus and BSBV - Beet soil borne virus) transferred by a fungal vector - the fungus Polymyxa betae. Simultaneously, we microscopically analysed the presence of cystosori of the fungus P. betae and also of filamentous fungi, which could naturally react against it in an antagonistic way. In greenhouse conditions, antagonistic effect of selected isolates of Trichoderma species against P. betae was tested. It was found that treatment of beet seeds by spores of Trichoderma can reduce the colonisation of sugar beet roots by P. betae and the amount of BNYVV in beet roots by 20-50 %.

Modelling of the interaction of elicitors from Phytophthora and its utilisation in biocontrol of fungal pathogens

Modelování interakcí elicitorů houby z rodu Phytophthora a jeho využití při biokontrole houbových patogenů

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Several plant pathogen models have been studied in detail. One of them is the tobacco – *Phytophthora* interaction in which *Phytophthora*-secreted proteins called elicitins seem to play a major role (for a review, see Ricci 1997). Elicitins

COLLOQUIUM FUNGI AS MODEL ORGANISMS: PHYTOPATHOGENIC FUNGI

are holoproteins which induce hypersensitive response and non-specific systemic acquired resistance. In cell suspension, they trigger classical events, such as calcium influx, alkalinisation of the extracellular medium, production of active oxygen species and cell wall modifications (Blein et al. 1991, Kieffer et al. 2000). We found previously that elicitins are sterol carrier proteins (Mikeš et al. 1998). The secondary structure of cryptogein, the most efficient elicitin, has been determined by Boissy et al. (1996). We compared the primary structure of about 50 elicitins in order to assess a relationship between their structure and reactivity. The amino acids participating in sterol binding are highly conserved. The amino acids responsible for the "toxicity" of elicitins are distributed uniformly on the protein surface so that the affinity to the receptor could be due to the property of the whole protein, such as isoelectric point, and not to a specific elicitin cluster. We studied the link between elicitor and sterol carrier properties using a site directed mutagenesis and heterologous expression of the cryptogein gene. Sterol binding kinetics was related to the biological effects of the mutated proteins. The mutation in tyrosin-87 involved in sterol binding altered both specific binding to high affinity sites and biological activities. The results strongly suggest that formation of the sterol elicitin complex is a requisite step before binding to the specific receptor.

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Culture characteristics of selected Fusarium species isolated from maize and their in vitro interaction with an antagonist

Kulturálne vlastnosti vybraných druhov rodu Fusarium izolovaných z kukurice a ich interakcia s antagonistom v podmienkach in vitro

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Diseases of maize (stem rot, ear rot, seedling damping-off) are frequent and economically important in Slovakia. They are caused by Fusarium species. The aim of this study was to investigate the culture characteristics of pathogens from the genus Fusarium isolated from maize fields. These fungi caused necrotic lesions on mesocotyl during seedling development and stem rot after flowering stage. We isolated fifteen Fusarium species from maize plants in Slovakia, three of them from the section Discolor (F. graminearum, F. culmorum, F. crookwellense), one species from the section Liseola (F. moniliforme), F. oxysporum from the section *Elegans* and *F. sporotrichioides* from the section *Sporotrichiella*. The temperature optima for colony growth ranged from 20 to 25 °C. We observed differences in growth rates among species from the sections Discolor, Liseola, Elegans and Sporotrichiella. The effect of nutrient media was also investigated. All species reached maximum growth rates on Czapek-Dox agar in comparison with potato-dextrose agar. Differences in mycelium pigmentation on five nutrient media were also observed. We further studied the effect of culture filtrates of selected fungi, Alternaria sp., Penicillium sp., and Trichoderma sp., isolated from maize and *Beauveria* sp. isolated from corn borer (Ostrinia nubilalis), on growth of *Fusarium* species in vitro. These fungi are commonly isolated together with Fusarium from maize tissue. We observed a stimulating effect of Alternaria, Beauveria and Trichoderma species on the growth of Fusarium colonies. Only the medium with Penicillium filtrate had an inhibiting effect on all Fusarium species. Interactions in dual cultures of Fusarium species and Trichoderma species were studied in vitro to determinate their antagonistic ability. T. harzianum and T. viride inhibited the growth of all Fusarium species.

This work was supported by Slovak Grant Agency VEGA, grant no. 2/6001/20.

Colloquium Fungi as Model Organisms: Phytopathogenic fungi

Interaction of elicitors from Armillaria with plant cells

Interakce elicitorů václavek s rostlinnými buňkami

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Armillaria (honey mushroom) is mentioned as one of the most dangerous pests in forestry. It causes the so-called "Armillaria root disease", which can damage large forested areas. Understanding the interaction of Armillaria with their hosts may help to improve protection against Armillaria root disease.

For the experiments, we used suspension-cultured tobacco cells (*Nicotiana tabacum var. xanthi*). Hypersensitive reactions elicited by water extracts from mycelia of *Armillaria* were monitored measuring the production of active oxygen species and pH changes.

Intensities of the hypersensitive reaction elicited by several *Armillaria* species were compared. The correlation between intensity of hypersensitive reaction of the host plant and virulence was not obvious.

The water extracts were subjected to various procedures to determine the active components. The experiments revealed that elicitor activity was heat stable, but it was drastically decreased when the lipophilic substances were removed by adsorption on a hydrophobic matrix. The presence of ergosterol was proved. On the other hand, a low elicitor activity resides in chitin fragments.

The experiments with inhibition of signal cascade, which is involved in recognising the elicitors from extracts and triggering the hypersensitive reaction, showed that both active oxygen species production and plasma membrane H⁺ ATPase inhibition take part in the alkalinisation of tobacco extracellular medium. Moreover, both intracellular and extracellular sources of calcium are involved in the elicitor induced signalling. Phospholipid/calcium-dependent proteinkinases were revealed as an essential element in elicitor-induced signalling.

This work was supported by FRVŠ grant no. 759/2002 from the Ministry of Education of the Czech Republic.

Growth, colony interactions and hyphal interference between fungal pathogens isolated from horse chestnut leaves and the fungus Trichoderma harzianum on different media

Rast, interakcie kolónií a interferencia hýf hubových patogénov izolovaných z listov pagaštana konského a huby Trichoderma harzianum na rôznych médiách

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Interactions in dual cultures of horse chestnut leaf pathogens and Trichoderma harzianum were studied in vitro to determine their antagonistic ability and their tolerance or antagonism. The growth of and interactions between horse chestnut leaf pathogens, Phyllosticta sphaeropsoidea, Phomopsis carposchiza, Diaporthe and the antagonistic fungus Trichoderma harzianum (three isolates) were examined on potato-dextrose agar (PDA), carrot agar (M), 2% water agar (V), Czapek-Dox agar (CzD) and malt extract agar (MEA). All pathogens had maximum growth rates on carrot agar, all Trichoderma isolates had maximum growth rates on PDA. Inhibition of the pathogen's development in dual culture was assessed according to two parameters: inhibition percentage of radial growth and width of the inhibition zone. The results were analysed by Fisher's least significant difference procedure. T. harzianum significantly inhibited the growth of Diaporthe sp. on PDA, M and MEA. Isolate TH02 significantly inhibited the growth of *Phomopsis carposchiza* on all media. For the majority of dual cultures, pathogen-antagonist combinations did not show the same colony interactions on all media. Mutual and extreme inhibitions were found in the Phomopsis-Trichoderma combination. T. harzianum produced the largest inhibition zones (8.5 mm) when grown in dual culture with Phomopsis carposchiza on MEA. Trichoderma harzianum grew superficially over Phyllosticta sphaeropsoidea and Diaporthe sp. and inhibited their growth. Hyphal interference was assessed microscopically for coiling of the antagonist on the surface of the pathogen, penetration, granulation, abnormal branching and lysis of the pathogen's hyphae. Granulation of the cytoplasm and lysis of the pathogen's hyphae were the most frequently observed effect of interaction on all media. These results have implications for use of such in vitro tests as part of a general screening for efficacy of action of antagonists against leaf pathogens.

This work was supported by Slovak Grant Agency VEGA, grant no. 2/7118/20.

Medical mycology

Another view of the lipophilic yeasts of the genus Malassezia

Lipofilní kvasinky Malassezia spp. trochu jinak BEDŘICH ERNEST AND NADĚŽDA BOHÁČOVÁ Nepomucká 27, CZ – 326 00 Plzeň, Czech Republic

This study of lipophilic yeasts was inspired by frequent confusion in the diagnosis of itchy, papulose skin affections. The study gave a new picture of the life of *Malassezia* yeasts on the skin and of their morphology. The communication is based on microphotographic documentation.

Besides well-known forms of yeasts, such as blastospores and simple filaments, other not yet documented forms were detected. These comprise clusters of dark cells of different sizes and shapes (7–90 μ m), tiny blastospores (0.3–1.9 μ m), dark thick filaments, very long black filaments (up to 12,000 μ m), dark ovoid blastospores and dark coarse orbicular blastospores. From these structures long thin filaments, bizarre networks of dark filaments and clusters of black cells originated. All lipophilic yeasts found had life cycles with a heterogeneous morphology, including previously undescribed forms. Tiny orbicular blastospores showed signs of fermentation and produced an unidentified gas. The production of yellowish and orange-coloured pigments was also observed. The yeasts and filaments of *Malassezia* spp. inhibited the keratinisation process of skin keratinocytes.

Spore germination: Aspergillus flavus, A. niger, A. ochraceus from drinking water after UV disinfection

Germinácia spór: Aspergillus flavus, A. niger, A. ochraceus z pitnej vody po UV dezinfekcii

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The results of laboratory tests focused on an evaluation of the effects of UV irradiation on germination of spores and growth of three species of *Aspergillus* isolated from distribution systems of drinking water in Slovakia are presented. The experimental model water samples were prepared from sterilised tap water

inoculated with one type of spores of the following pure cultures: Aspergillus flavus, A. niger and A. ochraceus. The final concentration of spores in water before the irradiation was approximately $10^{5}.1^{-1}$. The samples were irradiated in an encapsulated emitter, in which water by-passed a gas discharge lamp in a 3 cm layer. Irradiation doses from 25 W radiation source were 7,708 to 360,982 [μ W.s.cm⁻²]. The effects of various doses of UV radiation on spore germination and on the character of growth of mycelium (changes in the pigmentation, S-stimulation) were evaluated using cultivation methods. 1 ml samples were taken in 15 min. intervals and incubated on Sabouraud and Czapek-Dox agar plates in Petri dishes for seven days at laboratory temperature (20–22 °C).

The results indicated that UV doses necessary to eliminate fungal spores present in drinking water are very different – Aspergillus flavus 127,406 μ W.s.cm⁻², A. ochraceus 92,646 μ W.s.cm⁻², A. niger 339,48 μ W.s.cm⁻² and were several times higher than the bactericidal ones (6000–10,000 μ W.s.cm⁻²). UV radiation applied for water disinfection according to standard microbiological water quality criteria (*Enterobacteriaceae*) may worsen the quality of irradiated water from the point of view of hygiene, health and distribution. It is therefore necessary to determine the effective doses for each disinfected water source experimentally and individually in dependence on the character of present microbial species.

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Molecular genetic methods in the demonstration of invasive mycotic infections

Metody molekulární genetiky při průkazu invazivních mykotických infekcí

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Systemic mycoses caused by *Candida* spp. and *Aspergillus* spp. represent a serious worldwide problem, particularly in immunocompromised hosts, because in such patients they are connected with high morbidity and mortality rates. Therefore, it is necessary to identify these organisms in the human body as soon as possible. The aim of this communication is to present an overview of molecular genetic methods used for the detection of pathogenic fungi. They are mostly based on an analysis of chromosomal DNA. For direct detection of mycotic elements in clinical samples, various modifications of the polymerase chain reaction (PCR) are available. A lot of primer pairs for the amplification of highly conserved sequences of fungal genomes were developed; most of them were derived from the 18S or 28S subunits of the rRNA gene. Some amplicons are specific to single fungal genera or species, others are "panfungal" and the PCR product must be Colloquium Fungi as Model Organisms: Medical mycology

further identified using hybridisation with a specific gene probe, restriction with endonucleases or through two-step "nested" PCR. Because of its rapidity and specificity, PCR should be adopted as a part of routine mycology diagnosis in large hospitals, particularly for risk patients with neutropenia. Amplification of conserved sequences followed by restriction endonuclease analysis (REA) appears to be promising also for accurate identification of various fungal cultures at the species level. For an epidemiological analysis of outbreaks, various molecular methods are available. However, none of them has been accepted as a standard so far. In contrast to direct detection of fungal DNA in clinical samples, typing methods are based on evaluation of genetic variability among fungal strains. As the most frequently used techniques, REA followed by hybridisation with specific gene probe, karyotyping using pulsed-field gel electrophoresis and random amplification of polymorphic DNA (RAPD) were reported. As each method has some drawbacks, the simultaneous use of two of them has been recommended for verification of results.

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Effect of air cleaners on spores of microscopic filamentous fungi in the indoor air of a nursery school

Vliv čističů vzduchu na spóry mikroskopických vláknitých hub v ovzduší mateřské školy

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Air cleaners are recommended by commercial companies for reducing concentrations of moulds in indoor air to improve its quality and as a suitable remedy for patients with an allergy to moulds. Exposure to moulds in the indoor environment is usually assessed by monitoring of culturable total spore counts of microscopic filamentous fungi.

The levels of mixed populations of moulds in the air of a nursery school (two rooms with air cleaner, one room without air cleaner, and a cloakroom without cleaner) and outdoors were examined. Air was sampled by an RCS Plus aeroscope on YM agar strips (cultivation 5 days at 24.5 °C). The study was performed over a period of two years.

The mean values of concentrations of mixed mould populations in the air were (CFU.m⁻³): 66.1 \pm 60.3 (room with cleaner, third floor), 96.1 \pm 56.7 (room without cleaner, second floor), 102.7 \pm 119.4 (room with cleaner, ground floor), 218.7 \pm 190.6 (cloakroom, ground-floor) and 335.7 \pm 237.0 (outdoors).

No statistically significant differences in the concentration of moulds in the air between rooms were found. Mould concentrations were in correlation with air humidity (r = 0.66).

The concentrations of moulds in all examined rooms were at an acceptable level in accordance with values of European Union Recommendation. The highest mould concentrations were detected outdoors.

We conclude that air cleaners do not influence the concentrations of moulds in indoor air and therefore cannot improve the health of people with an allergy to moulds. Air cleaners should not be recommended as a means for these patients to alleviate their situation.

Nested PCR detection of Aspergillus species DNA in clinical samples

Detekce aspergilové DNA v klinických vzorcích uhnízděnou PCR.

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Invasive aspergillosis (IA) represents an important cause of morbidity and mortality in immunocompromised hosts. A fast and accurate diagnosis plays key role in efficient therapy of IA. PCR amplification of foreign DNA may provide a promising alternative to traditional culture methods. We have tested a previously developed system for nested PCR amplification of an *Aspergillus* species-specific fragment of 18S RNA gene in 70 clinical samples obtained from patients suffering from a serious underlying immunocompromising disease and showing signs of suspected IA. PCR showed 11 positive samples in contrast to only 1 sample with successful cultivation of *Aspergillus* species. The positive samples were represented by 4 blood samples, 4 samples of bronchoalveolar lavage (BAL) fluid and 3 sputum samples. Contamination of the sputum samples by airborne *Aspergillus* conidia could not be excluded. However, the cooperating clinicians insisted on examination of the samples to acquire complementary information. The underlying diseases in patients with positive samples were represented by 6 cases of myeloid leukemia, 1 case of lymphoid leukemia, 2 cases of non-Hodgkin lymphoma, 1 case of morbus

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Hodgkin and 1 case of pneumonia. Our results clearly show that nested PCR of *Aspergillus* species DNA in clinical samples can provide a sensitive, fast and accurate tool in diagnostics of systemic mycoses.

Hsp90 vaccination in systemic candidiasis

Hsp90 vakcína u systémové kandidózy

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Rationale. Inherited or secondary immunodeficiencies are frequently associated with increased incidence of different forms of candidiasis. The therapeutic effect of antifungal drugs is highly individual. These problems led to considering alternative preventive and therapeutic approaches. Protective anti-*Candida* immunity recognises, among other antigens, the *Candida albicans* heat shock protein (hsp90). Previous experiments used hsp90 antigen in the form of a protein vaccine. Other approaches can be used for the induction of immune response such as a DNA vaccine. The possibility to select a suitable Th1/Th2 immune response is the main advantage of the DNA vaccine. We compared the effectiveness of the hsp90 protein and DNA vaccines in a mouse model of disseminated candidiasis.

Methods. Hsp90 cDNA was isolated from the yeast form of *C. albicans*. Recombinant protein was expressed in an *Escherichia coli* system. DNA vaccine was prepared by cloning hsp90 cDNA into pVAX1 vaccination plasmid. Suitable challenge strain of *C. albicans* was passaged over mice to achieve a constant lethal dosis. Experimental mice (BALB/c) were divided into four groups of five mice and vaccinated with two doses in different ways. Groups I and II: intradermal injection of 0.4 and 0.16 mg of hsp90 protein in CFA/ICFA. Group III: intramuscular injection of 0.1 mg of DNA vaccine without adjuvans. Group IV (control) was vaccinated intradermally with CFA/ICFA. 15 days after the second dose all groups were challenged intravenously with 10^7 CFU of *C. albicans*. Protectivity was assessed by clinical appearance.

Results. Protein vaccination extends significantly the surviving of vaccinated mice in comparison to the control and DNA-vaccinated group. A low dosage of recombinant protein vaccine showed better protectivity than a high dosage. All long surviving mice (protein vaccinated) showed signs of spastic paresis in contrast to short surviving mice with no spastic syndromes.

Discussion. In contrast to our previous observations we did not confirm significant protective effects of DNA vaccination. It could be caused either i) by rapid killing of most *Candida* yeasts resulting in a systemic toxic shock or ii) by

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an inappropriate vaccination scheme. The better effect of vaccination with a low dosage of recombinant hsp90 protein could be explained similarly in two ways. To confirm our hypotheses we are currently performing similar experiments aimed at an evaluation of the immunity parameters (Th1/Th2 cytokine profile of specific T lymphocytes and specific serum antibodies level) in particular groups.

Acknowledgement. The project was supported by grant no. 14501104 of Palacký University.

Evaluation of the mycological quality of drinking water in the Slovak Republic in the year 2001

Hodnotenie mykologickej kvality pitnej vody v Slovenskej republike v roku 2001

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Microfungi in drinking water are an important group of microorganisms. Seemingly pure water can be a source of contamination with microfungi. Within the framework of the project no. 10.3. "Determination and identification of microfungi in drinking water in the Slovak Republic" we investigated a set of 2916 samples of drinking water according to the STN ISO 7954 norm.

73.30 % of tested water samples from Slovakia showed the presence of microfungi. 79.38 % of samples of drinking water showed less than 100 colony forming units of microfungi per 100 ml. In the current regulation, maximum tolerated levels are applied only to saprophytic species of microfungi. If the water contains species able to produce mycotoxins, drinking water is considered defective for health.

In the total amount of tested drinking water samples, 6% of samples contained potential producers of toxins and 4.6% of samples were positive for microfungi of the genus *Fusarium*.

It is necessary to pay steady attention to the problem of microfungi within the whole area of the Slovak Republic. Colloquium Fungi as Model Organisms: Medical mycology

Ecological studies in black yeasts

Ekologické studie u černých kvasinek

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Black yeasts are now ranked under the Ascomycetes, order Chaetothyriales, family *Herpotrichiellaceae*. Melanin in their cell walls is a virulence factor and enables them to survive in the phagolysosomes of neutrophils followed by cell penetration and tissue invasion. Different forms of phaeohyphomycosis, chromomycosis and mycetoma can develop.

The source of nosocomial phaeohyphomycosis is searched for in iatrogenic intervention in the environment, on skin and in the respiratory tract of the hospital staff.

However, it should be kept in mind that food, especially fruit which is part of the diet in hospitals or is brought in by visitors, is in contact with the environment containing many species of fungi. Thanks to the presence of sugars, fruits are a suitable milieu for fungal reproduction.

The greatest quantity of *Dematiaceae* (black yeasts and filamentous fungi) was found by our group on red currants and white plums. Only one species (*Aureobasidium pullulans*) was found on bilberries; cherries and bananas harboured only *Cladosporium herbarum*.

The contamination found on ten species of fruit is important because the present fungi can cause various forms of phaeohyphomycoses in immunocompromised patients.

Preparation of new antimycotic vaccines

Příprava nových antimykotických vakcín

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This contribution is focused on a review of contemporary knowledge on immunity against fungal infections. While the knowledge on immunity against bacterial and viral infections has developed relatively rapidly, the situation in fungal infections is more complicated. It is due to

1) the eukaryotic nature of fungi and their high morphological and biochemical variability

2) the complexity of host defence against fungal pathogens, based primarily on specific cell immune response.

Despite of this limitation a number of antifungal, specific and non-specific, effector mechanisms were discovered. These experiments paved the way for the development of several types of vaccines. The efforts on the preparation of various vaccines against *Candida albicans, Cryptococcus neoformans, Coccidioides immitis* and some dermatophytes were mentioned in detail. The experiments with candidate antigens, especially heat shock proteins, were presented in full, just as the progress toward preparation of a DNA vaccine against fungal pathogens.

Mycology of foods and mycotoxins

Micromycetes as "starter cultures", foodstuffs and the protection of public health

"Kulturní" vláknité mikromycety, potraviny a ochrana veřejného zdraví

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Some of the most important organisms used in biotechnology are micromycetes (moulds) – "starter cultures". Fermented foods and beverages have been manufactured for thousands of years. Traditional fermented foods and beverages with "starter cultures" around the world may be divided into the following categories:

- Fermented alcoholic beverages. The traditional alcoholic beverages in Japan are non-glutinous rice wine named sake or seishu and distilled sake called shochu. Sake is ripened by *Aspergillus oryzae*.

- Fish or meat fermented with enzymes derived from the cells of Aspergillus species together with lactobacilli in the presence of high salt concentrations. Katsuobushi is made in Japan by fermenting cooked bonito fish with Aspergillus glaucus until it dries out. Shavings of the resulting hard, dark substance are used to flavour other foods.

- Proteinaceous plant foods fermented with *Rhizopus* or *Actinomucor* species with or without salt. Tempeh, a soybean product, is an important food in Indonesia. It is an attempt to make the notoriously indigestible soybean both edible and tasty by exploiting fungal enzymes. Soybeans are cooked, then inoculated with *Rhizopus oligosporus*. Sufu is a Chinese version of soybean cheese, the fungus involved being *Actinomucor elegans*.

- Proteinaceous plant foods fermented with *Aspergillus* species, followed by yeast and lactobacillus fermentation in the presence of high salt concentrations. Shoyu (soy sauce) is a standard part of the everyday Japanese menu. Shoyu is made from a mixture of wheat and soybeans or soybean flour with *Aspergillus* oryzae, yeast and *Lactobacillus*.

- Soft-ripened Camembert-type cheeses, and blue Roquefort-type cheeses. The Camembert-type cheeses (e.g. Camembert, Brie, Hermelín) are ripened by *Penicillium camemberti* or *Penicillium caseicola*. These micromycetes form a dense white mycelial mat on the outside of the cheese, and their extracellular proteases give the cheese a wonderfully smooth, soft, almost buttery consistency.

P. camemberti produces a dangerous mycotoxin called cyclopiazonic acid. The blue cheeses (e.g. Roquefort, Gorgonzola, Stilton Danish Blue and Niva) are ripened by *Penicillium roqueforti*. *P. roqueforti* is able to oxidise fatty acids to methyl ketones, which are believed to give the cheese its penetrating smell and its unique, pungent flavour. Although under some conditions *P. roqueforti* can produce a dangerous mycotoxin called PR toxin, this is fortunately not formed during the cheese-making process.

- Fermented salami and fermented meat products. *Penicillium nalgiovense* is used as a starter culture for fermented meat products in Europe.

– Other fermented foodstuffs. The fleshy ascomata of $Cyttaria\ darwinii$ and $C.\ espinosae$ (Ascomycotina) contain more than 15 % of fermentable carbohydrates. Natural fermentation of ascomata produces a refreshing, mildly alcoholic drink called "chicha del llau-llau" in Chile. *Monascus*, also known under the name Angkak or red fermented rice is mostly produced on the basis of glazed rice and fermentation with *Monascus purpureus* or related species. Positive health aspects of foods and drinks prepared from or with *Monascus* have been well-known for centuries.

The food safety of "starter cultures" (elimination of production of mycotoxins and other toxic compounds) is very important in the protection of public health in the Czech Republic.

Penicillium expansum – an important contaminant of apples and producer of patulin

Penicillium expansum – významný kontaminant jablek a producent mykotoxinu patulinu

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Several species of *Penicillium* have been isolated from apples naturally infected with the blue mold but *Penicillium expansum* is the most common and economically important species.

"Apple blue mold", also known as soft rot or wet rot is the most prevalent of the postharvest rots in apples. The soft rot is characterised by a light brown discolouration in the early stage. The decayed tissue is completely mushy and can be separated from healthy tissue by flushing with water. Blue mold infections can occur even at 0 °C and usually originate from wounds. Lenticels on any part of the apple may also become infected, especially in over-mature or long-stored fruit. Colloquium Fungi as Model Organisms: Mycology of foods and mycotoxins

A large mass of blue green spores develop as the rot radiates from the point of infection. Spore production is accelerated at higher temperatures, and these spores become a source of infection for other fruit.

The frequent occurrence of *Penicillium expansum* on apples is probably due to growth of the mould on rotten matter in orchards, from where it could infect the trees and the apples. Indeed, with soil as its host substrate, *Penicillium expansum* is often isolated from the surface of healthy fruit tissue.

Penicillium expansum is a psychrophile. The minimum temperature that has been reported for this species is -3 °C. The optimum temperature is close to 25 °C and the maximum close to 35 °C. The minimum water activity (a_W) for germination is 0.82–0.83.

Penicillium expansum is able to produce patulin and citrinin. The optimum water activity a_W is 0.95 at a temperature of 25 °C for the production of patulin. A modified atmosphere of 3 % CO₂ and 2 % O₂ completely inhibited patulin production at 25 °C. Patulin is a heat-resistant mycotoxin, and pasteurisation at 90 °C for 10 seconds caused up to 20 % reduction. Patulin is gradually destroyed during storage in the presence of sulphites, – SH groups, and ascorbic acid. Fermentation of apple juice to produce alcoholic beverages results in a complete destruction of patulin.

Apples with this decay should not be used for processing. Poor quality control, i.e. the use of rotting fruit in juice or cider production can result in high concentrations of patulin in juice. Apple juice prepared from apples contaminated with *Penicillium expansum* could be a possible source of patulin in the human diet. Patulin has been found to occur at high levels (hundreds of ng/g) in some apple juice products.

The World Health Organization (WHO) has recommended a maximum patulin level of 50 ng/g in apple products. At least twelve countries regulate patulin at 30–50 ng/g. Patulin is limited by hygienic regulations in the Czech Republic in Decree No. 53/02 Coll. (apples 50 ng/g, in baby food 30 ng/g and in infant food 20 ng/g) issued according to Act No. 110/97 Coll. on foodstuffs and tobacco products. JECFA/WHO established a provisional maximum tolerable daily intake (PMTDI) for patulin of 0.4 ng/kg body wt/day. Patulin is not classifiable as to its carcinogenity to humans (IARC/WHO).

We studied the hygienic problem of the contamination of the apple cultivar Gloster with *Penicillium expansum* and the presence of patulin in our laboratory. The surface tissue of stored apples was not damaged. However, the core of 3 % apple samples was contaminated with spores of *Penicillium expansum* through the calyx fossa and open calyx tube. Patulin has been found at levels of tens to hundreds of ng/g in the apple samples. This demonstrates that an efficient control of patulin contamination primarily depends on careful fruit grading and handling practices prior to further processing.

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Experimental contamination of foodstuffs with spores of toxigenic micromycetes and the production of mycotoxins

Experimentální kontaminace potravin spórami toxinogenních mikromycetů a produkce mykotoxinů

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Foodstuffs are suitable substrates for the contamination, growth and reproduction of toxigenic micromycetes and, subsequently, for the production of mycotoxins. There are approximately 114 species of micromycetes that are very important in foodstuffs, 65 of them toxigenic. They are important factors that may have a potentially negative effect on human health. Therefore, foodstuffs contaminated with toxigenic micromycetes present a serious hazard by so-called "hidden mycotoxins". Mould foodstuffs containing toxigenic micromycetes and mycotoxins present a great hazard to the health of the Czech population, especially in terms of the so-called late toxic effects (e.g. carcinogenicity and developmental toxicity). The most important toxigenic micromycetes are the producers of aflatoxins.

A selection of commodities was based on data of a consumer food basket and focused on important groups of foodstuffs known to be contaminated with toxigenic micromycetes in the Czech Republic and in other parts of the world. Based on these facts, a study of experimental contamination of foods (bread, apricot jam and Edam cheese) by spores of *Aspergillus flavus* was prepared.

The production of aflatoxins after experimental contamination of bread was estimated after 72 hours of storage. The found values were 34.3 ng/g of aflatoxin B_1 , 34.2 ng/g of aflatoxin G_1 , and <0.35 ng/g of aflatoxins B_2 and G_2 . Conditions of the contamination: spores of *Aspergillus flavus* CCM F-108, the fall-out metod. Conditions of the storage: 21 °C, dark, plastic (PE) bag.

The production of aflatoxins after experimental contamination of apricot jam light (23.5 g sugar per 100 g jam light, 100 mg aspartame per 100 g jam light) is presented in Table 1. Conditions of the contamination: spores of *Aspergillus flavus* CCM F-108, the fall-out method. Conditions of the storage: 6 °C and 19 °C, 7 days, dark, consumer package (glass).

The production of aflatoxins after experimental contamination of Edam cheese (45 % fat in dry matter) is presented in Table 2.

Conditions of the contamination: spores of Aspergillus flavus CCM F-836, the fall-out method. Conditions of the storage: 21 °C, 14 days, dark.

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 Table 1. Results of production of aflatoxins after experimental contamination of apricot jam light.

Layer (mm)	6 °C / 7 days		19 °C / 7 days	
	Aflatoxin B1(ng/g)	Aflatoxin G1(ng/g)	Aflatoxin B1 (ng/g)	Aflatoxin G1 (ng/g)
0-8	4	14.8	7.6	26.6
8-16	<1	<1	<1	<1
1624	<1	<1	<1	<1

Table 2. Results of production of aflatoxins after experimental contamination of Edam cheese.

Layer (mm)	21 °C / 14 days		
	Aflatoxin B ₁ (ng/g)	Aflatoxin G1(ng/g)	
05	24	549	
5-10	7.5	67.5	
10-15	< 0.7	6	
15-20	< 0.7	<0.7	

Chemotaxonomy of aflatoxigenic species of Aspergillus section Flavi

Chemotaxonomie aflatoxinogenních druhů rodu Aspergillus, sekce Flavi

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Aflatoxigenic strains of Aspergillus species are important microorganisms capable of producing aflatoxins and other mycotoxins as aspergillic acid, cyclopiazonic acid etc. Aspergillus flavus, A. parasiticus, A. nomius, A. tamarii, A. pseudotamarii and A. bombycis are six morphologically similar species belonging to the Aspergillus section Flavi. The aflatoxigenic strains of the fungi are isolated from foods (cereals, pulses, oilseed, dried fruit, spices), soil, air and water.

Mycological analyses are based on valid standards and recommendations of the International Commission for Food Mycology (ICFM). Identification of the isolated aflatoxigenic fungi in foodstuffs and feedstuffs is possible by using:

1. Classical mycological cultivation methods (morphological and culture criteria).

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- 2. Diagnostic nutrient media (Aspergillus flavus and Aspergillus parasiticus agar (AFPA) medium from Oxoid and Aspergillus Differentiation Medium Base (ADMB) from Himedia).
- 3. Chemotaxonomy, carried out by high performance thin-layer chromatography (HPTLC) determination of selected mycotoxins in YES (Yeast Extract Sucrose) medium after incubation at 30 °C for 14 days.
- 4. Molecular methods, e.g. Polymerase Chain Reaction (PCR), have recently been used to assess toxigenicity. They represent independent confirmatory methods able to detect specific genes (omt-1, ver-1, afl R /apa-2/) that encode enzymes participating in the biosynthesis of aflatoxins.

The functional system approach to the identification of aflatoxigenic fungi has to combine results of the classical mycological cultivation methods, diagnostic nutrient media, chemotaxonomy and molecular biological methods.

Chemotaxonomy is a specific method for the determination of a metabolic profile of toxigenic fungi based on the identification of their secondary metabolites – mycotoxins. It enables to carry out their species identification. If a strain of the microscopic fungi species is found to produce a specific mycotoxin, then all the strains of this species are considered to be potentially toxigenic, i.e. capable of producing a specific mycotoxin. Uncertainties of the method occur in case:

 the isolated fungus does not produce aflatoxins or other mycotoxins, even if it is a toxigenic strain.

– the isolated strain produces other mycotoxins than those typical of the given species.

Thirty strains of aflatoxigenic fungi obtained from the Culture Collection of Fungi (CCF) were tested. Three strains differed from the chemotaxonomic profile of the species published in the literature.

Aspergillus taxonomy is based on morphological and physiological similarities. This approach is, however, very-time consuming and may lead to misclassification. Rapid and more objective methods for the identification of aflatoxinogenic fungi are necessary for an evaluation of microbiological risks of the given food and feed. Interpretation of secondary metabolite data is very difficult. That is why molecular methods could be a possible alternative approach for an accurate, sensitive, and specific identification and confirmation of the aflatoxinogenic fungi in foods and feeds.

The significance of toxinogenic strains in foodstuffs must not be underestimated nor overestimated. It is necessary to pay attention to their study and maintain their research. Colloquium Fungi as Model Organisms: Mycology of foods and mycotoxins

New findings in mycotoxin research

Aktuální novinky v oblasti studia mykotoxinů a jejich producentů

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The recent news can be summarized according to the following topics:

The occurrence of ochratoxin A (OTA) in foodstuffs (grapes, musts, wines and raisins).

Leitner et al. (2002) compared different analytical methods for the determination of OTA in wine, Pietri et al. (2001) found the amount of OTA to range from less than 1 to 3856 ng/l for red wines, Castelari et al. (2001) evaluated a variety of fining agents for their abilities to remove OTA in fortified wines, Sage et al. (2002) investigated grapes and musts used in red table wines for the occurrence of potential OTA producing micromycetes.

The development of analytical methods for the determination of biomarkers of mycotoxins in human biological material (blood plasma and serum, urine). Škarková and Ostrý (2000) evaluated an HPTLC method for the determination of ultra-trace amounts of aflatoxin M_1 in human urine. Several authors (Peraica et al. 2001, Thuvander et al. 2001, Gilbert et al. 2001, Malir et al. 2001) determined ochratoxin A in blood, resp. urine of a healthy population to assess a dietary exposure to this toxin.

Fusarium toxins and their occurrence. Great effort is devoted to the research of all groups of fusarium toxins, that usually occurr together in contaminated materials (Visconti et al. 2001, Keblys et al. 2001, Radova et al. 2001). Bakan et al. (2001) studied the toxigenic potential of *Fusarium culmorum* strains isolated from wheat.

The development of molecular methods for the identification of toxigenic micromycetes – Aspergillus, Penicillium and Fusarium species. Besides classical identification methods molecular methods are being developed, e.g. polymerase chain reaction (PCR) and chip technology. They are independent confirmatory methods able to detect specific genes that encode enzymes participating in the biosynthesis of mycotoxins (Chiou et. al. 2002, Färber and Geisen 2001, Seo et al. 2001).

Interaction between GM plants (maize) and *Fusarium moniliforme*. Many authors are concerned in a comparison of fungal growth and fusarium mycotoxin contents in isogenic traditional maize and transgenic Bt-maize hybrids (Bakan et. al. 2002, Valenta et al. 2001).

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Mycotoxins observation after the harvest of grains of naked oat

Sledování mykotoxinů po sklizni obilek nahého ovsa

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As part of a number of small-scale experiments we examined the biopreparation Supresivit S 2 containing the propagules of the antagonistic fungus Trichoderma harzianum. The small-scale experiments, which were carried out in the experimental plot of the Department of Crop Production (University of South Bohemia in České Budějovice, Faculty of Agriculture), were aimed at impairing the biotic fungistasis by biological means applied to grains of naked oat, variety Adam. Our objective was to accomplish a surface microbial analysis of stored naked oat grains, variety Adam, after application of the biofungicide Supresivit S 2 in combination with surface treatment of the grains and biological screening applied during the vegetation period. The harvest of naked oats, variety Adam took place at the beginning of full ripeness in phase 91 DC, during sunny, warm weather. The harvest itself was carried out in individual small plots. Particular variants were hand-mown and individual yields were thrashed by the stationary thrasher Veb Fortschritt K-119. In the presence of Dr. H. Lew and Dr. A. Adler (Bundesanstalt für Agrarbiologie, Linz), 1 kg of oat specimens of each observed variety were taken and transported to Linz for microbial analysis of fungi colonising grain surfaces in stored variants as well as for assessment

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of the occurrence of secondary Fusaria metabolites. The fungi of the genus Fusarium were tested by cultivating them on a modified nutritional substance according to Papavizas (1985). In particular variants of the experiment we analysed the contents of some mycotoxins produced by the fungi of the genus Fusarium in surface parts of the stored grains. The fungi of the genus Fusarium were most numerous in the variant oats – bioagent, namely 250 spores per 1g of grain. In all other variants the amounts of mycotoxins were considerably below the known effective doses for animals and plants. The largest amount of vomitoxin was found in the variant chemical standard (Rovral TS, effective substance Carbendazim 17.5 % and Iprodion 35 %) – 18 μ g. Zearalenon was found in all variants to an amount of up to 5 μ g.

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Detection of aflatoxinogenic fungi in feed using the PCR method

Detekce aflatoxinogenních hub v krmivech metodou PCR

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This work deals with the possibility of using the polymerase chain reaction (PCR) method for acceleration and a more accurate identification of aflatoxinogenic fungi isolated from feed.

The method was optimised on pure cultures (*Aspergillus flavus* CCM F-108 and *Aspergillus parasiticus* CCM F-550). The specificity of the optimised PCR method was verified using various fungal strains.

50 samples of feed were examined, 18 of which were positive for the presence of aflatoxinogenic fungi on AFPA medium. Isolated *Aspergillus* strains were examined using the PCR method. The obtained results almost always agreed with the results of conventional identification on AFPA medium.

This method is a possible starting point for accelerating the detection of aflatoxinogenic fungi, but it will be necessary to solve certain non-specific reactions, which are caused by a complex sample matrix.

The PCR technique itself has proved to be useful in the detection of aflatoxinogenic fungi isolated from feed.

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