

**Colloquium „Fungi as Model Organisms in Research
and Biotechnology – III“ Olomouc, Czech Republic,
2 September 2005**

The colloquium was a continuation of the previous scientific meetings that took place in Olomouc in 1999 and 2002 (Czech Mycology 52: 139–178, 2000 and 55: 103–149, 2003). It was organised by the 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 aim of the colloquium was to provide a platform for a broad discussion on experimental mycology in all branches of basic and applied research. Besides two plenary lectures, 8 short communications and 8 posters were presented. In total 32 researchers from the Czech and Slovak Republics took part in the colloquium and discussed various topics important for the further development of experimental mycology. Abstracts of the contributions are given below.

Jiří Kunert and Vladislav Raclavský

Occurrence of *Fusarium* in food and feed

Plísňe rodu *Fusarium* v potravinách a krmivech

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Two PCR methods were evaluated for the successful identification of *Fusarium* species. The PCR was performed using a primer specifically targeted to *Tri5* gene. The DNA extraction technique by Cenis (1992) and the PCR procedure according to Edwards et al. (2001) were compared with the DNA isolation and amplification using the Plant PCR Kit. The sensitivity of the latter method was determined using the reference strain of *Fusarium graminearum* CCM F-683 and the selectivity was examined using reference cultures of different genera of fungi naturally occurring in food and feed. Fifty food and feed samples were examined for the presence of trichothecenes-producing *Fusarium* species. In total, seven *Fusarium* species were isolated, five of which reacted positively with the primer, resulting in an expected size amplicon of 260 pb. Macroscopic and micro-

scopic features of pure cultures of *Fusarium* and of other isolated fungi were observed during growth in different laboratory media. Morphological features are important for species identification whereas the PCR can distinguish between toxigenic and non-toxigenic species of *Fusarium*. These fungi could be detected within 2 days, while their identification using macroscopic and microscopic features takes 2–3 weeks. Thus the PCR technique proved to be a reliable and rapid method for the detection of toxigenic *Fusarium* species.

This work was supported by GA ČR Grant no. 203/05/2106 and the Ministry of Education, Youth and Sports of the Czech Republic, project no. 0021627502.

Identification of *Aspergillus niger* isolates (with the PCR method) and their catalase production as a response to pollutant stress by metals

Identifikácia izolátov *Aspergillus niger* (pomocou PCR metódy) a ich katalázová aktivita ako odpoveď na stres prostredia znečisteného kovmi

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For this particular study, four isolates of *Aspergillus niger* were used. *A. niger* strain 1 was taken from a collection and was used as a reference. *A. niger* strain 2 was isolated from coal dust near a mine (As 400 mg/kg). *A. niger* strain 3 was an isolate from river sediments near the same mine (As 1651 mg/kg, Sb 362 mg/kg) and *A. niger* strain 4 was adapted on Sabouraud medium supplemented with As⁵⁺ (5 mg/l).

Morphological and biochemical identification was confirmed by the PCR method. PCR uses small differences in chromosomal DNA for 5.8S rRNA (coding sequence and two intergenic spacers ITS1 and ITS2) of two strains of *A. niger* and *A. tubingensis*, major species in the *A. niger* aggregate. PCR showed that all cultures belonged to the same species *Aspergillus niger*. For a negative check, *Aspergillus niger* var. *tubingensis* (Mosseray) Kozakiewicz CCF 2818 was used.

The isolates of *Aspergillus niger* isolated from coal dust of a mine containing arsenic and from river sediments of mine surroundings growing in a minimal nitrate medium and developing hyphae and spores, exhibited much higher levels of total catalase activity than the strain of the same species from the culture collection and the culture adapted to medium supplemented with As. Electrophoretic separation of catalases from cell-free extracts revealed three isozymes of catalases. Production of individual isozymes was not significantly affected by stress environments. Exogenously added stressors (As⁵⁺, Cd²⁺, Cu²⁺) at final concentrations

of 25 and 50 mg/l and H₂O₂ (20 or 40 mM) mostly stimulated production of catalases only in isolates from mine surroundings. H₂O₂ and Hg²⁺ caused the disappearance of catalases with the smallest molecular weight. Isolates from the mine environment exhibited a higher tolerance to toxic effects of heavy metals and H₂O₂ (monitored by growth) than the strain from the culture collection did.

The study was supported by grant VEGA no. 2/5069/25 and APVT project 51-024804.

Control strains of filamentous fungi and yeasts in the Czech collection of microorganisms

Kontrolní kmeny vláknitých hub a kvasinek v České sbírce mikroorganismů

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The Czech Collection of Microorganisms (CCM) is a non-profit organisation established in 1963. At present, the CCM holds well over 3,000 strains of more than 810 species of bacteria and about 700 strains of more than 500 species of fungi in pure cultures, covering a very wide range of applications. The CCM provides specialised collections of 144 quality control strains for microbiological laboratories. These control strains are especially intended for diagnostic laboratories in human and veterinary microbiology as well as microbiological laboratories of quality control of food and water. The control strains of filamentous fungi belong to the hyphomycetes and yeast cultures to the saccharomycetes. The quality control strains are supplied freeze-dried in sealed glass ampoules and in the form of gelatin discs. Actual information about cultures is available on our web site (<http://www.sci.muni.cz/ccm/>).

List of control strains of filamentous fungi and yeasts:

CCM	Strains	Applications
8186	<i>Candida albicans</i>	media, sterility and disinfectants testing
8189	<i>Aspergillus niger</i>	media, fungus resistance and wood preservation testing
8191	<i>Saccharomyces cerevisiae</i>	media testing, assay of antibiotics
8215	<i>Candida albicans</i>	media, sterility and antimicrobial preservative testing
8222	<i>Aspergillus niger</i>	media, antimicrobial preservative testing
8223	<i>Candida tropicalis</i>	media testing, assay of antibiotics
8224	<i>Zygosaccharomyces rouxii</i>	antimicrobial preservative testing

CCM	Strains	Applications
8226	<i>Candida albicans</i>	media, sterility and fungicides testing
8260	<i>Candida parapsilosis</i> (ATCC 22019)	antifungal susceptibility testing
8261	<i>Candida albicans</i> (ATCC 90028)	antifungal susceptibility testing
8359	<i>Penicillium hirsutum</i>	media testing
8363	<i>Aspergillus flavus</i>	media testing
F-108	<i>Aspergillus parasiticus</i>	media testing
F-550	<i>Aspergillus parasiticus</i> var. <i>globosus</i>	media testing

Heavy metal contents in fruit bodies of wood-rotting fungi collected in the Kłodzko region, Poland

Obsahy těžkých kovů v plodnicích dřevokazných hub z oblasti Kłodzka (Polsko)

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Fruit bodies of *Fomes fomentarius*, *Fomitopsis pinicola*, *Stereum sanguinolentum*, *Gloeophyllum sepiarium* and *Osmoporus odoratus* were collected in June 2005 in an area delimited by Międzyzylesie, Międzizórze, Králický Sněžník and Klecienko. A total amount of 28 samples was collected. Dried fruit bodies were mineralised in a microwave digestion unit (nitric acid / hydrogen peroxide 5:1) and contents of Cd, Cu, Mn, Pb and Zn was determined with atomic absorption spectrometry. Average contents in all samples were 14.04 ± 12.02 ppm Cu, 2.17 ± 1.11 ppm Cd, 93.49 ± 136.82 ppm Mn, 6.00 ± 2.69 ppm Pb and 73.85 ± 50.01 ppm Zn. The samples were then divided into four groups, corresponding to the collection regions. The highest Cu and Cd contents was found in the area of Jodłów, while Mn, Zn and Pb content reached maximum in samples from Králický Sněžník. Average metal contents in the samples of *Fomes fomentarius* were 14.67 ± 8.20 ppm Cu, 2.31 ± 1.03 ppm Cd, 89.45 ± 176.76 ppm Mn, 5.45 ± 2.45 ppm Pb and 60.50 ± 59.48 ppm Zn. The results for *Fomitopsis pinicola* were 7.07 ± 2.09 ppm Cu, 2.20 ± 1.22 ppm Cd, 54.50 ± 65.90 ppm Mn, 7.63 ± 2.02 ppm Pb and 82.23 ± 33.47 ppm Zn. Cadmium and lead contents in samples collected in Poland were higher than those in samples collected in the nearby Jeseníky Mts. in the Czech Republic in 1995.

The use of fungi in folk and official medicine

Využití hub v lidové i oficiální medicíně

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This introductory lecture described the use of fungal fruit bodies in folk and official medicine. At first, attention was paid to hallucinogenic properties. Differences in the use of fungal species in Europe, Northern Asia (*Amanita* sp.) and Central America (*Psilocybe* sp., *Panaeolus* sp.) were mentioned. Toxic properties of fungi, especially *Amanita phalloides* were discussed and examples of well-known intoxications (Euripides, Tiberius Claudius, pope Clemens VII etc.) were given. Then a short survey was given of fungal species used in folk medicine. Among others, *Laricifomes officinalis*, *Fomes fomentarius*, *Phallus impudicus*, *Elaphomyces granulatus*, *Calvatia utriformis*, *Langermannia gigantea* and *Inonotus obliquus* were discussed. Attention was also paid to fungal species used in folk medicine in Asia (e.g. *Ganoderma lucidum*, *Trametes versicolor*, *Hirneola auricula-judae*, *Lentinus edodes*). Examples of fungal preparations used in homeopathy (*Russula emetica*, *Boletus satanas*, *Amanita muscaria*) were given and then examples of fungal metabolites with antibacterial, antifungal, virostatic and immunomodulatory effects were given. *Oudemansiella mucida*, the fungus from which mucidin (the only antibiotic used in human medicine, discovered in the former Czechoslovakia) was isolated, was also discussed.

Testing of the virulence of *Cryphonectria parasitica*

Testovanie virulencie huby *Cryphonectria parasitica*

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Cryphonectria parasitica, chestnut blight fungus, occurs in nature in two forms: virulent and hypovirulent. These strains have different physiological and morphological properties (Grente 1965). Hypovirulent isolates are discoloured (paler orange mycelia in cultures), practically without reproduction organs (lowered

sporulation), and with significantly lesser virulence to *Castanea sativa*, compared to the virulent isolates. The trees respond to the hypovirulent strains by promoting callus formation and healing of the cankers. Abnormal cankers from which the hypovirulent strains of the fungus are isolated consist of exposed sapwood bordered by a vigorous callus, with superficial infections radiating from the margins of the openings (Elliston 1985). Cytoplasmic hypovirulent strains consistently contain dsRNA (Anagnostakis and Day 1979). The final effect of the hypovirulent agent (dsRNA hypovirus) is a reduction of mortality of infected trees. Chestnut blight has recently been controlled by means of a biological method based on the use of these hypovirulent strains of *Cryphonectria parasitica*.

No hypovirulent isolates were detected in Slovakia. Slovak virulent isolates were transformed into hypovirulent forms with French hypovirulent isolates and were used for biological control of chestnut blight in Slovakia. In 2004, 53 isolates with morphological properties of hypovirulent strains were obtained from naturally healing cankers. Presence of dsRNA using a molecular method was confirmed in eight of them. This means that the hypovirus spread to normal cankers in a natural way, and an important success in chestnut protection against chestnut blight has been achieved.

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Endophytic fungi on European Horse-chestnut

Endofytické huby na pagaštane konskom

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Endophytic fungi are a group of fungi which live asymptotically inside plant tissues. The endophytic fungi *Phyllosticta sphaeropsoides* Ellis et Everh., *Asteromella*

aesculicola (Sacc.) Petr., *Phomopsis carposchiza* Fairm., its teleomorph *Diaporthe padi* Otth, *Alternaria alternata* (Fr.) Keissl., *Colletotrichum gloeosporioides* Penz., *Epicoccum nigrum* Link ex Wallr., *Botrytis cinerea* Pers. and *Chaetomium* sp. were isolated from symptomatic and healthy-looking leaves, flowers and seeds of European Horse-chestnut. In the study of leaf-inhabiting endophytes, coelomycetes form a dominant group. The three most frequent species were *Phyllosticta sphaeropsoides*, *Phomopsis carposchiza* and *Colletotrichum gloeosporioides*. In general, coelomycetous anamorphs are widespread colonisers of plant tissues, in which they can be present either as pathogens or endophytes (Sutton 1980, Petrini 1986). According to Espinosa-Garcia and Langenheim (1990) leaf endophytes are ubiquitous and their distribution patterns within and among plants, as well as the possible consequences of their presence, reinforce the idea that not only single endophyte species but whole endophytic communities may be very important for the plants that harbour them.

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Effect of light and temperature on tomato powdery mildew (*Oidium neolycopersici*)

Vliv světla a teploty na padlí rajčat (*Oidium neolycopersici*)

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Tomato powdery mildew (*Oidium neolycopersici*) affects the leaves and stems of tomatoes where the white superficial mycelium causes characteristic powdery mildew symptoms. The purpose of the present research was to determine the effect of temperature and light (spectrum, intensity and photoperiod) on germination, development and sporulation of this pathogen. Considering the temperature, development and intensive sporulation occurred within the range

15–25 °C and was the most intensive between 20 and 25 °C. At temperatures slightly below the optimum, mycelial development and appearance of the first conidiophores was delayed. The temperature range for conidia germination was wider than that for mycelium development and sporulation. Some conidia germinated at each of tested temperatures – 10, 15, 20, 25, 30, 35 °C, however at marginal temperatures (10 °C and 35 °C, resp.) the germination was strongly limited. Light influenced the pathogen development also markedly; a maximum sporulation and mycelium development was recorded at light intensities between 60–100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. At lower light intensity the pathogen development was delayed, and finally in the dark the sporulation was completely restricted. The results of the effect of light wavelength on *O. neolycopersici* were more controversial. The pathogen development was more rapid under red, blue and green plastic foil than under white light. The slightly delaying effect of white light compared with colour foils can be caused either by the effect of selected wavelengths on the pathogen or by reduced light intensity under the colour foils, because shading appeared to be more suitable for powdery mildews than direct light. The obtained information about the effect of environmental conditions on *O. neolycopersici* can help improve the strategy of plant protection and can also contribute to general knowledge on the biology and ecology of powdery mildews.

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Ascomycetes as causal agents of economically important crop diseases

Vreckaté huby ako pôvodcovia ochorení ekonomicky zaujímavých plodín

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The leaves, ears and seeds of cereals, including wheat, are liable to be attacked by different organisms during harvest and storage. About 72 % of organisms which attack the wheat seeds and leaves are microscopic fungi. Diseases of winter wheat (leaf spot, head blight, take-all) are frequent and economically important in Slovakia. They are caused by different species of ascomycetes. The aim of this study was to investigate the occurrence and distribution of these fungi on winter wheat fields. These fungi caused necrotic lesions on leaves, stems and ears during plant development and many of them were seed-borne. Most of these fungi were found also as fungi sporulating on glumes of ears.

In 2005, the major components of the leaf spot disease complex were two fungi: *Pyrenophora tritici-repentis*, the cause of tan spot, and *Leptosphaeria*

nodorum, the cause of *Septoria nodorum* blotch. Two minor components were *Cochliobolus sativus*, the cause of spot blotch, and another *Septoria* disease caused by *Septoria avenae* f. sp. *triticea*. All three species of the genus *Septoria* were found on wheat leaves, but only *S. nodorum* (teleomorph stage *Leptosphaeria nodorum*) and *S. avenae* (*L. avenae*) occurred frequently also on ears of winter wheat. The species *S. tritici* (teleomorph *Mycosphaerella graminicola*) was found on ears and leaves of wheat.

The species of the genus *Fusarium* belong to the most frequent species of parasitic mycoflora. *F. graminearum* (teleomorph stage *Gibberella zeae*) was isolated as the dominant species. The perithecia were often found on stalks and ears glumes of wheat at the end of the season.

On wheat debris we also found *Gaeumannomyces graminis* var. *tritici* as the fungus causing take-all of wheat, and further *Cochliobolus sativus*.

All ascomycetous stages of the mentioned fungi persist and spread from infested wheat residues to wheat and weed grasses in the following years.

This work was supported by the Science and Technology Assistance Agency under contract no. APVT-27-009904.

**Spread of introduced powdery mildew species on ornamental woody plants and their partial control by the hyperparasite
*Ampelomyces quisqualis***

Šírenie introdukovaných druhov múčnatiek na okrasných drevinách a ich čiastočná bioregulácia hyperparazitom *Ampelomyces quisqualis*

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Powdery mildew infections remain among the most important plant pathological problems world-wide, despite extensive research on their pathogenesis, epidemiology and control. An epidemic spread of the North American powdery mildew *Erysiphe flexuosa* on *Aesculus* spp. in Europe has been observed since 1999. *Erysiphe elevata* is a powdery mildew common on *Catalpa bignonioides* and *C. speciosa* in North America and was recently introduced in Europe. We have recorded massive occurrence of this powdery mildew diseases in Slovakia since 2003. The spread of both powdery mildew species in other European countries has also been recorded. *E. flexuosa* and *E. elevata* have adapted to ecological conditions of Central Europe. The hyperparasite *Ampelomyces quisqualis* was also observed. A total of 35

samples of *E. flexuosa*-infected Horse-chestnut leaves and 222 samples of *Erysiphe elevata*-infected *Catalpa* leaves were examined in 2004. Abundant pycnidia of *A. quisqualis* were found. The incidence of *Ampelomyces* in *Erysiphe flexuosa* was 41 %. Very obvious mycoparasitism was found in *Erysiphe elevata*. The incidence of *Ampelomyces quisqualis* in *Erysiphe elevata* ranged from 20 to 100 %. Pycnidia of *Ampelomyces* were found in approximately 79 % of samples. This parasite reduces growth and may eventually kill the powdery mildew colony. According to Kiss et al. (2004) the effect of *Ampelomyces* in the control of powdery mildew infections is weak, however, it is still important because it suppresses the sporulation rate of its fungal hosts, and the infected plants recover after *Ampelomyces* has killed the pathogens. No *Ampelomyces* product is registered in Slovakia. Application of the hyperparasite *A. quisqualis* may in the future become an alternative to the use of fungicides for the control of powdery mildews in our country.

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Molecular-genetic approaches to the identification of pathogenic fungi

Molekulárně-genetické přístupy k detekci a identifikaci houbových patogenů

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Generally, identification of pathogenic fungi can rely on the examination of either phenotype or genotype characteristics. Although significant progress has recently been made in standardisation of phenotyping systems, ultimate identification can be complex and time-consuming in some species. Therefore, a vast array of genotyping methods has been developed to provide rapid and accurate species identification. Many of these techniques are based on PCR-amplification of fungal DNA and enable not only accurate identification of isolates, but in many cases also rapid and sensitive direct detection of fungal DNA in clinical samples. We

summarise the most promising approaches to the detection and identification of pathogenic fungi by molecular-genetic techniques including nested PCR, PCR combined with species-specific probes (ELISA or real-time qPCR), PCR followed by other special techniques (capillary electrophoresis for accurate length examination, SSCP, or sequencing) and PCR-fingerprinting. We also present our experience with different nested multiplex PCR-techniques and elucidate the potential of the McRAPD approach newly developed by our group. In our opinion, high-resolution melting analysis of amplified duplex DNA represents the most promising post-PCR technique suitable for rapid and accurate identification of pathogenic fungi. To conclude, molecular-genetic approaches to the identification of pathogenic fungi represent a promising field of applied science. Although it has not yielded any gold standard technique yet, it is already firmly established in the diagnostics of human diseases caused by pathogenic yeasts.

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Fungicide resistance in cucurbit powdery mildew populations on the territory of the Czech Republic

Resistence k fungicidům v populaci padlí tykvovitých na území České republiky

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A survey was conducted on possible fungicide resistance or tolerance of cucurbit powdery mildews, *Golovinomyces cichoracearum* (*Gc*) and *Podosphaera xanthii* (*Px*), using 85 (62 *Gc*, 23 *Px*) Czech isolates dating from the years 2000–2003. The effectiveness of three frequently used fungicides, Rubigan 12 EC (fenarimol), Karathane LC (dinocap), and Fundazol 50 WP (benomyl), at five concentrations, was observed using a modified leaf-disc bioassay. Discs were prepared from adult plants of *Cucumis sativus* cv. Stela F₁. Significant differences among fungicides and even among years were found. Occurrence of resistant and/or tolerant isolates of both powdery mildew species in different locations were observed. Rubigan 12 EC showed a high level of effectiveness and control of powdery mildew at a dosage of 36 µg a.i./ml (optimal concentration). Karathane LC was less effective in 2001–2002, when 5 % of the isolates overcame the registered concentration (105 µg a.i./ml), but 100 % effective in 2003. Fundazol 50 WP

was the least effective of the fungicides, as 94 % of the isolates sporulated at every tested concentration and 75 of the isolates were resistant.

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***Aspergillus niger* as a model organism in the bioaccumulation of arsenic**

Aspergillus niger ako modelový organizmus v procese bioakumulácie arzénu

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In the present study, an alternative procedure has been examined based on bioaccumulation using the fungus *Aspergillus niger* strain 1 and 2 as a biosorbent for the removal of both trivalent and pentavalent arsenic compounds from arsenic-contaminated groundwater running through an abandoned mining area. The fungus *A. niger* strain 1 was isolated from eutric fluvisols FMm (Gabčíkovo region), pH H₂O/KCl = 7.7/7.4 and *A. niger* strain 2 was isolated from stream sediment of the Blatina river (Pezinok-Kolársky vrch region), pH H₂O/KCl = 5.2/4.8; As = 363 mg/kg; Sb = 93 mg/kg.

The toxic and carcinogenic properties of inorganic and organic arsenic compounds make their determination in waters particularly important. The toxicity, reactivity and bioavailability of elements depend on their chemical forms. In general, trivalent forms of arsenic are more toxic than pentavalent forms, and their inorganic forms are more toxic than the organic ones. Due to the physiological and chemical properties of arsenic compounds, the quantification of individual species is required, not simply the determination of the total arsenic concentration. The problem of arsenic contamination in groundwaters has been subjected to extensive study because of its adverse effects on human health, primarily due to the consumption of arsenic-contaminated drinking water. As(V) can replace phosphate in several biochemical reactions whereas As(III) may react with critical thiols in proteins and inhibit their reactivity.

Several treatment technologies, such as coagulation, filtration, ion exchange and adsorption, have been applied to remove excess arsenic from water. The application of fungi to solve the problem of water contaminated with toxic compounds has received increasing attention, as fungi are often dominant organisms in soils.

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Mechanism of biologic methylation of arsenic and its possible application in bioremediation

Mechanizmus biologickej metylácie arzénu a možnosti jej využitia v bioremediácii

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Biological transformation and formation of organometallic compounds are part of the biogeochemical cycle of metals (metalloids). A specific mechanism of biological transformation is biomethylation. This is a natural intracellular enzymatic mechanism, which was theoretically described by Challenger in the first half of the 20th century. Challenger identified trimethylated species of arsenic and antimony and dimethylated species of tellurium and selenium produced by the fungus *Scopulariopsis brevicaulis*. The mechanism of biomethylation is not related only to a metal(loid) metabolic pathway, but is also a part of further physiological processes in prokaryotic and eukaryotic cells. Challenger's mechanistic theory of biomethylation of arsenic interprets biomethylation as a redox reaction which transforms trivalent arsenic to its pentavalent form. The rationale for this reaction is the potential toxicity of trivalent arsenic. Through biomethylation the cell probably manages to eliminate or transform free trivalent arsenic in the cytosol into stable or relatively non-toxic macromolecules, or to exclude arsenic from the cell.

Later on, other fungal species that were able to biomethylate arsenic into mono-, di- or tri-methylated species were isolated. The formation of each arsenic species depends on specific As(III)-methyltransferases that are present in the cell. Production of dimethylarsine and trimethylarsine is important for bioremediation, because these arsenic metabolites are volatile. Gas that contains trimethylarsine is called Gosio gas and is characterised by a garlic odour. In our research we have quantified the volatilisation of inorganic arsenic under laboratory conditions by microscopic filamentous fungi, originally isolated from the locality Pezinok – Kolársky vrch. Along with biomethylation we investigated biosorption as a possible method of application of living mycelia for bioremediation.

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Occurrence of potentially toxigenic fungi in foodstuffs

Výskyt potenciálně toxinogenních plísní v potravinách

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The occurrence of fungal spores, especially those of potentially toxigenic species, was monitored in different cereals and bakery products. Consumption of such a food could affect the human health due to presumptive mycotoxin production. The air is a significant reservoir of fungal spores and may be responsible for their transmission to foods being contaminated during processing, transporting and storing. Although cleaning procedures with common disinfectants could successfully avoid bacterial contamination, fungal spores resist. Hence food products are supplemented with chemical substances which could suppress the growth of fungi.

The objective of this study was to isolate and identify fungi from bakery products and cereals. When potentially toxigenic fungi belonging to the genera *Aspergillus* and *Fusarium* were found, an optimised PCR technique was applied to detect the presence of genes coding for aflatoxin B1 and trichothecenes. Primers specifically targeted to *apa-2*, *ver-1* (*Aspergillus* sp.) and *Tri-5* (*Fusarium* sp.) were used. Different chemical substances were also evaluated for their fungicidal properties. *Cladosporium* sp. and *Aspergillus niger* were the most frequently isolated species, followed by members of the genera *Penicillium* and *Acremonium*. *Aspergillus flavus* and a variety of xerophilic fungi (especially *Eurotium* sp.) were also isolated. From all conservation substances tested, propionic acid and potassium sorbate were found to be the most effective inhibitors of growth of the isolated fungi. A pH dependence of antifungal properties was observed. Results indicate that occurrence of potentially toxigenic fungi in bakery products represents an issue which should be routinely investigated.

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A new cryopreservation method for the maintenance of filamentous fungi

Nová kryoprezervační metoda uchovávání vláknitých hub

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A new alternative method using perlite as a particulate solid carrier in the growth medium with a cryoprotectant was developed for cryopreservation of several basidiomycete species after several partially or completely unsuccessful attempts to preserve them by routinely used cryopreservation procedures. Expanded perlite is a unique volcanic aluminosilicate mineral holding and retaining substantial amounts of water, which can be released as needed. Fungal cultures are grown directly in sterile plastic cryovials with perlite moistened with growth medium, enriched with glycerol as a cryoprotectant and inoculated with an agar plug. The cryovials with perlite overgrown by the mycelium are frozen in a programmable freezer and then placed in liquid nitrogen. Frozen strains are kept in cryovials submerged in liquid nitrogen and when needed they are thawed and checked for viability, purity and changes in growth, morphology and biochemical characteristics. All tested cultures survived the cryopreservation procedure and no negative effects were observed after 6 months of their storage in liquid nitrogen (Homolka et al., *J. Microbiol. Methods* 47: 307–313, 2001). The improved cryopreservation procedure has several additional advantages. One cultivation step is saved by using the cryovials directly for the cultivation of cultures; the transparency of the cryovials makes it possible to check the growth of the culture inside and to prevent problems with insufficient inoculation and contamination. The mycelium grows continuously on perlite and its damage caused by punching it from the agar plate and by subsequent handling is prevented. Specific mycelial structures (rhizomorphs etc.) can be preserved more easily. The described method is evidently suitable for different fungal strains requiring special treatment and seems to be generally applicable to most fungal cultures. This expectation is supported by the promising results of preliminary tests on another 351 basidiomycete strains from 80 different genera and 173 species. Moreover, 40 micromycete (Ascomycota and Zygomycota) strains were at least able to grow on perlite and survive the process of freezing and thawing (at present under further study).

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Evaluation of antibody response in suspected candidosis

Hodnocení protilátkové odezvy u suspektních kandidóz

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In a set of 546 patients (287 with infections of the upper respiratory tract, 102 with alveolitis, 76 with infections of the digestive tract and 76 patients with leukaemia or other malign diseases) the level of antibodies against *Candida albicans* was determined. Four serologic methods were used, namely immunodiffusion (ID), countercurrent immunoelectrophoresis (CIE), agglutination (AGL) and complement fixation (CF). The non-corpuseular antigen was used for ID, CIE and CF. The corpuseular antigen was used for AGL. The greatest percentage of *Candida*-specific antibodies were found in all four groups of patients by AGL, lower amounts using CF, still lower with CIE (29.4 %) and only negligible amounts with ID (1.2 %). In complement fixing and agglutinating antibodies the titres could be estimated. The highest titres found were 1: 8,192 in the agglutination reaction and 1: 96 in complement fixation.

Candida albicans is an opportunistic pathogen, often present among the normal flora of the mucous membranes. Therefore the finding of this yeast by microscopic examination or cultivation is no proof of a pathogenic process. More significant is the specific humoral response of the host, however, a moderate antibody level can be probably elicited even by mere colonisation. Consequently, it is necessary to employ for each specimen several serologic reactions with antigens prepared with different methods and the patient has to be sampled repeatedly.

Contemporary trends in antimycotic vaccine preparation

Současné trendy v přípravě antimykotických vakcín

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In the world, more than two hundred thousand different species of fungi exist. However, only several tens of them are considered to be pathogenic to man and animals and only a few species can be regarded as primary pathogens. The re-

maining species are opportunistic pathogens which invade only immunologically altered hosts. With respect to problems with antimycotic therapies, the preparation of different effective vaccines is a great challenge for the mycologist and immunologist.

Intensive experimental work on vaccine preparation is focused on *Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*. The new trends in vaccine construction are aimed at a preparation of subunit vaccines exploiting recombinant proteins or purified cell-wall extracts. Most widely used candidate antigens with immunological protective potential for the construction of subunit vaccines are glucuronoxylomannan in *Cryptococcus neoformans*, the mannan complex, Hsp 90 protein and the receptor for the *Pichia anomala* toxin in *Candida albicans*, the Hsp 65 protein in *Histoplasma capsulatum*, the 42 kDa protein of the soluble conidial cell wall fraction in *Coccidioides immitis* and the 43 kDa protein (exo- β -1,3-D-glucanase) in *Paracoccidioides brasiliensis*.

Completely new and very promising seems to be the construction of DNA vaccines. Some aspects of experimental antimycotic DNA vaccines prepared in our laboratory were discussed in detail.

Two DNA vaccines were prepared in our laboratory: the heat shock protein Hsp 60 kDa expressing DNA vaccine from *Trichophyton mentagrophytes* and the Hsp 90 kDa expressing DNA vaccine from *Candida albicans*. Both vaccines were compared with recombinant Hsp 60 kDa or Hsp 90 kDa protein vaccines in experimental animals (guinea pigs and calves) as models. The results indicated that immunological effects of the DNA vaccines are dose-dependent. In higher doses, DNA vaccines could induce humoral as well as cellular immune response. Protective effects of the prepared DNA vaccines are to be evaluated.