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MATING TYPE GENES POSITIVELY REGULATE ASEXUAL SPORULATION AND CAROTENOGENESIS IN *FUSARIUM*

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Filamentous ascomycetes, including mitotic holomorphs have constitutively transcribed *MAT* (mating type) genes. These genes encode transcription factors and are considered to be the major regulators of sexual communication. The proven targets of the MAT transcription factors are pheromone precursor and pheromone receptor genes. However, recent studies [1, 2] showed that the *MAT* genes may also affect other genes not involved directly in the mating process. Studies on *Fusarium veriticillioides*, a mycotoxin producing plant pathogenic fungus revealed that *MAT* genes, besides regulating mating may also play an important role in the asexual part of the fungal life cycle. Light inducible carotenogenesis was partially blocked in hyphae of a *ΔMAT1-2-1* knock-out mutant of *F. veriticillioides* grown under nutrient limiting conditions, on carrot agar or minimal medium. Asexual sporulation of the *ΔMAT1-2-1* mutant cultured either under continuous illumination or in the dark was reduced by ~ 60% in a light-independent manner. Transcript levels of *con10,* a late-stage conidiation specific gene was also greatly reduced in the mutant as compared to the wild type after five days growth indicating that microconidium formation is developmentally regulated.

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SALMONELLA CONTAMINATION OF LAYING FLOCKS, EGGS AND EGG PRODUCTS IN HUNGARY

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Salmonellosis is still the most important foodborne zoonosis in Hungary altough the number of human cases has shown decreasing tendency in the last few years. The main sources of infections are foodstuffs containing inadequately heat treated poultry meat or eggs. Illnesses can arise from incorrect food handling during and after food preparation in the household and in catering trade. In Hungary more than 80% of human cases are caused by *Salmonella enteritidis,* while *Salmonella typhimurium* and *Salmonella infantis* can be detected from 5-10% of the cases. Recently *Salmonella infantis* became far the most frequent in broiler flocks and in chicken meat, and the third most frequent in human cases. According to the Hungarian Statistical Office the most frequent consumed meat is poutry meat and egg consumption was about 167-195 eggs/person/year between 2002 and 2006. The aim of these studies was to analyse our data about *Salmonella* contamination of Hunagarian laying flocks, eggs and egg products and try to relate these data to the ones on human *Salmonella isolations* in Hungary. Regarding *Salmonella* contamination rate of laying flocks, the most reliable source for EU countries is the "EU Baseline Study" (2004-2005). According to these data, the *Salmonella prevalence* in Hungarian laying flocks was 43,7% (putting Hungary to the no. 5th-6th place among the coutries with relative high rates). The dominant serovar - as in all EU countries - is *Salmonella enteritidis* (SE). Most of the Hungarian SE strains belong to phage type PT4

and PT6 (Colindale system) which is correlating to those PTs from Hungarian human cases. Furthermore, according to the baseline studies, the prevalence of *Salmonella infanti*s was more frequent in laying flocks (4%) than that of *Salmonella typhimurium* (2,5%). Strains of *Salmonella infantis* were more multidrug resistant, similarly to those described by Nógrády at al, (2007). Although the Hungarian official laboratory system examined eggs, liquid egg, egg powder and dried pasta between 2000 and 2007, the systematic microbiological monitoring of egg products has started in 2006. The rate of *Salmonella* positive samples was between 0-8,3%. In general there is a decreasing tendency of *Salmonella* contamination rate in eggs and egg products: there were no Salmonella positive samples from the 474 batches of egg yolk from table eggs in 2007. Most of the isolated strains represented serovar *Salmonella enteritidis*, but *Salmonella infantis* became also relatively increased. The above decrease in frequency and phage types of SE infections and in the prevalence and types of *Salmonella infantis* have been reflected - with slight parallel changes - in a general decrease of human infections of the same years, indicating the links between *Salmonella* contamination of eggs and egg products and human salmonellosis in Hungary.

FOOD-BORNE VIRUSES AND FOOD SAFETY CONSIDERATIONS

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Food-borne illness caused by microorganisms is a large and growing public health problem. Enteric viruses have been increasingly recognized as an important cause of food borne diseases lately, because of the increasing consumption of shellfish, ready-to-eat foods, and raw or/and minimally processed fruits and vegetables. Today we ensure food safety through Hazard Analysis Critical Control Point (HACCP) and its supporting programs (prerequisite programs or good practices) from producer to consumer through entire food supply chain, but viral infections are still very common in many parts of the world despite the internationally recognized approaches for assuring food safety already in place to reduce bacterial contamination. Establishment of critical control points which are necessary for successful HACCP implementation is one of the crucial goals of current food supply systems in particularly in areas where fresh products are produced for direct market supply. The major source of contamination seems to be from infected people, who handle food which is not heated or otherwise treated and contaminated water. As human enteric viruses are present in water samples at very low concentrations, we have focused on development of a concentration step before virus detection. A novel method, using CIM® monolithic chromatographic supports has already been successfully applied for the concentration of several plant and human infecting viruses from water samples. In our study we applied the method to the concentration of hepatitis A virus (HAV) and the norovirus surrogate, feline calicivirus (FCV). Since most enteric viruses are negatively charged at ambient pH, positively charged CIM® quaternary amine (QA) monolithic columns were applied for concentration of the two different viruses. Two-step real time RT-PCR was used for quantification of virus in load and elution fractions. Adsorption of viruses to the CIM matrix using phosphate buffer pH 7 and elution using 1M NaCl resulted in recoveries of close to 50% for HAV and about 100% for FCV. Both viruses were spiked together into 1.5 L of bottled water reaching different final concentrations and afterwards concentrated using CIM® QA 8 mL columns to about 15 mL of final volume. This volume was further concentrated by ultracentrifugation. Better results were observed using the CIM/ultracentrifugation method in comparison to the concentration with positively charged membranes and ultrafiltration, which is the most commonly used method for concentration of viruses.

TAXONOMIC DIVERSITY OF CULTURABLE BACTERIAL COMMUNITY IN COMPOSTS

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Composting is an aerobic process, during which organic waste is biologically degraded to humus kind of material. Pathogens or seeds with germinability should not be found at the end of the process. To improve the composting process it is important to know which microbes have the functional importance in the composting process. Traditional methods that based on cultivation are currently considered to identify only a small fraction, 0.01-10%, of the microorganisms in natural environments. After isolation of culturable strains, DNA-based techniques allows a more detailed investigation of soil microbial community structure. In our experiments, three various mixtures of compost were studied. Compost A was prepared from straw, biogas fermentation-residue, rabbit-manure, turkey-manure and inoculum A added. In case of compost B, straw, biogas fermentation-residue, wood-ash were applied and inoculum A were used. Compost C was prepared from straw, turkey-manure, vinasse, wood-ash and a bacterial inoculum B added. During the compost-experiment, samples were taken five times from the three compost-piles. Altogether 68 bacterial strains were isolated by cultivation on TGE agar plates. The strains were phylogenetically identified by sequencing 16S rRNA gene. Based on the partial 16S rDNA sequences, the 68 strains were taxonomically classified to 41 different species of 19 different genera. The most tipically occuring species belong to *Bacillus, Acinetobacter, Pseudomonas, Pseudoxanthomonas, Microbacterium* and *Comamonas*. By the identification result, one opportonistic pathogen species was found in compost mixture A and another one in compost mixture B at the final stage of compost-maturity process. Both species were present at the level of 106 CFU/g. No opportunistic pathogen strain was cultured and identified from compost mixture C. These results show that the process of composting should be re-planned in the case of A and B composts. Alterations of the applied inocula could be a possible solution to make these composts safety for using in the practice. An additional achievment of our study was that 15 strains isolated cannot be identified as any described bacterial species. These strains can be potential candidates for new undescribed species, for which further investigations are needed.

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COMMUNICATION BETWEEN YEAST COLONIES ON SOLID AGAR

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Microorganisms do not live solitary reclusive lives but rather they communicate using diverse chemical languages. In recent years, there have been several reports of quorum-sensing-like phenomena in fungal species. It is believed that yeasts can develop a wide range of mechanisms for sensing and adapting changes in the environment, which can be termed as molecules of early response. The role of those molecules is to warn cells against nearby growing cells, nutrient limitation or upcoming stress situations. Their accumulation in the medium can lead to induction of gene expression. Quorum-sensing molecules were so far identified only in two yeast genera

Saccharomyces and *Candida* [1, 2]. It is expected that wine yeasts *Dekkera* ssp., *Hanseniaspora* spp., *Zygosaccharomyces* spp. and *Candida* ssp. have comparable mechanisms of communication. We are assessing colonies of *Saccharomyces cerevisiae* plated 1,5 cm apart on GM solid medium containing glycerol where a turbid path was formed which could be related to communication molecules. Proteins from that area are extracted and determined by 2-D gel electrophoresis. Possible communication molecules of volatile nature will be identified and quantified in chemically defined liquid media by using analytical methods (HPLC, GC-MS) or enzymatic methods with intention to understand intercellular communication (cross-talk) in yeasts.

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THE ROLE OF *LACTOBACILLUS BREVIS* L62 PLASMIDS DURING PROLONGED STATIONARY PHASE

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In the state of starvation bacteria spend most of their time in a natural environmental. Starvation makes bacterial evolution possible in scantily condition. Stressful environment can result in selection of mutants that express growth advantage during starvation period. Growth advantage in stationary phase (GASP) is the term used to describe this phenomenon.
The aim of this work was to carry out the curing of bacteria *Lactobacillus brevis* L62 which contains three plasmids, to examine the role of removed plasmids considering growth in rich MRS and minimal MML medium, as well as their coexisting with enterobacteria, *Escherichia coli* MG1655 or *Salmonella enterica* serovar *Typhimurium*, during long term cultivating.
Under the influence of lysozime in concentration of 10 mg per ml during 24 hours under the temperature of 37°C, two smaller plasmids have been removed from *L. brevis* L62, while the biggest plasmid remained intact. Monitoring the surviving strains in minimal MML medium, during the prolonged stationary phase, better growth of the *L. brevis* L62T cells, lacking two plasmids, has been determined in comparison to the parental cells. Furthermore, in mixed cultures bacterial cell of *L. brevis* L62T, as opposed to parental cells, do not show GASP phenotype with *E. coli*, while both strains show strong GASP phenotype during coexistence with *S. enterica*.

DEVELOPING OF GENETIC TOOLS FOR THE MODEL ORGANISM, *THERMOPLASMA ACIDOPHILUM*

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T. acidophilum is a thermoacidophilic archaeon grows optimally at 58 C, pH 1,5-1.8. This microbe can be employed as a model organism, because this species has a strong evolutionary relationship to eukaryotes. In 2001, the genome structure of *Thermoplasma acidophilum* DSM 1728 was sequenced. Despite the intensive and fruitful research on Thermoplasma genomics and proteomics, vectors and

transformation methods are still missing for genetic manipulations. Moreover, there is no solid media for cultivation the microbe, so obtaining clonal cultures, the primary requirement for any genetic system, is yet unresolved. Recently Japanese researchers published the isolation of the first *T. acidophilum* plasmid pTA1, and a mutated gyrase B producing novobiocin resistant cell line is also available. The aim of our research was to develop genetic tools for *T. acidophilum*. As growth on solid media is essential before genetic techniques such as transformation are developed. Firstly we constructed a silica based solid media for *T. acidophilum*. On silica media yellowish colonies were formed after 7 days of incubation at 58 C. Parallelly we built up vectors for transformation experiments. The first set of vectors was constructed for homologous recombination purposes. The novobiocin resistant gyrB operon was chosen and cloned to pUC18 vector for construction of pDTA (6401bp) plasmid. By further site directed mutagenesis step a higher novobiocin resistant gyrB containing plasmid pNTA was also created. Besides, a new *E. coli, T.-acidophilum* shuttle vector was developed using pUC18 and the ori region and three ORFs from pTA1 resulted pSTA1 plasmid. For elaborating transformation method different approaches were tried: electroporation, lipofection, magnetofection and gene gun techniques. Electroporation was totally inefficient while the combination of ballistic method and lipo-and magnetofection was finally successful. Transformation of *T. acidophilum* and *T. volcanium* by pDTA and pNTA vectrors yielded 13 novobiocin resistant cell lines in independent experiments. The highest novobiocin resistance was measured by *T. acidophilum* cells harboring pNTA vector. These transformants showed a 2 000 nanogramm/ml novobiocin tolerance compared to the 10 ng/ml tolerance of the wild type cells. Homologous recombination was verified by PCR in all the *T. acidophilum* transformants. Our work is the first detailed description of the development and use of new genetic tools for *Themoplasma acidophilum* and *T. volcanium*. Further research need to be done on the optimization of clonal work and transformation protocol but based on our results now we can start to develop KO mutagenesis and His-tagged constructs for support joint research at MPI.

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WEST NILE VIRUS INFECTIONS IN ANIMALS IN 2008 AND 2009 IN CENTRAL EUROPE

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An exotic, lineage 2 strain of West Nile virus (WNV) emerged in 2004 in Hungary, and caused sporadic, fatal encephalitis in goshawks (Accipiter gentilis). The same strain was detected in 2005 in encephalitic birds of pray and in a sheep, and in 2007 in geese, in birds of pray, and also in a horse at the same geographic region of the country (South-East). A consecutive monitoring programme for WNV was established to follow the occurrence and spread of this strain wild and domestic animals in

Central Europe. Wild birds succumbed in central nervous system (CNS) symptoms were submitted for laboratory investigations by the officers of BirdLife Hungary, and of National Parks, as well as by falconers. Serum and EDTA-blood, CSF, and tissue samples of horses suffering or succumbed in CNS symptoms were received for diagnostic investigations from veterinarians. Limited number of mosquitoes was collected in a red-footed falcon (Falco vespertinus) colony nesting place, where WNV-induced bird mortality was recorded in 2007. In Austria wild bird samples were collected by the BirdLife Austria, and samples of captive raptors were sent by falconers. Mosquito samples were collected in four federal states of Austria in WNV affected and non-affected regions. Direct virus detection methods were based on reverse-transcription polymerase chain reaction (RT-PCR) using universal flavivirus-, and WNV lineage 2 specific primers, TaqMan real-time RT-PCR using WNV lineage 2 specific primers and probe, and immunohistochemistry. Positive samples were subjected to virus isolation on Vero cells, chicken embryo and in suckling mouse brain. Viruses were identified by partial genome sequence determinations and sequence comparisons. Serological investigations were performed on human, horse and wild bird samples. WNV specific IgG and IgM antibodies were detected by indirect immunofluorescence assay (IFA), haemagglutination inhibition test (HIT), microneutralization test (NT), and competitive ELISA.

In Hungary the first WNV positive wild bird samples in 2008 were identified in the middle of August. Predominantly goshawks succumbed in encephalitis (15 diagnosed cases), but viral nucleic acid sequences were also demonstrated in two Harris hawk (*Parabuteo unicinctus*) and in three Gryfalcon (*Falco rusticolus*) samples. The samples were originated mainly from the central and western part of Hungary. In the beginning of September WNV nucleic acid and antigens were detected in goshawks, which were found dead in Lower-Austria, close to Vienna. A few weeks later the virus was also detected in other captive Austrian wild birds, including a Kea (*Nestor notabilis*) and a Bearded Vulture (*Gypaetus barbatus*). WNV was isolated from several bird samples. The number of reports on horses with CNS symptoms increased in August and in September in Hungary. Symptomatic horses (n: 30) were tested for WNV by direct and indirect methods. WNV nucleic acid was detected in one horse, while WNV-specific antibodies were found in 16 horses' samples (IFA titres between 1:80 and 1:2560). Serological surveys in spring and summer of 2009 in different Hungarian horse populations detected 15 to 35 % seropositivity; however, most of the horses were asymptomatic. The distribution of WNV in mosquito vectors was surveyed in the eastern region of Austria. Mosquito pools (n: 138) were tested by real-time RT-PCR. Six pools of adult *Culex pipiens* mosquitoes were found positive. In Hungary, one *Cx. pipiens* pool was found positive out of 12 investigated pools. The partial nucleotide sequences of the detected viruses were >99% identical with the WNV lineage 2 strain, which was first identified in 2004 in Hungary. In 2009, a goshawk was found dead in the first week of June, and the first investigations indicate WNV infection. Further details of the 2009 cases will be discussed in the presentation. In 2008 considerable increase and geographic spread of the previously detected, encephalitic WNV lineage 2 strain was observed in Hungary. The virus reached Austria and caused the first documented animal cases in the country. The favourable weather conditions might have supported the increase in virus activity; however, so far unidentified avian hosts are suspected to play a role in the spread of the virus. The goshawk mortality was an early indicator of the epizootic. Surveillance and monitoring systems for the early detection of cases, as well as immunization of susceptible horse populations are needed.

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ARBUSCULAR MYCORRHIZAL FUNGI OF SEMIARID SANDY AREAS ON THE GREAT HUNGARIAN PLAIN. WHAT THE SPORES CAN TELL US?

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The taxonomy of arbuscular mycorrhizal (AM) fungi is based on the ontogeny and morphology of their asexual spores. Nowadays molecular taxonomic methods have also been used in the descriptions of AM fungal (AMF) taxa. It has been proven that there is no significant correlation between spore diversity and abundance in soil and fungal colonization of roots and there are much more distinct phylogenetic lineages („molecular taxonomic unit" or „virtual taxa") than the ca. 200 described species in Glomeromycota. The main aim of my research project was to gain information about the diversity of AMF of semiarid sandy grasslands of the Great Hungarian Plain. Thirty soil samples were collected around the roots of Juniperus communis from our three sampling sites (Bugac, Fülöpháza, Tatárszentgyörgy) in the Hungarian Plain. Pot cultures using *Plantago lanceolata* as trapping plant have been established from these soil samples. Altogether sixty soil samples were checked: AMF spores from those established pot cultures and the original soil samples were isolated and compared. Reference slides were made for common and/or interesting species. Representative spores have been mounted in PVLG (polyvinyl alcohol-lactic acid-glycerol) and Melzer's reagent to make diagnostic staining reactions of the spore wall layers visible. The spores of twenty-eight known AMF species and two probably new species were isolated from the samples. The most abundant species, which have been found in all three sites (Bugac, Fülöpháza, Tatárszetgyörgy) are: *Glomus aureum*, *G. aurantium*, *G. constrictum*, *G. microcarpum* and *G. versiforme.* There are some species which can hardly be found in field samples but sporulate well later in pot cultures (like Archaeospora trappei), or can be found often in the soil sample but cannot be isolated in pot cultures (e. g. *Glomus aureum*). Spore based data have been compared with the results of the in planta AMF diversity studies carried out in the area.

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THE MONOKINE INDUCED BY INTERFERON-GAMMA SHOWS ANTI-CHLAMYDIAL ACTIVITY

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The spectrum of illnesses caused by *Chlamydophila pneumoniae (C. pneumoniae)* ranges from severe community acquired pneumonia to bronchitis, pharyngitis, laryngitis, or sinusitis. The immunological factors which contribute to the development of the pathological conditions remain poorly understood. Monokine induced by IFN-γ (MIG/CXCL9) belongs to the group of CXC chemokines and has the ability to signal through CXC chemokine receptor 3, which is present on T cells and NK cells. The binding of ligand to the receptor results in the activation and recruitment of the cells to the sites of inflammation. Our aim was to evaluate the role of CXCL9 during the *in vitro* and *in vivo C. pneumoniae* infection. In *in vitro* experiments $2x10^3$ IFU *C. pneumoniae* were incubated together with various concentrations of MIG for 2 hours at 37°C. To quantitate bactericidal activity of MIG the incubation mixtures were placed onto HEp cells and the culturable *C. pneumoniae* was detected

by indirect immunofluorescence test. The kinetics of the anti-chlamydial effect of MIG was also studied. In *in vivo* experiments Balb/c female mice were infected intranasally with *C. pneumoniae*. Mice were sacrificed at different times after inoculation. Lungs were removed and homogenized and total RNA was extracted from half of the lungs to determine the expression of MIG mRNA by Real time PCR. From the supernatant of the lungs MIG and IFN-γ proteins were detected by ELISA assay. In the bactericidal assay, recombinant MIG showed dose-dependent antibacterial activity against *C. pneumoniae*. At 10 μg/ml concentration MIG reduced the number of the elementary bodies by 96 %, and even 1,25 μg/ml concentration of MIG caused 80% reduction. The heat inactivated recombinant MIG, which was used as control, did not show any antibacterial activity. Significant anti-chlamydial activity of MIG was observed after 15 minutes incubation period. The expression of MIG mRNA in the *C. pneumoniae* infected mouse lung showed 156-fold increase compared to the non-infected mouse lung on day 7. MIG was also detected on protein level from the first day with the highest concentration on day 7 in the supernatant of the infected lung. The expression of IFN-□, which is the main inducer of MIG, showed similar kinetics. The results of our *in vitro* and *in vivo* experiments suggest that MIG might have an important role during *C. pneumoniae* infection.

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PSEUDOMONAS AERUGINOSA IN THE WATER DISTRIBUTION SYSTEMS

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Pseudomonas aeruginosa is widespread in all water environments and generally persist in drinking water distribution systems. Under favorable conditions, *P. aeruginusa* heavily colonizes the pipelines and the water outlets of the water systems and proliferates in the biofilms. *P. aeruginosa* is an opportunistic human pathogen; it infects predominantly immunocompromised patients. It is an important indicator organism in water hygiene that characterizes the overall microbial status of the water system. *P. aeruginosa* in drinking water is determined in routine water microbiology using a culture based membrane filtration method (MSZ EN ISO 16266:2008 standard). The standard identifies *P. aeruginosa* based on the following biochemical and morfological characteristics: (1) it produces a fluorescent pigment under UV light and/or a green diffusive pigment on cetrimide-naladixic acid agar (2) oxidase positive and (3) releases ammonia from acetamide. The above tests theroretically provide unambiguous confirmation for the target species. However, the environmental microbial communities are a lot more diverse than medical ones, therefore the presence of false positive isolates is possible. The aim of the present study was to compare the conventional tests to a DNA-based and a fatty-acid based confirmation method. Over 100 *P. aeruginosa* strains were isolated from drinking water samples with standard methods. The strain collection contains both typical isolates and atypical ones that are nevertheless considered *P. aeruginosa* by the standard. The strains were analysed by genus and species-specific PCR and a commercial PLFA-based identification system. All isolates gave positive signal in the genus and species-specific PCR. Most strains were identified as Pseudomonas by PLFA profile as well. However, the similarity index was often lower than required for unambiguous identification, and the species-level identification was also problematic. The time demand and financial considerations also make taxon-specific PCR the method of choice if additional confirmation is needed. A subset of the strains was also characterized by rep-PCR. The strains isolated from different sampling locations were compared. High sub-species

level variability was observed. In most cases, a water outlet was colonized by a single strain, though more types within one distribution system were observed.

FUNGAL INFECTIONS IN IMMUNE SUPPRESSED PATIENTS

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Candida species belong to the normal flora of the gastrointestinal tract and of the vaginal mucosa in humans. They cause infections in immune suppressed individuals after chemotherapy, when the number of functional polymorphonuclear leucocytes decreases significantly. Organ transplant recipients, persons with leukemia, lymphoma, and individuals with metabolic disorders are at risk. In our study we investigated 107 immune suppressed patients suffering from oropharyngeal thrush. All were hospitalized, 61 in the Hematology and 33 in the Hematology- Bone Marrow Transplantation unit. Their specimens were tested in the Microbiology Unit of the Emergency County Hospital in Tg. Mures, from 01.01.2009 to 30.07.2009. Pharyngeal swabs and tongue scrapings were examined microscopically, cultivated on Sabouraud and Candiselect agar plates and identified with the API Candida (Bio- Merieux s.a.) and Vitek yeast identification systems. Antifungal susceptibility was checked by Etest (Bio- Merieux s.a) on modified RPMI-1640 agar. Fluconazole (Flu), voriconazole (Vor), posaconazole (Pos), caspofungin (Cas) were tested. Our results show: 10 samples of Candida (C) spp. (9,35%) and 13 C. non- albicans (12,15%). Further identification of these cases was not possible. 61 patients had *C. albicans* (57,01%), 8 *C. glabrata* (7,48%), 4 C. *krusei* (3,74%), 3 *C. famata* (2,80%), 3 C. *lipolytica* (2,80%), 2 *C. kefyr* (1,87%), one *C. tropicalis* (0,93%) and 2 *Saccharomyces* (S.) *cerevisiae* (1,87%) infections. Associated fungal infections were diagnosed in 7 cases: 2 *C. albicans* + *C. non-albicans*, one *C. albicans* +*C. glabrata*, 1 *C. albicans* + *S. cerevisiae*, one *C. krusei* + *C. kefyr*, one *C. famata* + *C. krusei and* a triple infection with *C. famata*, *C. glabrata* and Geotrichum sp. The in vitro antifungal sensibility test showed sensibility of *C. glabrata* in 100% to Cas., in 50% to Vor. and in 25% to Pos. All of the *C. krusei* isolates were sensible to Vor., Cas and Pos. *C. famata* was sensible to Vor., Cas. and Flu. but resistant to Pos. *C. lipolytica* showed sensibility to Vor. but 50% resistance to Pos. The single *C. tropicalis* isolate was sensible to Vor. and Cas. but resistant to Pos. *C. kefyr* was sensible to Pos. and Vor. 2 of the 3 S. cerevisiae isolates were sensible to Vor., one was sensible to Cas, but resistant to Pos. One isolate was resistant to Pos and Vor. In conclusion, the number of non- albicans fungal infections is increasing. It is important to check in vitro the antifungal drugs` efficiency, because yeasts frequently acquire resistance.

LONG TERM FOLLOW UP OF GIARDIASIS IN INSTITUTIONALIZED PHYSICALLY AND MENTALLY DISABLED PERSONS

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Giardia lamblia also called *Giardia intestinalis* or *Giardia duodenalis* is a unicellular flagellated protozoan that causes diarrheal infections all over the world. In countries like Romania, it is

considered to have a high prevalence, and data suggest that institutionalized persons are more exposed to the infection leading to chronic giardiasis associated in long-term with growth retardation. In areas where water is contaminated with viable *G. lamblia* cysts, tourists may suffer from travelers-diarrhea. The aim of our study was to follow the prevalence of giardiasis in a closed Institute in Brancovenesti, county Mures, which provides shelter and care for 334 physically, and mentally disabled women and children. This institute was founded in the former castle of baron Kemény in 1990 and, in the last years, a great concern of the medical stuff was to detect and eliminate the parasitic infections of the inhabitants. The study started in 1997 when all the inhabitants of the institute were examined for the presence of the parasitic protozoan *Giardia lamblia*. Stool samples were collected and transported to the Parasitological Laboratory of the University of Medicine and Pharmacy in Tg. Mures, where they were examined by using the wet mount preparation technique and the lugol staining method. The positive samples were treated, and in the case of the negative specimens, the examination was repeated twice. After applying the anti-*Giardia* treatment with albendazole, its efficiency was also tested. The study was repeated after 5 and 10 years, in the same conditions. Our results show the following. After our first screening in 1998, we detected 13 Giardia infections, representing 3,88% of the inhabitants. All of them were room-mates. After application of the anti- parasitic treatment, the number of *Giardia*- cases decreased to 3, but 43 (13,73%) cases were newly discovered. None of the cases were associated with other parasitic infections. In 2003, the number of *Giardia*- infections was 25 (7,48%), 7 of them being associated with other intestinal parasitic infections. Following treatment, the number of *Giardia*- patients decreased to 13, situation which needed a second medication. The third study was applied only to 287 inhabitants; the children were moved to other institutes. We identified only 5 (1,74%) *Giardia*- infections, and after the treatment it remained only one positive case, which was treated again. In conclusion, the follow up of the *Giardia*-cases was successful, the number of the positive cases decreased significantly.

PREVALENCE OF *LEGIONELLA* IN WATER DISTRIBUTION SYSTEMS, SPAS AND COOLING TOWERS IN HUNGARY

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Two years after the implementation of the EGWLI Guidelines in Hungary the legislation of environmental Legionella monitoring is still incomplete. There is no regulation for cooling towers or hot water systems, only the monitoring of hot whirlpool spas is mandatory. To emphasize the need for a more extensive regulation, the *Legionella prevalence* in different man-made aquatic environments was investigated by standard culture methods in the 2006-2009. The Legionella isolates were typed using monovalent sera, rep-PCR and PFGE. The majority of the investigated water distribution systems (102 objects) was colonized by Legionella (66 %), 44 % of the samples were positive, 25 % had Legionella counts above 1000 CFU/L. Legionella titers up to 106 CFU/L were observed. In the cooling towers and spa pools (21 and 19 objects, respectively), the prevalence of *Legionella* was lower than expected.

HEAT ADAPTATION OF *BACILLUS CEREUS* CELLS

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Mild treatments and environmental effects often present sub lethal stresses to microorganisms that either lead to their death or cause stress reactions in the surviving cells increasing their resistances to similar or other kinds of stresses. These reactions can be adaptation processes ceasing with the termination of the stress, or can lead to mutations resulting in permanent changes in the genome of the microbe. In case of mild treatments there is a greater chance for the microbe to survive and strains with new characteristics (resistances) to arise. According to the literature among other things similar reasons had lead to the emerging of „new pathogens" in the last decades. Mild preservation treatments desired by the consumer represent a danger to food safety because of the stress adaptation abilities of microorganisms. We have studied the effect of sub lethal heat treatment on the survival, the growth parameters and the protein composition of *Bacillus cereus* cells (indicator microbe for food industry). We have studied the survival of 24 hour culture *Bacillus cereus* T vegetative cells following heat treatment at 55°C for 10 minutes. Another 24 hour culture was prepared with the surviving cells and it was heat treated again. In spite of the repeated treatments no signs of development of resistance could be detected. In the protein composition of control and treated bacterial samples no difference could be found with 2D electrophoresis. Heat destruction of cultures of *Bacillus cereus* T grown at 25 and 30°C was examined with tradition plating and conductance measurement. We have found that heat sensitivity of the vegetative cells grown at lower temperature was greater than of the ones grown at higher temperature, though surviving cells from the 25ºC culture proved to be less injured. When cells were exposed to 37ºC for 10-120 minutes prior to heating at 52ºC for 6 minutes, the survival of the bacteria increased with the increasing time of preconditioning, after 120 minutes the survival has increased from 0.1% to approximately 100%. We have compared the colony forming ability of the surviving cells after heat treatment on general TGY Agar used in the experiments and on *Bacillus cereus* Selective Agar used for the selective enumeration of *Bacillus cereus*. On the selective agar 1.5-3 orders of magnitude less surviving cells were able to form colonies than on the general agar. According to the results it can be stated the efficiency of the heat treatment depends greatly on the pre life of the microbes. The detection of surviving but injured *Bacillus cereus* cells with the standard method is not reliable.

EPIGENOTYPING OF LATENT EPSTEIN-BARR VIRUS EPISOMES IN BREAST CARCINOMA CELL LINES

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Epstein-Barr virus (EBV) is a ubiquitous human herpes virus, most notable for causing infectious mononucleosis. EBV is associated with a number of malignancies, such as Burkitt's lymphoma, Hodgkin's disease, B-cell lymphoma in immunocompromised individuals, and nasopharyngeal carcinoma (NPC). EBV is also linked with some other malignancies, including gastric carcinoma, leiomyosarcoma in immunocompromised individuals, T-cell lymphoma, carcinoma of the salivary glands, and thymic carcinoma. In the mid 1990-s, EBV has been detected in some breast tumors, too. Breast cancer is one of the most prevalent malignancies in Western countries. Although there are several well- established risk factors for breast cancer, other factors contributing to the development of breast cancer likely exist. Breast cancer is a multistep disease in which a virus could play a role.

Researchers so far, have been focusing on the presence of EBV in breast carcinomas, or the effect of EBV on the tumor's drug resistance. The exact role of EBV in the development of breast carcinomas has not been elucidated yet. Epigenetic control mechanisms, like DNA methylation and histone modifications play an important role in the regulation of latent EBV promoters. In our study we focused on the epigenotyping of the EBV episomes persisting in breast carcinoma cell lines C2G6 and C4A3. We observed the Q promoter (Qp) where transcripts for the nuclear antigen EBNA1 are initiated was unmethylated and active in both breast carcinoma cell lines. In contrast the C promoter (Cp) where transcripts for the nuclear antigens EBNA1 to 6 are initiated in B lymphoblastoid cell lines but not in the breast carcinoma cell lines, was found to be highly methylated at CpG dinucleotides in C2G6 and C4A3 cells. The LMP2A promoter (LPM2Ap) where transcripts ancoding the transmembrane protein LMP2A are initiated was unmethylated in C2G6 cells but highly methylated in C4A3 cells. The analysis of histone modifications effecting chromatin structure and promoter usage is in progress.

CORRELATION BETWEEN POSTANTIFUNGAL EFFECT AND THE EFFICACY OF SINGLE 5 AND 10 MG/KG CASPOFUNGIN DOSES FOR TREATMENT OF DISSEMINATED CANDIDIASIS CAUSED BY *CANDIDA KRUSEI* IN A NEUTROPENIC MOUSE MODEL

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Postantifungal effect (PAFE) of caspofungin against *C. krusei* was measured and the efficacy of traditional and single-dose caspofungin regimens were compared in an immunosuppressed murine model of disseminated *C. krusei* infection. Three *C. krusei* isolates were tested. PAFE of 0.12-16 mg/L caspofungin was determined. Immunosuppressed female BALB/c mice were intravenously challenged with 1.2-2x10^7 and 1-2x10^6 cfu/mouse in lethality and in tissue burden experiments, respectively. Mice were assigned to four treatment groups; no treatment, 1 mg/kg caspofungin daily for five days, a single 5 mg/kg caspofungin dose and a single 10 mg/kg caspofungin dose. All regimens were started 24 hours postinoculation and were administered intraperitoneally. The experiments were terminated at day six. For tissue burden experiments all mice were sacrificed and fungal tissue burden in kidney pairs was determined. Survival was analyzed by logrank test and logrank test for trend, tissue burden was compared by ANOVA or Kruskal-Wallis test.PAFE was never observed. All regimens improved the survival; 1 mg/kg daily caspofungin regimen was superior to single-dose regimens against two of three isolates ($p=0.035$ and $p=0.003$). Against the remaining isolate all regimens performed comparably ($p=0.254$). However, none of the regimens achieved 100% survival. Fungal tissue burden was decreased significantly by any caspofungin regimens in case of only one isolate ($p=0.020$). In contrast to some earlier observations with *C. albicans* and *C. glabrata*, single large dose caspofungin regimens were inferior to the traditional daily-dose regimen, predicted by the lack of in vitro PAFE in *C. krusei*.

SIMPLE AND RAPID METHOD FOR MONITORING METALS TOXICITY USING SIDEROPHORE- AND LYTIC ENZYME-PRODUCING MICROORGANISMS

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World-wide industrialisation has produced a higher demand for chemicals and increased environmental problems. Methods to cleanse the environment directly, such as bioremediation are imperative, but able to measure the extent of environmental pollution. The monitoring of environmental pollution has to be simple, rapid and precise. In the present study optical density and viable count techniques describe a simple, inexpensive and rapid method for detecting the heavy metal pollution in different soil extractions due to the high sensitivity of the investigated strains of siderophore- and lytic enzyme-producing microorganisms against various heavy metal concentrations. Concentrations of metal were determined using an atomic absorption spectrophotometer. The results from the optical density (absorbance at 540 nm using a spectrophotometer) and viable count (colony forming units per ml) techniques were very similar. All samples that gave high viable cell numbers also returned high optical density values. *Pseudomonas, Saccharomyces* and *Bacillus* strains were more heavy metal detectable than *Enterobacter, Rhizobium, Azotobacter, Flavobacterium and Escherichia*. Our results indicate that the biological detection using the optical density may be useful for routine environmental monitoring of metals (Ag^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Mo^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Tl^{2+}, V^{3+}, and Zn^{2+}) toxicity at different anion forms.

INFLUENCE OF MICROENCAPSULATION AND FREEZE-DRYING ON FUNCTIONALITY OF PROBIOTIC BACTERIA

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Bacterial strains *Lactobacillus helveticus* M92, *Lactobacillus plantarum* L4 and *Enterococcus faecium* L3 have been previously characterised as probiotic strains according to strong probiotic selection criteria in Laboratory for Antibiotic, Enzyme, Probiotic and Starter Culture Technology on Faculty of Food Technology and Biotechnology University of Zagreb. Besides their traditional applications as functional starter cultures for various food commodities, there is also a great interest for an investigation of these bacteria as biotherapeutic agents. The major criterion for the selection of probiotics is their survival during the transit through gastrointestinal tract. In order to exert their health benefits, probiotics need to be delivered to a host at high viable cell number, as a concentrated powder, that could be performed by freeze-drying. Hence, the objective of this work was to investigate the effects of different lyoprotectants on the survival of probiotic strains *L. helveticus* M92, *L. plantarum* L4 and *E. faecium* L3 during freeze-drying. Fresh and freeze-dried bacterial cells were microencapsulated in alginate and the effect of the microencapsulation on a viability of these probiotic bacteria in the simulated gastrointestinal tract conditions was investigated. The highest viable bacterial cells counts were reached when skim milk with glycerol were applied as lyoprotectants. The better survival of microencapsulated than non-microencapsulated probiotic cells, in simulated gastrointestinal tract conditions, indicates the protective effect of the microencapsulation in alginate. The presence of pure probiotic culture in each examined freeze-dried preparation was confirmed by RAPD (Randomly Amplyfied Polymorphic DNA).

MOLECULAR TYPING OF *CAMPYLOBACTER COLI* AND *C. JEJUNI* ISOLATES BASED ON *FLA-IGS* PCR

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Numbers of illnesses caused by food-borne pathogenic campylobacters are significant. Ratios of infections in Hungary caused by thermophilic Campylobacter species are higher than the average in the European Union, though the number of reported cases shows decreasing tendency. Differentiation of the species in Campylobacter genus is done only by phenotyping methods and the tests resulted in wrong results in many cases. These facts contributed to the increasing demand on rapid and efficient DNA-based techniques for detection, identification, typing and determination of transmission's rout of Campylobacter species.The aim of this work was the characterisation of food safety relevant Campylobacter isolates by three different PCR-based typing methods. Intergenic sequence (IGS) of flaA and flaB genes was investigated by denaturing gradient gel electrophoresis (DGGE) analysis. The optimal denaturant concentration was determined by perpendicular DGGE method, and applying the optimal concentration the differences between amplicons with nearly same size were defined by parallel DGGE. Electrophoretic mobility of homoduplex and heteroduplex molecules of the fla-IGS was determined in heteroduplex mobility assay (HMA). Heteroduplexes were formed during the hybridisation of fla-IGS sequences of the test and reference strains. Determining the exact nucleotide sequence of fla-IGS region in case of all isolates the results of DGGE and HMA were estimated. Based on the results obtained by parallel DGGE analysis the 20-40 % denaturing concentration proved to be applicable in case of fla-IGS region. Using this denaturing gradient interval the applicability of DGGE analysis on typing of Campylobacter isolates proved to be applicable, though its Simpson's index of diversity was 0.859. In the case of the heteroduplex mobility assay (HMA) most of the isolates formed heteroduplex molecules with the type/reference strains which refers to the sequence polymorphism of the 180-200 base pair DNA sequences. Diversity index of the heteroduplex mobility assay was 0.872, so the method is able to characterise isolates at strain level, but similarly to the DGGE analysis it is not applicable for species identification. Although considerable sequence polymorphism of intergenic region between flaA and flaB genes were observed in case of Campylobacter isolates, sequence similarities and differences of the strains did not show species characteristics of the two most significant *Campylobacter* species (*C. coli* and *C. jejuni*). Comparing the results of direct sequencing with results of DGGE and heteroduplex mobility assay it was established that the DGGE and HMA are good techniques for detection of sequence differences within shorter gene sequences, but their applicability for typing is limited.

EXTRACELLULAR SIGNALING IN THE REGULATION OF CELL DIFFERENTIATION AND ANTIBIOTIC PRODUCTION IN *STREPTOMYCES GRISEUS*

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Members of the prokaryotic genus *Streptomyces* produce over 60% of all known antibiotics and a wide range of industrial enzymes. A leading theme in microbiology is which signals are received and transmitted by these organisms to trigger the onset of morphological differentiation and antibiotic

production. The small gamma-butyrolactone A-factor is an important autoregulatory signaling molecule in streptomycetes, and A-factor mutants are blocked in development and antibiotic production. In this study we showed that heterologous expression of the 324-amino acid secreted regulatory protein Factor C resulted in restoration of development and enhanced antibiotic production of an A-factor-deficient bald mutant of *Streptomyces griseus*, although the parental strain lacks an facC gene. Proteome analysis showed that in the facC transformant the production of several secreted proteins that belong to the A-factor regulon was restored. HPLC-MS/MS analysis indicated that this was due to restoration of A-factor production to wild-type levels in the transformant. This indicates a connection between two highly divergent types of signaling molecules and possible interplay between their regulatory networks (Birkó et al., Mol. Cell. Proteomics 2007, 6(7):1248-56).Starvation is a major trigger for development, and nutrients are provided by degradation of the vegetative mycelium via a process of programmed cell death, reusing proteins, nucleic acids and cell wall material. The A-factor regulon includes many extracellular hydrolases. We also showed via proteomic analysis that many nutrient scavenging and stress-related proteins are overexpressed in an A-factor nonproducing mutant (AFN) of *Streptomyces griseus* B2682. Transcript analysis showed that this is primarily due to differential transcription of the target genes during early development. The targets include proteins relating to nutrient stress, environmental stress and an orthologue of the *Bacillus* sporulation control protein SpoOM. The enhanced expression of these proteins underlines the stress that is generated by the absence of A-factor. Wild-type developmental gene expression is restored to AFN by the signaling protein Factor C, in line with our earlier observation that Factor C triggers A-factor production (Birkó et al., Mol. Cell. Proteomics, in press).

THE EFFECTS OF HERBICIDES ON THE GROWTH OF *S. MELILOTI* STRAINS

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Biological nitrogen fixation has an important role in sustainable agriculture. In order to increase the utilization of biological nitrogen fixation, the inoculation of alfalfa seeds with efficient Sinorhizobium meliloti strains is recomended. For successful production of commercial inoculum it is necessary to use highly efficient rhizobial strains which are, among other things, herbicide tolerant. In this study, the effect of herbicides imazamox, thifensulfuron-methyl and fomesafen on growth of ten *S. meliloti* strains was analysed in order to improve alfalfa pre-sowing seed inoculation. The filter-disc method and four different herbicide concentration (10 ppm, 100 ppm, 1000 ppm, 10 000 ppm) was used in this study. The results showed negative effects on growth of some rhizobia, but only when the highest herbicide concentration were used (10 000 ppm). Thifensulfuron-methyl inhibited growth of strains *S. meliloti* C16 and *S. meliloti* S26 and herbicide fomesafen inhibited the growth of *S. meliloti* strains 2001 and Os5. The highest herbicide concentrations used in this study were multiple increased than the herbicide rates used in agriculture practice. The application of highest concentration of herbicide fomesafen resulted in decreasing and very slow growth of strains *S. meliloti* Z21, Os6 and 9930, as well as herbicide thifensulfuron-methyl decreased the growth of strain *S. meliloti* 9930. Imazamox application in all four herbicide concentration, resulted in increasing the bacterial growth of all tested *S. meliloti* strains. The results of this study revealed that strains of nitrogen-fixing bacteria differently tolerate herbicides application, so for future work it is necessary to test the growth and symbiotic efficiency of rhizobial strains after herbicides treatment in field conditions.

COMPARATIVE DIVERSITY INVESTIGATIONS ON PLANKTONIC BACTERIAL COMMUNITIES OF AN EXTREMELY SHALLOW SODA POND DURING A PHYTOPLANKTON BLOOM

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Water of soda lakes represent highly productive naturally occurring alkaline environments dominated by prokaryotes. The soda ponds of Kiskunság National Park are extremely shallow, alkaline and moderately saline water bodies which may dry out completely to the end of the summer.

The aim of this study was to reveal the aquatic bacterial diversity of Böddi-szék during an early summer phytoplankton bloom. For comparison traditional cultivation-based technique and cultivation-independent molecular biological analysis were applied. Water samples were taken from the 5-10 cm deep pond in June 2008. Serially diluted samples were plated onto different media and bacterial strains were investigated for their ecological tolerance characters. Representatives of ARDRA groups were identified by 16S rDNA sequence analysis. The phylogenetic diversity of bacteria was also studied by molecular cloning of 16S rRNA gene.

Altogether 93 strains characterized with aerobic chemoorganotrophic metabolism were isolated. Most of them were identified as alkaliphilic or alkalitolerant and moderately halophilic *Bacillus, Gracilibacillus* (Firmicutes)*, Algoriphagus, Aquiflexum* (Bacteroidetes)*, Alkalimonas, Nitrincola, Marinospirillum, Halomonas* (β-Proteobacteria) species. Other strains were closely related to alkaliphilic purple nonsulfur bacteria members of genera *Erythrobacter* and *Rhodobacter* (δ-Proteobacteria). Screening of the 16S rRNA gene library of the 96 Böddi-szék clones resulted in 71 different ARDRA groups. Sequence analysis revealed a Proteobacteria, Bacteroidetes, Spirochaetes dominated phylogenetic diversity. Other clones belonged to Firmicutes, Lentisphaerae, Cyanobacteria, Actinobacteria, Fibrobacteres and Tenericutes.

Most of our clones showed the highest sequence similarities to uncultured environmental clones described mainly from soda lakes of different parts of the world. The high prokaryotic phylogenetic diversity of the water of Böddi-szék, revealed by both of cultivation based and independent methods may hypothesize a very intensive sulfuretum type community metabolism. It can be based on the primary production of aerobic and anaerobic phototrophic bacteria (e.g. species of *Prochlorococcus*, *Anabaena*, *Rhodobaca, Rhodobacter, Rhodovulum, Ectothiorhodospira*) and connected with the decomposition of chemoorganotrophic microorganisms (e.g. species of *Desulfonatronum, Geobacter, Desulfobotulus, Anoxynatronum, Spirochaeta*) adapted to the water chemistry of Böddi-szék.

MATHEMATICAL MODELLING OF KINETICS AND PHARMACOKINETICS OF MICROBIAL PROCESSES IN VIVO

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Due to the complexity of microbial processes developing in infected macroorganisms the different approaches to their prevention and study are usually applied. Mathematical models can be an efficient tool for the study of process relationships. In this work, the mathematical model (defined system of a series of differential equations) convenient for a simultaneous study of pharmacokinetics and kinetics of possible microbial process events in infected body of hypothetical live organism, thought to be a mammalian of assigned relevant properties applicable for studies of possible events in the real human body, was developed. Computer simulations were applied to evaluate the convenience of the model in explaining the process relationships. In the model, infected organism pharmacokinetics, microbial growth, enclave formation, death and autolysis kinetics, organism immunity development, changes of organism body viability and non-viability, microbial production of toxic substances, pharmacokinetic substance effects on microbial cells and organism tissue were taken into account in developing the corresponding mathematical model. Results of computer simulations suggest that the developed model could be successfully applied in explaining a series of events in real live organisms expressing the properties analogous to those of hypothetical live organism.

IN VITRO ACTIVITY OF OLIVE OIL POLYPHENOLS AGAINST *LISTERIA* SPP.

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Listeria monocytogenes, gram positive, facultative intracellular rod, is found ubiquitously in the environment and is capable to grow and survive in a wide range of environmental conditions. However, the current knowledge of its ecology and in particular, the mode of its environmental survival and transmission remains limited. It has been suggested that free-living protozoa serve as an environmental reservoirs and vehicles for the transmission of several obligate and facultative intracellular pathogens, but the ability for intracellular multiplication and survival of *L. monocytogenes* in *Acanthamoeba* spp. is still unclear. The aim of this study was to investigate the interaction between the protozoan *Acanthamoeba castellanii* (ATCC 30234) and EGD strain of *Listeria monocytogenes*. The results indicate that *L. monocytogenes* does not reside within *A. castellanii*, as we were unable to recover any intracellular bacteria after 4 hours of co-cultivation. However, extracellular bacteria benefit from co-cultivation with amoeba showing increased growth and multiplication.In conclusion, *Listeria monocytogenes* is not able to survive inside *Acanthamoeba castellani*, but is quickly degraded under the laboratory conditions tested. *A. castellani* is not able to act as environmental reservoir for *L. monocytogenes* but may provide a survival advantage for

extracellular listeria during co-cultivation of the two organisms.

PRODUCTION AND PURIFICATION OF EXTRACELLULAR PHYTASE FROM *THERMOMYCES LANUGINOSUS*

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Thermomyces lanuginosus is a widely distributed thermophilic filamentous fungus commonly isolated from self-heating masses of organic debris. Characteristic feature of thermophiles is that their enzymes are more heat stable than that of mesophiles. Phytase from *Aspergillus* has most commonly been employed for commercial purposes, but the temperature optimum of this enzyme is about 50-55°C. Enzymes that are used as animal feed supplements should be able to withstand temperature of feed pelleting process. Thermostable phytase of high catalytic efficiency have commercial interest because the supplementation of seed-based poultry and pig feed with phytase increases the availability of phosphorous. During the enzymatic hydrolysis of phytic acid, inositol phosphates are also released. These intermediers with various chemical structures have numerous beneficial effects such as reducing the risk of the development of cancer of the large intestine and having function in transmembrane signalling and within the control of Ca^{2+} concentration. As a result of a selection procedure *Thermomyces lanuginosus* IMI 096218 strain was found to be the most promising one to produce phytase. The composition of medium for the production of phytase enzyme was optimized. Among the tested substrates, the highest activity was measured in medium containing rice flour. The optimum concentration of rice flour was found to be 5%. The fermentation medium prepared with Tris-maleat/NaOH buffer (pH=7.5) promoted the increase of phytase activity. When complementing the medium with different additives (Tween-80, citric acid, yeast extract) it was found that citric acid inhibits the enzyme production. Yeast extract had no effects, but Tween-80 which affects the permeability of cell wall had enhanced the phytase activity. Using Tween-20 or Tween-40 as surfactant during fermentation it was found that production of phytase enzyme was increased. After 3-day fermentation the mycelia were collected by filtration. Extracellular proteins were precipitated by iso-propanol and concentrated by ultrafiltration. Enzyme purification was achieved by combination of numerous chromatographic steps. Ion exchange chromatography (DEAE-Sepharose CL-6B) followed by hydrophobic interaction chromatography (Phenyl Sepharose Fast Flow) and gel filtration (Superose 12). All chromatographic steps were performed with fast performance liquid chromatography system (FPLC) at 4°C. The homogeneity of the purified phytase enzyme was estimated by 10% SDS poliacrylamide gelelectrophoresis. The characterization of purified phytase enzyme is in progress. *Thermomyces lanuginosus* IMI 096218 strain synthesised high level of phytase enzyme in medium containing rice as main carbon source. This enzyme is very promising to stable at higher temperature and has potential to apply in feeding applications.

MYELOID CELLS STIMULATED BY MELANOMA ANTIGENS EXPRESS THE ANTICANCER CYTOKINE IL-24 AND MIGRATE IN RESPONSE TO IL-24

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IL-24 (melanoma differentiation associated gene 7 product) is a member of the IL-10 cytokine family with anti-tumor activity. Immune tissues produce IL-24 and its expression can be induced in human peripheral blood mononuclear cells by pathogen-associated molecules. Because IL-24 can induce selective apoptotic responses in melanoma cells, we investigated the ability of melanoma cell supernatants, exosomes, and gp100 (melanoma antigen) to induce IL-24 protein production by immature dendritic cells, which might be in the tumor environment. Our results indicate that both native and synthetic melanoma cell products stimulate the production and secretion of both glycosylated and unglycosylated forms of IL-24 by bone marrow derived dendritic cells. While immune cells are known to produce IL-24, the response of immune cells to IL-24 is unclear. Using a recombinant form of human IL-24, we demonstrated that IL-24 induces human monocyte migration. To further classify the IL-24 response and type of receptor utilized by IL-24 we treated monocytes with several receptor signaling pathway inhibitors. The migration induced by IL-24 was sensitive to pertussis toxin treatment, thus implicating G-protein coupled receptor(s) in this process.
Additionally, MEK and JAK inhibitors significantly decreased monocyte migration toward IL-24. The results of inhibition studies suggest that IL-24 mediates cross talk between IL-10 receptor family members and G-protein coupled receptors in immune cells.

COMPARISON BETWEEN OPERATIONAL SPECIES CONCEPTS FOR YEASTS: GENUS *HANSENIASPORA* AS A CASE STUDY

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Even though the species is one of the most controversial definitions in microbiology we operatively need this basic unit for construction a taxonomic classification system which provides a stable identification framework for microbiological disciplines. The diversity of organisms with their specific characteristics (e.g. lack of mating, parthenogenetic or allopartic microorganisms) has lead to a plethora of different species concept which change with developments of new tools for their descriptions. In this view the discovery of DNA as a molecule containing historical data about the evolution of the species enabled the most useful concepts for definitions of microbial species.
In this view we have implemented different concepts for definition of new species of apiculate yeast genus *Hanseniaspora*: phenetic species concept (dissimilarity in phenotypic characteristics), biological species concept (indirect approach by measurement of DNA-DNA homology), phylogenetic species concept (by sequencing the „chronometers" of evolution and placing the new taxa in phylogenetic frame) and multilocus genealogical concept (by comparing different gene trees to detect reproductive barrier between populations of apiculate yeasts). In view of these results five new species and a new variety of *Hanseniaspora* had been described [1, 2].

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ATPASE INHIBITORS AS NEW EFFLUX PUMP INHIBITORS OF *ESCHERICHIA COLI*

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Antibiotic resistance in Gram-negative bacteria can be increased by extrusion of the antibiotic through efflux systems. In *Escherichia coli*, the major efflux pump (EP) is the AcrAB which is mainly driven by energy coming from the proton motive force (PMF). Environmental factors such as Calcium (Ca^{2+}), pH or glucose (GLU) (energy source) have major influence in retention or efflux of compounds by the cell. Our previous results suggest that efflux and accumulation of EB by *E. coli* AG100 is pH and energy dependent and influences the performance the AcrAB pump. This EP is dependent upon protons present in the periplasm for their activation. Hence, when *E. coli* faces stress conditions caused by a noxious agent, its extrusion would be preferentially performed by an ABC type transporter at pH greater than 7. A better understanding of the role of different energy sources on the efflux mechanism was the aim of this study. Three well characterized *E. coli* strains were used: (i) the parental wild-type *E. coli* K-12 AG100 which has intact and functional AcrAB; (ii) *E. coli* K-12 AG100A whose genes that code for the AcrAB have been deleted; (iii) *E. coli* K-12 $AG100_{TET}$ that was adapted to increasing concentrations of tetracycline and over-expresses the AcrAB system and others EPs. It was used a semi-automated fluorimetric method that detects the efflux of the fluorochrome ethidium bromide (EB), on a real time basis at 37ºC under different environmental conditions: (i) energy source, (ii) pH, (iii) presence and absence of known inhibitors of energy biosynthetic pathways at concentrations that do not affect the cellular viability, (iv) presence and absence of Ca^{2+}. The compound 8-hydroxyquinoline (HQ), was used as inhibitor of the electron transport chain, sodium azide and sodium orthovanadate (Na_3VO_4) as inhibitors of ATPase, carbonyl cyanide m-chlorophenylhydrazone (CCCP), chlorhexidine and 2,4-Dinitrophenol (DNP) as un-couplers of oxidative phosphorylation, chlorpromazine (CPZ) as inhibitor of calcium channels and EDTA as chelator agent. It was previously observed that efflux is GLU dependent at pH 8 but this effect is not noticed at pH 5. CPZ increases the retention of EB especially in absence of GLU, but not in the presence of Ca^{2+}. The addition of Ca^{2+} to an EDTA containing medium nullifies the accumulation promoted by EDTA. The simultaneous presence of CPZ and EDTA synergistically increases accumulation. At pH 5 the effects of CPZ, EDTA and Ca^{2+} are minimal.

IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY OF *LISTERIA MONOCYTOGENES* FROM ROW MEAT

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Listeria spp. are Gram positive, short, non-sporing rods, facultatively anaerobic. Of the six species currently recognized, *Listeria monocytogenes* is the most important causing a range of infections in humans and animals. The organism can be found in a wide variety of habitats including the soil, food processing environments and row foods. The ability of the organism to grow at refrigeration temperatures is of importance in food production. The aim of our work was isolation, enumeration and identification *Listeria monocytogenes* in row poultry and pig meat. Enumeration and isolation of bacteria were performed according to ISO procedure (ISO 11290-1 and ISO 11290-2). Vitek 2

Compact System (Biomérieux) was used for identification of isolated strains and their antibiotic susceptibility. Isolated strains were identified as *Listeria monocytogenes*, *Listeria innocua* and *Listeria welshimeri*.

DETECTION OF HUMAN PARVOVIRUS 4 RELATED PORCINE HOKOVIRUSES IN HUNGARY

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Parvovirus infections of swine, with the classical porcine parvovirus (PPV) strains, are widespread worldwide and are responsible for reproductive disorders, especially in those herds where vaccination protocols are not followed correctly or, where vaccine efficacy is decreased due to immunosuppressive factors. Thanks to the rapidly improving nucleic acid amplification technologies new porcine parvoviruses had been described during the last years. An unusual parvovirus was detected in pig sera in Myanmar, and provisionally named PPV2, as the genome was distantly related to previously known porcine parvovirus sequences. Later during the year 2007 yet another parvovirus was described in pigs in Hong Kong and hence named porcine hokovirus (PHoV). PHoV was found to be genetically similar to the also recently described human parvovirus type 4 and 5 (PARV4, 5) and the bovine hokoviruses were also described. The worldwide occurrence of these newly discovered swine parvoviruses is not known yet. The purpose of this study was to determine if hokoviruses were also present in Hungary, or they were only characteristic to the Hong Kong area, and if positive cases were identified, to determine the prevalence of PHoV in Hungary and compare the sequences with those available in the Genbank. Organ samples (lungs, liver, kidneys, spleen and lymph nodes) were collected randomly at slaughterhouses and at the Department of Pathology, between 2006 and 2009. The organs were processed following standard nucleic acid purification methods and tested for the presence of hokovirus genomes by polymerase chain reaction (PCR) and sequencing. Primer pairs for amplification and sequencing, were synthesized and a different set of primers comprising a short region of 130 bases was designed for diagnostic purposes. PCR was carried out as a standard amplification procedure. PCR results with the diagnostic primers indicated that 37% of the samples were positive for PHoV, similar to the prevalence described earlier. No geographic differences could be detected in the prevalence of the virus, and comparing samples according to the year of collection, only a minor increase could be shown from 2006 to 2009. Phylogenetic analysis indicated that the hokovirus genomes present in Hungary carried only single nucleotide differences in the sequenced areas, and they also showed high homology with the ones available in the Genbank. Attempts to culture the virus *in vitro* in continuous porcine kidney (PK-15) and testicle (ST) cell lines were not successful, but when ST cells were co-infected with PHoV from infected organs and a porcine adenovirus, *in vitro* replication of PHoV isolates could be detected by PCR until the third passage of the progeny.

INFLUENCE OF TEMPERATURE, PH AND WATER ACTIVITY ON *TRICHODERMA PLEUROTUM* AND *T. PLEUROTICOLA*, THE CAUSATIVE AGENTS OF OYSTER MUSHROOM GREEN MOULD

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The green mould disease of edible oyster mushroom (*Pleurotus ostreatus*) caused by filamentous fungi from the genus Trichoderma has been recently reported to cause great crop losses world wide. The fungi responsible for the disease were described as the new species *Trichoderma pleurotum* and *Trichoderma pleuroticola*. Molecular identification of Trichoderma strains isolated from samples of oyster mushroom cultivation substrate (wheat straw from natural resources) collected in Hungary and Romania revealed the presence of both pathogenic species in both countries. The effect of environmental parameters (temperature, pH, water availability) on the linear mycelial growth of the Pleurotus-pathogenic Trichoderma strains was examined on synthetic minimal medium and oyster mushroom powder containing medium. The growth of *T. pleurotum* showed narrower temperature range (15-30°C) than that of *T. pleuroticola* (10-35°C). Acidic and neutral pH values (pH 5-7) and higher water activities were favouring the growth of both pathogens. Conclusions from this study provide useful information for mushroom growers to optimize the environmental parameters during Pleurotus cultivation in order to avoid economic losses due to green mould outbreaks.

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CHARACTERIZATION OF *MUCOR CIRCINELLOIDES* TRANSFORMANTS CARRYING AUTONOMOUSLY REPLICATING PLASMIDS

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Genetic analysis and biotechnological utilization of the microorganisms require transformation systems that result stable transformants. Genetic transformation of Mucoral fungi often generates transformants harbouring the introduced DNA as autonomously replicating elements without any integration event [1]. In most of the previous studies, such transformants of the Zygomycetes have been proven to be mitotically unstable rapidly losing the introduced foreign DNA. The aims of the present study were: to transform the zygomycete fungus *Mucor circinelloides* with different autonomously replicating plasmids and to investigate the transformants (e.g. to determine the transformation frequency and the mitotic stability of the transformants and to examine the copy number and the expression of the introduced genes). Expression vectors containing different isoprenoid-pathway genes were constructed. These genes were also placed under the control of *Mucor gpd1* regulatory sequences. A bacterial gene was also involved in these experiments. Single and co-transformation experiments with a uracil and leucin auxotrophic *M. circinelloides* strain were carried out by the PEG-mediated protoplast transformation method [2]. All transformations resulted similar numbers of transformant colonies and there was no significant difference in the transformation frequencies. The transformation events were verified by PCR and Southern analysis. All transformants were stable under selective conditions. Some randomly selected transformants were analysed under non-selective condition too, where all of them proved to be stable. Real-time PCR analysis revealed a relatively high copy number of the plasmids in the transformants and an imbalanced proportion of the plasmids in many of the co-transformants. Expression of the introduced genes was also examined. Real-time PCR analyses verified that elevation of the copy number and/or induction of the *gpd1* promoter by higher glucose concentration in the media increased the expression level of the tested genes.

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REAL CASE APPLICATION OF COMMERCIAL MYCORRHIZA PRODUCT „SYMBIVIT" IN THE HORTICULTURE

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Arbuscular mycorrhizal fungi (AMF) give a significant contribution to plant nutrition promoting mostly the uptake of phosphorus and water thereby mycorrhiza inoculation can make cultivation cost-efficient in the horticulture. It is well required to provide more information about the applications of AMF because the high levels of phosphorus commonly used in commercial potting media often limits mycorrhizal formation. Our aim was to study the circumstances of using one commercial mycorrhizal product „Symbivit" and to improve our knowledge on critical environmental factors using *Pelargonium*, the most common annual ornamental plant of Hungary. Rooted cuttings of geranium (*Pelargonium hortorum* 'GRECO') were grown in a peat-perlite mix substrate (pH 6.47) in pots of 500 ml and inoculated with Symbivit containing a mixture of *Glomus intraradices*, *G. claroideum*, *G. microaggregatum*, *G. mosseae* and *G. etunicatum*. Pots either were not fertilized in one treatment or slow release fertilizer was applied at a reduced rate of 2 g/l (15N+9P+12K+2.5MgO+microelements). Mycorrhiza was added both to non-fertilized and fertilized pots. Plants were grown in a greenhouse for 72 days then plants were transplanted and inoculated again with AMF. No further fertilizer was added. Greatest growth responses occurred after application of both Symbivit and fertilizer however, no or very low colonization could be detected during the first 4 weeks. Visual differences were apparent mainly by inoculation treatments which were often adverse to root colonization levels. Mycorrhiza improved leaf colour of all treatments as well, the greatest differences of this parameter between inoculated and non-inoculated plants were found particularly in the treatments without fertilizer. Our results show that mycorrhizal inoculation could replace nutrient supply partially without active colonization of AM fungi suggesting the role of other beneficial microorganisms which are connected to mycorrhizal fungi. Because of the lack of high root colonization, new AMF inoculation was carried out after 72 days. Interestingly the late application of Symbivit decreased the growth of shoots and roots at nutrient supply showing a complex interaction among plant-mycorrhiza-soil microorganisms system. The results obtained in this experiment highlight the great importance of microorganisms which are connected to mycorrhizal fungi however more research is required to explore the reasons of the observed effects.

HUMAN HERPESVIRUS 6 INFECTION IN RENAL TRANSPLANTED PATIENTS

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Immunosuppression associated with transplantation is a risk for opportunistic infections, reactivations and reinfections. The usage of more effective immunosuppressive therapy leads to decreasing graft rejection, but increasing susceptibility to infections. Besides the most prominent human

cytomegalovirus there are a growing list of viruses which can cause serious, even life threatening problem among transplanted patients. HCMV infection is diagnosed daily by monitoring pp65-antigenemia from peripheral blood samples of renal transplanted patients in our department. The aims of our study to reveal the possible role of active HHV-6 infection in post-transplantation syndromes and the association between HHV-6 and HCMV infection in renal transplanted patients. We would like to prove the need for HHV-6 diagnosis in renal transplanted recipients, the best sample and the best methods for it. We would like to determine whether blood samples or urine are the best for diagnosis; to define the most sensitive and specific diagnostic method for this, which can be antigenemia, nested PCR or quantitative real-time PCR. We would like to survey the prevalence of the two distinct variants of HHV-6 among the patients. Up to the present 160 peripheral blood samples of renal transplanted patients were examined for HCMV and HHV-6 infections. 15 samples were positive for HCMV pp65 anigen. HHV-6 DNA was detected in white blood cells of 27 samples: 14 HHV-6 A and 13 HHV-6 B variant. In case of plasma samples HHV-6A DNA was detected by nested PCR in 12 samples, while HHV-6B was only in 2 plasma samples. HCMV and HHV-6A (in cells and plasma) was presented together in 3 samples. HCMV and HHV-6B was presented together in 2 samples, however in these cases plasmas were negative for HHV-6B DNA. 26 urine samples were also examined for HHV-6 DNA. 4 samples were positive for A variant, 3 samples for HHV-6B and 1 for both variants. 4 urine samples were positive for viral DNA while blood samples from the same patients at the same time were negative. Clinical symptoms of HHV-6 and/or HCMV positive patients were similar. Real-time PCR is optimalized, quantitaive examination of the samples for HHV-6 DNA is planned. HHV-6 antigenemia is under optimization.

IN MEMORIAM ERVIN K. NOVÁK - OBITUARY

T. DEÁK

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Ervin Károly Novák passed away on August 9, 2009 at the age of 77. He was an eminent scholar in the broad field of mycology interested in various aspects of activity of filamentous fungi and yeasts alike. He was born in Budapest in 1932. He entered the Budapest University named after Eötvös Loránd where he trained biology and chemistry and specialized in microbiology. Having graduated in 1955 he remained at the Department of Microbiology for a short while as junior trainee, and in 1956 he joined to the Mycology Department of the National Public Health Institute, and became its head until his retirement in 1992. At this position he had carried out great development as to the instrumentation, the routine practice and the scientific research.

Working within the frame of a public health institution, He dealt primarily with medical and hygienic mycology, the detection and identification of fungi in clinical specimens, and the methodology of diagnostic tests. However, he always felt strong motivation for research work, and soon achieved remarkable results in mycology. He has a strong background in chemistry and biochemistry, and his first publications discussed the metabolism of malonic acid. This was the subject of his university doctorate in 1962. His research was further extended to the diagnostic of dermatopytes and the mechanism of action of antifungal antibiotics, but his favourite subject was the physiology, identification and taxonomy of budding yeasts. The number of his publication soon reached 170, and honouring his remarkable achievement, in 1981 He was given by the Hungarian Academy of Sciences the PhD title on the basis of a summarizing thesis without requiring a full dissertation. At these years he also started teaching as invited lecturer at several Hungarian universities, and

eventually he obtained a honorary associate professor title. He indeed was an enthusiastic and skilled teacher with charm and a sense of humour.

In the years of 1970th and 1980th his activities developed entirely. The overall theme of his research can be summarized as the prevention of fungal diseases and development of methods for detection, isolation and identification of pathogenic fungi. Although he made important theoretical contribution, his research always retained a practical aspects. His most loved subject had, however, always remained the yeasts. Probably, the two publications he would most like to be remembered are the reviews he wrote together with János Zsolt from the University of Szeged, are the New system proposed for yeasts (1961) and the Physiological rules of yeast taxonomy (1962). He described seven new yeast species, some of them still valalidly accepted while two species were described bearing his name (*Candida novakii*, *Geotrichum novakii*).

E. K. Novak's total publication counts an incredible number of almost five hundred, namely 484 including a dozen of books, textbooks and chapters. By topics these can be grouped as shown in a tabulated form. The majority of papers was published with co-authors. E.K. Novak only felt co-authorship was justified when he had made definite contribution to the results. Moreover, he was scrupulous about the credit of work made in his lab. Otherwise He allowed his fellows a great deal of freedom and independence while always being critical and in the same time generous with help and advise when it was required.

We, colleagues and students around him, maintained and easy and friendly relationship with him. He was just called 'EK' for the initials of his given names. EK liked to work with a small, intimate group of colleagues and placing high importance of friendliness. I had the good lack and honour to work with EK closely for some 40 years. I remember a number of late evening work in his lab while running some experiments when the incubation lasted for long hours and meanwhile hearing some of his records of opera singers or full operas of which he was a fan and determined collectors. He often asked if we could recognize the singers by sound, and those of us who succeeded in, was eligible a glass of beer or wine. In between taking samples or making readings, we often play a deal of bridge. It was typical of EK's character that he started to develop a new system of bidding in bridge. Those who have worked with EK are proud to have known him, and will occasionally raise a glass of beer in memory of his strength, sense of humour, and non-assertive mode of leadership.

Outside the lab EK was also an active member of the scientific community. His discipline was firmly rooted in biochemistry, and he was a leading member of the Association of Hungarian Chemists and its Biochemical Section and organised several of its annual meetings. He was elected life member of this Society. Beside the Biochemical Section, EK was also involved in founding the Mycological Section of the Hungarian Microbiological Society. The HMS Mycological Section was an organization of particular importance to EK who was its president for many decades. His last participation on the Annual Meeting of HMS in 2006, when for health reason he was able to present a poster instead of lecturing, but the unusual subject of it („Mycologia criminalis") was still typical for his manner. At the international scene, he was a respected scientist in the field of mycology, frequently invited speaker of conferences, and for decades he was the Hungarian representative of the International Commission of Yeasts. He also organized international conferences in Hungary. Notable was the 5th ISSY in Keszthely in 1977. This was only followed after 26 years with the 23rd ISSY held in Budapest in 2003, in the organization of which he was a scientific adviser.

In the last years of his life, he suffered for a disease but struggled against it. He did not give up the struggle, and planned to write a series of publication summing up the world of fungi from physiological and ecological point of view and their harmful and beneficial activities to mankind. One day, a year ago, he was able to visit me at my Department to discuss some part of the subject, and on the way returning home he suffered an accident and became hospitalized. His underlying disease strengthened, and after ten months he unfortunately died.

His great achievements in the field of mycology will be remembered and retained by his students and

followers. I am grateful for help in writing this obituary from Dr. Judit Zala, Head of the Mycology Section, Johan Béla National center for Epidemiology, and Professor Anna Maráz, Head of the Department of Microbiology and Biotechnology, Corvinus University Budapest.

MOLECULAR INVESTIGATION OF NOSOCOMIAL HEPATITIS C VIRUS OUTBREAKS IN HUNGARY

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Since the screening of blood donors has been introduced, transfusion-associated hepatitis C infections have become rare events. However, a part of newly acquired HCV infections are still health-care related. By the phylogenetic analysis of viral sequences the relatedness of new infections can be demonstrated, but the investigation may also provide information on the source of infection, the number and time of transmission events. In this study we aimed to find molecular evidence for a nosocomial transmission and to ascertain the source of infections at a haemodialysis center and an oncology ward in Hungary. Regions of the HCV genome with different levels of variability were utilized for the investigation. A sensitive nested PCR specific for the conserved 5'UTR was applied to detect viral RNA. The more variable NS5B and E1/E2 regions were also amplified and sequenced for genotyping and phylogenetic analysis. Unrelated control sequences from Hungary were used for comparison. At the dialysis center 16 out of 25 seropositive patients were HCV RNA positive, they all carried subtype 1b viruses. Phylogenetic analysis of NS5B sequences showed that newly infected patients formed a separate group from control sequences with high statistical support, showing that they were probably related. Three of the putative source patients carried viruses unrelated to the outbreak. The viral sequence of the fourth putative source patient, however, clustered together with the sequences of the patients infected during the outbreak, which strongly suggested that the virus of this patient was the source of the nosocomial infections. At the oncology ward 12 newly infected patients were RNA positive, all infected with HCV subtype 1a. The only recognized chronic carrier treated at the ward carried subtype 1b virus and was excluded as a possible source of infections. In the phylogenetic analysis, the sequences of the outbreak patients clustered into two groups, each containing genetically closely related sequences. Evolutionary distance between the groups suggest a more distant common ancestor.

The grouping and genetic distances between isolates from the haemodialysis center show that the newly infected patients included in the phylogenetic analysis were infected from a common source, which was identified as the virus of a HCV carrier patient also haemodialysed at the same unit. Evidence of nosocomial HCV infections was found also in the oncology ward, however the source of infections could not be identified. It may have been a chronic carrier patient with highly divergent variants of subtype 1a. This is also supported by the genotype distribution observed among controls.

FATAL SEPSIS CAUSED BY HETEROGENEOUSLY VANCOMYCIN-INTERMEDIATE SENSITIVE *STAPHYLOCOCCUS AUREUS* (HVISA) STRAINS IN TWO HUNGARIAN PATIENTS

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The incidence of invasive MRSA isolates is 25% in Hungary. Frequent use of vancomycin resulted in selective pressure on *Staphylococcus aureus*, which eventually led to resistance among MRSA strains. The first appearance of vancomycin-intermediate sensitive *S. aureus* (VISA) was reported in 1996 in Japan, which was followed by reports of a number of cases of hetero-VISA. Until now only one case report of hVISA infection has been published in Hungary. Here we describe two additional cases of fatal hVISA infections at the Departments of the University of Debrecen. The first patient was a 55-year old man. He had parapharyngeal abscess, which was surgically removed. After surgery he developed sepsis, bronchopneumonia, and acute renal insufficiency which necessitated haemodialysis. He had been repeatedly treated with haemodialysis, haemofiltration and transfusions, but he had reoccurring high fever. At first he received a third generation cephalosporin, then amikacin, but his condition became worse. Routine nasal and throat cultures and hemocultures showed MRSA which was susceptible to vancomycin, therefore amikacin had been combined with vancomycin. After two weeks of continuous intensive therapy the patient died. Postmortem haemoculture showed MRSA with reduced vancomycin susceptibility. This strain was confirmed as a hVISA by macro E-test and population analysis. The second patient was a 24-year old woman, suffering from severe inborn diseases. She was admitted to the hospital with acute respiratory insufficiency after an epileptic shock. Endotracheal intubation became necessary, and tracheostomy was performed. Due to suspected aspiration combined empiric antibiotic therapy (clindamycin, ceftriaxone) was started. Despite the treatment the patient remained febrile, which was explained by fungal and *Pseudomonas* infections. The antibiotic profile was changed to ceftazidime, amikin, clarythromycin and the antimycotic fluconazole was administered. The patient responded well, but one month later her temperature elevated again. At that time enteral nutrition had to be instituted. Hemoculture and bronchial cultures showed MRSA with reduced susceptibility to vancomycin. This strain was also confirmed as a hVISA. Routine nasal culture from the left nostril proved MRSA colonization. Due to polyresistance, combined specific antibiotic regime was started. Hereafter her hemocultures showed only polyresistant Gram-negative pathogens and cultures from her upper respiratory system showed vancomycin susceptible MRSA. The patient died after 3 months of hospitalization due to sepsis and circulatory-respiratory failure.

THE STUDY OF THE METAL CONTENTS OF SOME DOMINANT PLANTS AND PHYLLOSPHERIC MICRO-ORGANISMS ON UPPER-TISZA AREA

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As an effect of last years' the industrial contaminations, were reached the critical ecological level on the flood-basin of Upper Tisza some times. The large content of pollutants (heavy metals, cianids, other industrial pollutants), the dispersion and accumulation infected the plants and animals in rivers and them flood-basin. The aim of study in 2007-2009 were investigated the relationship of soil-plant-

metal content in dominant plants and soils on the Tisza river and flood-basin. The sampling sites were Tiszabecs, Vásárosnamény, Dombrád, Tokaj. We measured the range mg/kg: Chrome (Cr) 61-581, Cadmium (Cd) 1,7-1,8, Nickel (Ni) 24,9-39,3, Copper (Cu) 23,1-35,8, Zink (Zn) 113-191,6 in soils of flood basin. The soils content of Cd and Cr exceeded the limit value according to 10/2000. VI.2. KöM-EüM-FVM-KHVM regulation. The dominant plants were *Rubus caesius*, *Humulus lupulus*, *Atremisia vulgaris*, *Ranunculus repens*, *Taraxacum officinale*, *Galium aparine* and *Solidago canadensis*.We investigated the Cr, Cd, Ni, Cu- and Zn-contents these plants. The metal-contents (mg/kg) of roots were: Cr 90-474, Cd 1,6-8,1, Ni 23,3-32,5, Cu 22,7-31,5 Zn 106-186. The leaves range were Cr 7-390, Cd 1,2-1,6, Ni 0,7-44,1, Cu 0,6-42,8 Zn 24,9-203,5. The metal- contents of steam were: Cr 4,2-5.5, Cd 1,2-4,6, Ni 0,4-1,9, Cu 4,4-13,9, Zn 27,1-69,1. The highest metal-content were of all investigated metals in roots and leaves of *Rubus caesius* and *Solidago canadensis*. Our study showed, that the high level of metals as soil contaminations and the high plants metal contents harmful in river compartment. Dominant bacteria in the phyllospheric were mostly Gram-negative micro-organisms. The surfaces of leaves were colonized by the *Pseudomonas, Enterobacter*, *Bacillus* and *Coryneform* genera. We investigated the highest amount number of bacteria on sequentially the leaves surface of *Rubus caesius*, *Solidago canadensis* and *Artemisia vulgaris*. The plants accumulated the metals on the investigated flood-basins. The highest amount of metal was in roots and leaves of *Rubus caesius* and *Solidago canadensis* on all sampling sites. The metal contents and number of bacteria showed positive correlation colonizing the leaves surfaces of plants.

APPLICABILITY OF TOPO-OPTICAL REACTIONS IN MICROBIOLOGY

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Polarisation optical technique gives an opportunity to perform sub-microscopic investigation on structures containing sterically highly ordered molecules like the cell envelope of microbes. Such structures can evoke optical anisotropy and birefringence i.e. the refractive indices are significantly dissimilar in different axes of the structure. This natural birefringence can be enhanced or modified by different dyes or reagents yielding further possibilities to specifically investigate the composition and structure of bacterial and fungal surface components. The aim of this study was to give an account on the applicability of classic and more recently developed topo-optical staining methods for microbiological purposes using an isogenic pair of encapsulated and non-encapsulated *Klebsiella pneumoniae*, and various fungal species. The classic aldehyde-bisulfite-toluidine blue (ABT) reaction, sialic acid specific topo-optical reactions and the chlorpromazine-eosin charge transfer reactions were applied. As expected, the non-encapsulated *Klebsiella* cells were phagocytosed in a higher number than the encapsulated counterpart. The intracellular majority of non-encapsulated bacteria produced only weak or no birefringence while encapsulated bacteria stained with strong intensity regardless of being engulfed or not. At two hours of incubation most of the phagocytosed non-encapsulated bacteria reversed the orientation of their birefringence. Similar changes were observed on phagocytosed encapsulated cells but only after four hours of incubation. The alterations could be observed in all the three types of topo-optical reactions. In case of yeasts *Cryptococcus neoformans*, *Saccharomyces cerevisiae*, and various *Candida* species were studied. We could show a highly fashioned organisation of the sugar moieties on the yeast cell surface. Sialic- and O-acyl sialic

acid residues could permanently be found in a terminal position on the carbohydrate chains. The capsule of *C. neoformans* exhibited a very strong metachromasy and anisotropy. It is especially remarkable that the sterical orientation of sugar chains, and the terminal sialic acid and 9-O-acyl sialic acid molecules was opposite in the inner and outer layer of the capsule.
Extension of the investigations to further prokaryotic and eukaryotic entities. Analysis of the molecular orientation of microbial cell surface structures under perturbing conditions (chemicals, antimicrobials, etc.). Application of topo-optical reactions on possible targets in drug research.

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GLUTATHIONE METABOLISM OF CARBON STARVING *ASPERGILLUS NIDULANS* CULTURES

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The high glutathione content of fungi can serve as sulfur, nitrogen or even carbon (energy) source during starvation. In *Aspergillus nidulans* cultures, we found an intensive decrease in the glutathione content after glucose depletion, which was accompanied with the induction of γ-glutamyl transpeptidase as a glutathione hydrolyzing enzyme. Interestingly the addition of weak carbon sources like yeast extract, BSA or Glu even increased the γ-glutamyl transpeptidase activities and did not decelerate the depletion of glutathione pools. Moreover, carbon starvation also enhanced the glutathione efflux and the secretion of an enzyme possessing γ-glutamyl transpeptidase activity. These data suggest that glutathione metabolism of carbon starving cultures is more complex than we assumed earlier and the observed changes can not be explained simply with the utilization of glutathione as a storage compound.

FERMENTATION CHARACTERISTICS OF THE YEAST STRAIN *KLUYVEROMYCES MARXIANUS* CBS712

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The exhaustion of fossil energy sources, the growing importance of economical considerations as well as the protection of our environment give an ever growing role to the utilization of renewable energy in our life. Bioethanol could be one of the renewable energy sources. A profitable way of production of it is the application of thermotolerant yeasts. *Kluyveromyces marxianus* is a yeast species with outstanding thermotolerance. The „IMB" strains of this species are used for ethanol production at 45 C in the industry. The final goal of our project will be the genetic modification of the *Kluyveromyces marxianus* CBS712 strain in order to produce the possible highest amount of ethanol at a temperature higher than that applied currently in industrial production. To achieve this

goal, it is crucial to characterize the original strain in detail. Determination of the temperature limit of growth, the ethanol production of the strain, and the conversion rates are especially important. Experiments were carried out in shaked flasks, in MYFM medium at 45 C to 48 C, temperatures that are close to the limit of growth of the strain. Ethanol production, the amount of the residual glucose, the viability of the strain and biomass production were investigated in experiments in which increasing amount of glucose (160-240g/l) was applied in the fermentation medium. The highest conversion rate: 55.3% was achieved at 45 C, producing 5.6% (v/v) ethanol. At 46 C the amount of produced ethanol was 4.99% which did not differ from the maximum production greatly, but the conversion rate was only 48.1% in this case. Further increase in the temperature caused a huge drop in the ethanol production, viability, and the amount of produced biomass. It is important to note that increasing the concentration of glucose in the fermentation medium did not influence fundamentally the investigated characteristics (ethanol production, viability, biomass production); the determining factor was the temperature. In summary, we conclude that the basic characteristics of the original *Kluyveromyces marxianus* CBS712 strain that we are going to further improve with genetic manipulations are comparable to those strains which are applied in industrial ethanol production nowadays.

CHARACTERIZATION OF BEECH ECTOMYCORRHIZAE RELATED TO THE *PACHYPHLOEUS-AMYLASCUS* LINEAGE (ASCOMYCETES, PEZIZACEAE)

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Though some extensive work has been carried out lately on the ectomycorrhizae (EM) formed by ascomycetes, further detailed morphologial and anatomical investigations are required to broaden our view. The EM-forming ability of the hypogeous genus *Pachyphloeus* has been proposed previously, however, only the EM of Pachyphloeus cf. virescens has been described yet. This genus forms a common lineage together with the hypogeous *Amylascus* and the epigeous *Scabropezia* within Pezizaceae. During a six-year-long study on the EM community of the montane beech stand of the „Õserdõ" forest reserve (Bükk National Park, Hungary), we found ten EM specimens belonging to the Pachyphloeus-Amylascus lineage. The morphology and anatomy of the EM were characterized by stereomicroscopy and differential-interference-contrast microscopy (Nomarski-DIC). Molecular identification was carried out by the phylogenetic analysis of the ITS and LSU regions of the nrDNA. All the EM specimens shared common morphological and anatomical traits. The EM form densely ramifying whitish-yellow to light-brown, pyramidal systems with short, stout ends. The surface is densely woolly with white or brown, curly hyphae. The mantle layers are pseudoparenchymatous with angular cells; the outer layer is covered by the network of thick-walled hyphae. The emanating hyphae are densely septate, without clamps. Based on their anatomy, the EM specimens can be sorted into three different morphotypes (Mt), differing each other concerning their colour, certain morphometric traits (cell-wall thickness, diameter of emanating hyphae, septal distance) and also anatomical characters (structure of the surface net). According to the results of the rDNA-sequence analyses, the three morphotypes are formed by three different species of the lineage. One of them can be identified as P. melanoxanthus and another can be regarded as an unidentified Pachyphloeus species. In case of the third morphotype, we can only tell that it is closely related to the Pachyphloeus-Amylascus lineage, without identifying even the genus. However, the ectomycorrhizae of neither

species was observed previously.

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PRODUCTION OF PURIFIED RECOMBINANT PROTEINS OF *CHLAMYDOPHILA PNEUMONIAE*

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Low calcium response proteins E (LcrE, CPn0324) and H (LcrH, CPn0811) are part of a type III secretion system (TTSS) in *Chlamydophila pneumoniae.*, TTSS allows this obligate intracellular bacterium to secrete effector molecules into the inclusion membrane and the host cell cytosol. Since LcrE is surface exposed in *C. pneumoniae* elementary bodies (EB), it can be regarded as a candidate component of a potential vaccine against this significant human pathogen. LcrH is a TTSS chaperone protein, expressed in the middle to late stages of the developmental cycle of *C. pneumoniae*.
In order to produce highly pure LcrE and LcrH proteins that are suitable for functional and immunological studies *lcrE* and *lcrH* were amplified by PCR and cloned into pET vector carrying His tag. The proteins were overexpressed in *Escherichia coli* HB101 and purified from the supernatant using the HIS-select TALON CellThru Resin. The purity was checked by mass spectrometry. The majority of the purified proteins were LcrE and LcrH respectively, some housekeeping proteins of *E. coli* were also present. The mass spectrometry showed that the full-length LcrE and LcrH were expressed. BALB/c mice were immunized s.c. with the purified LcrE and LcrH proteins mixed with Freund's adjuvant. The identity and immunogenicity of these proteins were proved by Western blot. The immunized mouse sera recognized the purified proteins and the same proteins in *C. pneumoniae* EB-s. The methods we applied are useful tools for production of other *C. pneumoniae* proteins, to study their immunogenicity.

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VOLATILE COMMUNICATION IN *TRICHODERMA*

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Conidiation in *Trichoderma* spp. can be induced by light or by starvation. We found that volatiles produced by conidiating colonies of *Trichoderma* spp. elicited conidiation in colonies that had not been previously induced to conidiation by exposure to light. The inducing effect of volatiles was both intra- and interspecific. Chemical profiles of the volatile organic compounds (VOCs) produced by the non-conidiated colonies grown in the dark and by the conidiating colonies were compared using solid-phase micro extraction of headspace samples followed by tandem gas chromatography-mass spectrometry. The conidiation was accompanied by pronounced changes in the spectrum of the produced VOCs with increased proportions of eight-carbon compounds 1-octen-3-ol and its analogues 3-octanol and 3-octanone. When the vapors of the authentic compounds were applied individually to dark-grown colonies, they elicited their conidiation already at sub-micromolar concentrations. It is concluded that the eight-carbon VOCs act as signaling molecules regulating

development and mediating the inter-colony communication in *Trichoderma*. Since *Trichoderma* is known antagonist of phytopathogenic fungi, there is growing interest in production of inocula for biocontrol purposes. The stimulating effect of volatile compounds on sporulation may be exploited at large-scale inocula production for increasing the yields of conidia.

THE OCCURENCE OF CRYPTOCOCCOSIS IN AN INDIAN HOSPITAL - IDENTIFICATION AND CHARACTERISATION OF THE STRAINS

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The basidiomycetous yeast *Cryptococcus neoformans* causes a serious and potentially lethal infection named cryptococcosis, the most common manifestation of which is meningitis. *C. neoformans* is an opportunistic pathogen; infection usually develops in patients with defective cell-mediated immunity (AIDS, organ transplants and immunosuppressive therapy). The prevalence of cryptococcosis has been increasing during the last decades along with the emergence and worldwide distribution of AIDS. Despite extensive antifungal therapy the mortality rate of cryptococcosis among AIDS patients is very high and still represents a life-threatening infection.
In this study, the identification and characterization of 13 clinical yeast isolates derived from HIV positive heterosexual patients with meningitis from an Indian hospital (Coimbatore Medical College, Coimbatore, India) was carried out. Fungal culture and serology of the patients was positive. All isolates produced melanin on sunflower seed extract media. The lack of fermentation and the carbohydrate assimilation patterns were characteristic to *C. neoformans* which was verified by the partial nucleotide sequence determination of D1/D2 region of the 26S rDNA. Eight of the isolates were mating type α while the remaining five were infertile. They were characterized by different sensitivities to five yeast killer toxins. Analysis of PCR-fingerprints generated with minisatellite-specific primer revealed that these isolates belong to the *C. neoformans var. grubii* serotype.

DIFFERENTIATION OF *CANDIDA ALBICANS* ISOLATES ON THE BASIS OF POLYMORPHIC MTDNA

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The imperfect yeast *Candida albicans* commonly belongs to the human microflora, nevertheless this species is the most frequent pathogen isolated from human fungal infections. Infection usually develops in patients with decaying balance of microflora or defective immunity. The incidence of *C. albicans* infections has been increased during the last decades because of the widespread use of antibacterial antibiotics and growing number of immunocompromised patients.
The genetic variability of *C. albicans* isolates originating from a Hungarian hospital was examined in our previous study using mtDNA RFLP, RAPD analysis and electrophoretic karyotyping. The isolates could be clustered into four groups on the basis of their mtDNA RFLP profiles. The physical map of the mtDNAs of the representative strains belonging to the four types (I: 10930, II: 9132, III: 5796, IV: 17471) was generated by using *Eco*RI and *Eco*RV restriction endonucleases. Partial genetic

map was also constructed with the following hibridization probes: cox1, cox2, cox3, cob, nad1, nad2, nad4, nad5 and atp6. The results revealed the same gene order in all the four types. The *Eco*RI fragments of Type I, II and IV were cloned into pBluescript SK vector for determination of the nucleotide sequence. We can conclude that point mutations, resulting the distinctive *Eco*RV patterns (e.g. GAT/ATC–GGT/ATC) are responsible for the polymorphism in the mtDNA within this species.

A specific region differing remarkably corresponding to the *Eco*RV sites in the four types was chosen in order to analyze all the 44 clinical isolates. This region was amplified by specific primers and digested with *Eco*RV, henceforward the groupings of all the 44 isolates proved to be right with PCR RFLP method. Sequencing of the smallest *Pvu*II fragment revealed 55 bp deletion in Type I., II. and IV. compared to Type III, in the upstream (intergenic) sequence of the cox3 gene. This fragment contains the previously described EO3 region. Four mtDNA types were separated by analysing this region: L, MI, MII and S. We manage to group our representative strains into this clusters: 5796 (III) belongs to group L, 9132 (II) and 10930 (I) fit into MII, while 17471 (IV) stand for MI. Group S do not have representatives in this population of *C. albicans*.

CLOSED AND OPEN ENDED GENOME-WIDE MOLECULAR APPROACHES TO STUDY DYNAMIC HOST-PATHOGEN INTERACTION

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Powdery mildews (PMs) are obligate plant pathogenic fungi of the order Erysiphales which includes a broad range of genera and species. Each PM species is pathogenic on a well-defined range of plants. *Erysiphe necator* (Schwein) causes PM disease on members of the Vitaceae family. *E. necator* is highly virulent on most varieties of the cultivated grapevine and, therefore, it represents a major agricultural problem worldwide. The identification of PM susceptibility or defense genes through induced mutagenesis and the positional cloning of mutated genes are not practical in woody perennial plants such as grapevine. An alternative approach is to find candidate genes based on features that indicate potential involvement in pathogenesis. Methods developed within functional genomics, including large-scale transcriptional profiling have opened up new possibilities for examining the global patterns of genes expressed in the interactions between plants and their pathogens. To better understand this plant/pathogen interaction, first we conducted comparative mRNA abundance measurements in two grapevine species using the Affymetrix Vitis GeneChip array. The two grapevine genotypes (highly resistant and disease-susceptible) which we used in this study were *V. aestivalis* 'Norton' and *V. vinifera* 'Cabernet Sauvignon' respectively. Comparative microarray analysis in young leaves revealed considerable variation in gene expression between *V. vinifera* and *V. aestivalis*. However, we were unable to identify any broad functional category in which transcript abundance was overall different in any one species. Statistical exploration of the data suggested that sequence divergence between the predominantly *V. vinifera* -derived GeneChip probes and the *V. aestivalis* cRNA did not confound the hybridization data and that the reliability of the microarray results was similar in the two grapevine species. Furthermore, to test the hypothesis that gene expression differences would be apparent when *V. aestivalis* and *V. vinifera* were mounting a response to PM, we conducted a comprehensive Vitis GeneChip analysis. We examined the

transcriptome at 0, 4, 8, 12, 24, and 48 hpi with PM. These results suggested that resistance to PM in *V. aestivalis* was not associated with overall reprogramming of the transcriptome. However, PM induced defense-oriented transcriptional changes in *V. vinifera*. To further analyze disease-susceptible plant and pathogen interaction, we employed suppression subtractive hybridization (SSH), differential hybridization and quantitative real-time (qRT) PCR for the identification of grapevine genes that were specifically up-regulated in response to the grape powdery mildew. These transcripts expanded the list of previously identified E. necator-responsive grapevine genes and facilitated a more comprehensive view of the molecular events that underlie this economically important plant/pathogen interaction.

POLYPHASIC BACTERIAL COMMUNITY ANALYSIS OF AN AEROBIC ACTIVATED SLUDGE REMOVING PHENOLS AND THIOCYANATE FROM COKE PLANT EFFLUENT

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Biological purification processes are effective tools in the treatment of hazardous wastes such as toxic compounds produced in coal coking. In this study, the microbial community of a lab-scale activated sludge system modeling coke wastewater treatment (removing thiocyanate and phenols) was investigated by cultivation-based (strain isolation, identification and biodegradation tests) and culture-independent methods (sequence-aided T-RFLP and taxon-specific PCR). *Comamonas badia* was identified as a key microbe, since it was the predominant member of the total bacterial community, and the phenol degradation capacity of this floc-forming microorganism was also demonstrated. Metabolism of phenol, even at elevated concentrations (up to 1500 mg/L), was also presented for many other dominant (e.g. *Pseudomonas*) and minor (e.g. *Alcaligenes*, *Microbacterium*) groups, while some activated sludge bacteria (e.g. *Sphingomonas*) did not tolerate it even in lower concentrations (250 mg/L). Thiobacilli were detected in the activated sludge, and were supposedly responsible for the intensive thiocyanate degradation in the system.

HIGH-THROUGHPUT SEQUENCING PROVIDES INSIGHTS INTO GENOME VARIATION, EVOLUTION AND PATHOGENICITY OF *PROPIONIBACTERIUM ACNES*

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The human microbiota contributes to our normal postnatal development and plays a significant role in defining our physiology. Inflammatory acne, a highly prevalent disease of humans is a chronic inflammatory disorder primarily involving skin, with distinctive clinical characteristics. Although its etiology still needs to be defined, a bacterial factor has been suggested in the bevelopment of the disease. In fact, antibiotic therapy targeting *Propionibacterium acnes* (*P. acnes*) has been the basic treatment for more than 30 years. Thus, given that bacterial colonization can intensify local inflammation, it is important to determine the exact involvement of *P. acnes* in the pathophysiology of acne. For this, we generated whole-genome sequences for 15 *P. acnes* isolates using next-

generation sequencing (NGS) technology. Isolates, including non-acne derived isolates, were selected to represent all the currently known phylogenetic clusters.
Our analysis highlighted variations, such as SNPs and deletions, in a number of genes encoding putative virulence factors and in enzymes that are potentially involved in degrading skin-derived substances. We have also identified genes with phase variation signitures, leading to the challenging suggestion that *P. acnes* is capable of adopting its surface structure in response to environmental challenges such as immunological reactions.

ANALYSIS OF MICROBIAL HAZARDS AND CCP DETERMINATION IN THE MEAT FROM TIBLICA PRODUCTION PROCESS

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Small and medium enterprises face a number of difficulties in implementinfg HACCP. Thus, in this work, hazard analysis and CCP determination for production of traditional product from Croatia „meat from tiblica" (vacuum-packed cooked cured meat) in a medium sized meat industry, is made using qualitative UGent method. Microbiological changes during production, from raw material through individual production steps to the final product, were investigated. Total viable count (TVC), nuber ol lactic acid bacteria (LAB), sulfite-reducing clostridia, enterobacteria, yeast and moulds, *S. aureus*, enterococci, micrococci, as well as presence of *L. monocytogenes* and *Salmonella* spp., were determined using standard microbial methods.Microbial hazards related with raw pork meat and back fat are: *Salmonella* spp., *Campylobacter* spp., *Y. enterocolitica*, *L. monocytogenes*, *S. aureus*, *Cl. botulinum* and *Cl. perfringens*. In spite of antimicrobial effect of individual processes in the „meat from tiblica" production, there is a risk of surviving and growth of pathogens especially when integrated time and temperature regime is insufficient. Moreover, temperature abuse during storage causes growth of pathogens, which also can contaminate product during packaging and handling.
In our investigation *Salmonella* spp. and *L. monocytogenes* were not detrminet in samples of raw material and meat and fat during production, as well as in the final product, while *S. aureus* and sulfite-reducing clostridia were below detectable level. Enterococci were below detectable level in all phases, but after curing, when their number reached 2 log.
Enterobacteria where detected only in raw material (1 log cfu/g). TVC ranged from 2-3 log cfu/g in raw material, to 5-6 log after curing, 3 log after baking, and 4 log in meat and 7 log cfu/g in fast after first month orf storage. In raw material number of LAB were below detectable level, yeast and moulds 2-3- log, and micrococci 2-3 log cfu/g. After curing number of LAB were 3-4 log, micrococci 2-3 log, enterococci 2 log, yeast and moulds 2-4 log cfu/g. After baking number of LAB and micrococci were below detectable level, while number of yeast and moulds were 2-4 log cfu/g. After first month of storage, 4 log cfu/g of LAb in fat, 2/5 (meat /fat) log cfu/g of yeast and moulds, and 3/5 log (meat/fat) cfu/g of micrococci were determined.In the „meat from tiblica" production process 6 CCPs are determined using UGent method: 1. preparing the brine (sodium nitrite concentration), 2. cooling of meat after thermal tratment in the chamber, 3. baking, 4. cooling after baking, 5. smoking of pork back fat and 6. pork back fat cooking.

COMPARATIVE GENOMICS OF PRIMARY CARBON METABOLISM IN ASPERGILLI USING EIGHT GENOME SEQUENCES

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Aspergillus constitutes a large genus of filamentous fungi with significant impact on mankind, comprising opportunistic human pathogens such as *A. fumigatus* and agricultural spoilage organisms such as *A. flavus* but also potent industrial cell factories such as *A. niger*. The homothallic organism *A. nidulans* has traditionally served as a model for the economically more important *Aspergilli* which were, until quite recently, considered imperfect species. Principally saprophytic microorganisms, *Aspergilli* are reputed for their versatile and efficient catabolism of soluble carbon sources as well as raw polymeric materials and are important producers of extracellular glycosyl hydrolases and proteases, organic acids and other food-grade additives, and pharmaceutical secondary metabolites such as antibiotics, sporins and statins. Adaptive enzyme variation is of key interest in understanding metabolic versatility and the differences in physiological behavior observed among the *Aspergilli*. The genomic and evolutionary basis for this versatility was addressed for the most central sections of primary metabolism using eight publicly accessible genome sequences [1]. Some 150 genes were manually re-annotated, primarily in *A. nidulans* (deposited in the Central *Aspergillus* Data Repository (CADRE) – http://www.cadre-genomes.org.uk) [2] and subsequently, in the other species.
While, unsurprizingly, the enzymatic set-up is essentially the same, *Aspergilli* appear to have re-enforced certain metabolic conversions and several areas of their primary carbon metabolism, mainly by gene duplication(s). However, horizontal gene transfer and functional gene clustering events could also be revealed. *Aspergilli* thus appear to have the tendency to better maintain genetic variation acquired during evolution and it can be assumed that this had aided their proliferation in specific habitats. Interestingly, *Aspergillus*-specific paralog proteins have been encountered of which the encoding genes do not occur (anymore) in other fungal genera and in some cases, even have disappeared from a number of *Aspergillus* species. Extended phylogenetic analysis of the enzymes encoded by duplicated genes also suggests that some gene products may have acquired new (physiological) functions that would render primary carbon metabolism of the *Aspergilli* more complex than generally thought.

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MYCOTOXINS: THE IMPORTANT FUNGAL SPECIES THAT PRODUCE THEM

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Mycotoxins, fungal secondary metabolites that can evoke a toxic response in vertebrates when introduced via a natural route, are produced by certain plant pathogenic and storage fungi. Among the plant pathogens *Fusarium* and *Alternaria* species play a major role, and *Fusarium* species in

particular can produce several different mycotoxins, including trichothecenes, zearalenone, fumonisins, beauvericin and enniatins. Prevention of formation of such mycotoxins requires knowledge on plant growth and stress and plant - fungus interactions. The taxonomy of *Fusarium* is still being debated, and one of the major problems is whether an important species such as *Fusarium graminearum* should be subdivided into 13 „phylospecies" or remain as one species that is phenotypically distinct. Population studies and phenotypic characterization suggest that *F. graminearum* is only one species, but an in depth poly-gene cladistic analysis indicates the 13 „phylospecies". A polyphasic approach to taxonomy, favoured here, would be in line with *F. graminearum* being one species. The major genera that can grow in foods and feeds during storage are *Penicillium* and *Aspergillus*. Species in these genera produce specific profiles of mycotoxins and other secondary metabolites and a polyphasic approach to biosystematics has been successful in showing that species in these genera exist and are distinct and non-overlapping. However, even in these genera there are cases where seemingly closely related species would be regarded by some taxonomists as only „phylospecies". One example is *Aspergillus flavus* and *A. minisclerotigenes*, both very important producers of aflatoxin B1 and aflatoxins of both the B and G type, respectively. Apart from the difference in aflatoxin profile, these two species can be distinguished by the former producing larger sclerotia than conidial heads, and the latter (*A. minisclerotigenes*) producing larger conidial heads than sclerotia. Since there are other differences between those two taxa, a polyphasic approach would indicate two distinct species, that otherwise have been called *A. flavus* L (large sclerotia) and *A. flavus* S (small sclerotia). In *Aspergillus* section *Nigri*, two very common species, *A. niger* and *A. tubingensis*, have been recognized based on AFLP, RAPD, beta-tubulin sequence differences etc., but they are indistinguishable from a morphological point of view. Fortunately there are actually phenotypic differences, including the ability to produce kotanins, ochratoxins and fumonisins B2, B4 and B6 by *A. niger* and the ability to produce asperazine by *A. tubingensis*. In *Aspergillus* section *Circumdati*, the two species *A. ochraceus* and *A. westerdijkiae*, could be separated using beta-tubulin sequencing, and there were few, distinct phenotypic differences. One was a difference in temperature growth curves and another a secondary metabolite family only found in *A. westerdijkiae*. Some of the most important mycotoxins are produced by a number of common species. This clear connection between a limited range of species and their mycotoxins has unfortunately been obscured somewhat by misidentified fungi or mycotoxins. The carcinogenic aflatoxins are produced by several species in *Aspergillus* section *Flavi*: *A. flavus*, *A. parasiticus*, *A. minisclerotigenes*, *A. arachidicola*, *A. nomius* and the less common *A. bombycis*, *A. pseudotamarii* and *A. parvisclerotigenus*. The production of aflatoxins by *A. ochraceoroseus*, *A. rambellii* (both in *Aspergillus* section *Ochraceorosei*) and *Emericella venezuelensis*, *E. olivicola* and *E. astellata* are of less importance for food safety. Ochratoxin A is produced by *Penicillium verrucosum* (in cereals), *P. nordicum* (dried meat products) and by most Aspergilli in section *Circumdati*, by *Petromyces alliaceus* in section *Flavi* and by *A. niger*, *A. carbonarius*, and *A. sclerotioniger* in section *Nigri*. Whereas *Fusarium verticillioides* produces fumonisins B1, B2 and B3, most isolates of *A. niger* produce fumonisin B2, B4 and B6. The production of fumonisins by *A. niger* is particularly important as this species occurs in maize, peanuts, grapes, mango, dried fruits, onions, and many other foods. *A. niger* was reported to be present in mould fermented tea (puerh tea), but recent research has shown that the species present in black and puerh tea is *A. acidus*, which cannot produce ochratoxins or fumonisins. Patulin is produced by *Penicillium expansum* (pomaceous fruits), *P. griseofulvum* (rice), *P. sclerotigenum* (yams), *Byssochlamys nivea* (pasteurized fruit juices), *Aspergillus clavatus* (malt), *A. giganteus* and several other Penicillia and Aspergilli. The nephrotoxin citrinin is produced by *Monascus ruber*, *P. expansum*, *P. verrucosum*, *P. albocoremium*, *P. citrinum* and several soil-borne species of *Penicillium* and *Aspergillus*. Several other mycotoxins from *Aspergillus*, *Penicillium* and other genera have been involved in mycotoxicoses.

ISOLATION AND CHARACTERISATION OF AUTOCHTHONOUS POPULATION OF ENTEROCOCCI IN ISTRIAN CHEESE

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Istrian cheese is produced from unpasterized milk and without adition of starter cultures. Its specific organoleptic characteristics are therefore result of activity of autochthonous microbial community during the ripening. They diversity vary during the ripening thus species and strains which are dominating during the first ripening phase are not necesarily present in the final one. Genus Enterococcus is widely distributed in traditional Mediteranian cheeses. Using physiological and molecular methods we found enterococci in samples of milk, fresh cheese and cheeses after 30, 60, 90 and 120 days ripening. Number of enterococci were between 1,5 x 10^3 do 8,0 x 106 CFU/g cheese. Species *E. faecalis* was dominant in our samples which demonstrated small inter species variety of genus Enterococcus in istrian cheese. The main number of isolates was resistant to clyndamicin, ampicillin, penicilin, tetracycline and rifampin, while most samples indicate sensibility on eritromycin and vancomycin. Further analysis of enterococci isolated and characterized in this work is required to determine their potential as starter cultures in technological process of Istrian cheese production.

GENETIC CHARACTERIZATION OF MULTIDRUG RESISTANT MAJOR NOSOCOMIAL PATHOGENS IN HUNGARY

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Antibiotic resistant genes were characterized and their way of dissemination established in major nosocomial pathogens in Hungary. Molecular techniques applied in the investigation included sequencing of antibiotic resistant genes and integrons, cloning of resistance determinants, Southern blotting, multi locus sequence typing (MLST), „spa" typing, pulsed-field gel electrophoresis (PFGE) and random amplification of polymorphic DNA (RAPD). A comprehensive review is given on findings obtained with methicilin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamasevb(ESBL) Gram-negative pathogens, multidrug-resistant *P. aeruginosa* with special emphasis on metallo-β-lactamase producing isolates and vancomycin-resistant enterococci (VRE).

IN VITRO ANTIFUNGAL EFFECT OF CYSTEINE AND CYSTEINE DERIVATIVES ON DIFFERENT ZYGOMYCETES

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Opportunistic fungal infections caused by the members of Zygomycetes have increased dramatically over the past several years. Nowadays, zygomycosis is one of the most frequent mycotic diseases caused by non-*Aspergillus* moulds. These fungi have a substantial intrinsic resistance to most of the widely used antifungal drugs or show high MIC values for certain agents in *in vitro* tests. Therefore, there is a substantial demand for new types of compounds with antifungal activity against Zygomycetes. The *in vitro* antifungal effect of cysteine (D- and L-cysteine) and its derivatives (L-

cysteine-methyl-ester, N-acetyl-cysteine, N-isobutyryl-D-cysteine and N-isobutyryl-L-cysteine) were examined on 20 zygomyceteous isolates representing 16 genera (*Absidia*, *Actinomucor*, *Backusella*, *Gilbertella*, *Micromucor*, *Mortierella*, *Mucor*, *Mycotypha*, *Phycomyces*, *Rhizomucor*, *Rhizopus*, *Saksenaea*, *Syncephalastrum*, *Thamnostylum*, *Umbellopsis* and *Zygorynchus*). The inhibitory potential of different concentrations of these compounds (ranging from 0.625 to 10 mM) was investigated on the germination of sporangiospores as well as on hyphal extension by broth microdilution method and agar plate test. Cysteine and its derivatives exerted a strong inhibitory effect in case of the most studied strains. Treatment with 10 mM of compounds resulted in complete blockage of growth, in some isolates. Severe changes in colony morphology and hyphal growth were observed in presence of 10 mM L-cysteine, N-acetyl-cysteine and N-isobutyryl-L-cysteine when a strain was sensitive to them. Microscopic observations revealed that 10 mM N-acetyl-cysteine induced dramatic modifications in the structural organization of the hyphae in case of *Rhizopus stolonifer*. Results suggest that cysteine and its derivatives have a therapeutic potential against fungal infections caused by Zygomycetes species, and urge the need of further studies to prove the practical efficiency of them, for example in plant and animal model experiments.

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IN VITRO INTERACTION BETWEEN SURAMIN AND AMPHOTERICIN B AGAINST CLINICALLY IMPORTANT ZYGOMYCETES

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The incidence of zygomycosis (opportunistic fungal infections caused by the members of the class Zygomycetes) has increased during recent years. Unfortunately, zygomyceteous fungi have a substantial intrinsic resistance to most of the widely used antifungal drugs, and also show high *in vitro* MIC values for a variety of other agents. The most efficient antifungal drug for the treatment of such infections is amphotericin B (AMB) and its lipid complexes; however, these are quite toxic and may have serious side-effects. Combined application of AMB with other effective antifungal agents would be advantageous as a basis of a less toxic therapy. Therefore, there is a substantial interest for drugs, which act synergistically with AMB, and allow the lowering of its therapeutic concentration. Suramin, a hexasulfonated naphtylurea, was originally applied as an antiparasitic agent and antitumor drug. It was demonstrated previously, that suramin have an inhibitory effect on the growth of several zygomycetes [1]. The *in vitro* antifungal activity of suramin (25-300 μg/ml) and its combinations with AMB (0.024-12.5 μg/ml) were tested in this study against 15 Zygomycetes isolates representing 8 clinically important genera (*Absidia*, *Micromucor*, *Mortierella*, *Mucor*, *Rhizomucor*, *Rhizopus*, *Saksenaea* and *Syncephalastrum*), using broth microdilution method. The investigated compounds acted antagonistically, synergistically and/or additively on the growth when a strain was sensitive to both compounds (*Mucor circinelloides* f. *lusitanicus*, *M. hiemalis* f. *luteus*, *Rhizomucor miehei*, *R. pusillus* and *Rhizopus microsporus* var. *rhizopodiformis*) or resistant to suramin and sensitive to AMB (*Mucor mucedo*, *Micromucor ramanniana*, *Mortierella wolfii*, *Rhizopus oryzae*, *Syncephalastrum racemosum*, *Saksenaea vasiformis*), at the same time, synergistic interactions were detected if strains were insensitive to both drugs (*Absidia corymbifera*). Only the inhibitory effect of only the suramin was measured, when a fungus was sensitive to suramin but resistant to AMB (*Absidia glauca*).
Combined antifungal therapies compared with monotherapy would create new potentials in the treatment of mycotic diseases. The observed activities of AMB-suramin combinations support the

assumption that these compounds may be regarded as promising candidates in future antifungal drug research.

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THE UNBALANCED OXIDOREDUCTION STATE OF THE RESPIRATORY DEFICIENT MUTANT *SCHIZOSACCHAROMYCES POMBE*

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The petite-negative *S. pombe* growth is highly dependent upon respiration. To maintain proper functioning in the mitochondrial respiratory chain of petite-negative fission yeast *S. pombe*, the presence of an abc1Sp gene is required. This gene's inactivation with homologue gene complementation results in a drastic decrease in bc1 complex and cytochrome aa3 activity but causes a slow growth phenotype [1]. The objective of this study was to see the influence of the elimination of abc1Sp gene on the mutant's oxidative stress and heavy metal resistance. The NBp17 mutant proved to be 25-fold more sensitive to cadmium, and accumulated 20 times more Cd2+ in an eight-hour treatment, than the parental strain. The mutant is sensitive to chromate (Cr(VI)) anion, and oxidative stressors such as hydrogen-peroxide and menadione as opposed to the parental strain. The two strains exhibited the same concentration of reactive oxygen species (ROS) peroxide and superoxide radical in complete media, but in minimal media the concentration of ROS was higher in the mutant strain. A decrease was observed in the specific enzyme activity of glutathione reductase, catalase and 6-phosphogluconate dehydrogenase, but the specific enzyme activity of glucose-6-phosphate dehydrogenase was higher in the mutant. The Cr(VI) reduction ability of the parental strain was 60 times higher than in the NBp17 mutant. The $^{\bullet}$OH induction defined on disrupted cells by electron spin resonance spectroscopy was 2.5 times higher in the parental strain than in the mutant. Adding NADPH and Cr(VI) to the disrupted cells simultaneously resulted in a 15-fold increase in the Cr(V) level in the mutant, while there was no increase in the parental strain. The intracellular medium of the mutant contained a high amount of glycerol, ethanol, acetate and piruvate. In the course of defense against Cd, the cells synthesize phytochelatins and upregulate the glutathione production. These processes are ATP-dependent. The synthesis of antioxidant enzymes, the repair of damaged proteins and nucleic acids and the detoxification of lipid peroxidation products similarly are all energy requiring processes. The respiratory deficient mutant is an energy deficient organism, and probably this is the reason for detected oxidative stress and heavy metal sensitivity of the mutant.

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HUMAN TRICHINELLOSIS: TOPICALITIES IN HUNGARY

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A summary of the available clinical epidemiological data on all the cases of human trichinellosis in the last decade, and a summary of laboratory results on the subject in the period concerned was made on the occasion of an outbreak in Hungary this year. From 1999 to June 2009, at the Department of Parasitology of the National Centre for Epidemiology (NCE), 1319, 535, 624, and 162 examinations were performed using microprecipitation (MP), a home-made ELISA (used up to June 2002), a commercial ELISA (used from 2002), and a commercial Western Blot (WB, used from August 2004). The clinical and epidemiological data were taken from databases of the NCE. Positive reactions were obtained from sera of altogether 81 patients: 65 patients using ELISA (from 12 patients with home-made ELISA and 53 with commercial ELISA) and 47 patients using MP or WB (36 with MP and 29 with WB). Comparing the results from commercial ELISA (for the last five years) with those from WB and MP as confirmatory techniques, the relative sensitivity of commercial ELISA was found to be 96%, but only 23 of 52 (43%) ELISA-positive samples were found positive by MP or WB. Comparing the results from commercial ELISA with those from WB, global agreement was found only 30% (27 of 90 reactions), while global agreement between those obtained from MP and WB was found to be 76% (101 of 133 reactions). The incidences of doubtful results were 1.9%, 4.3%, 9.9%, and 6.8% using MP, the home-made ELISA, the commercial ELISA, and the WB techniques, respectively. When testing sera of 9 infected patients affected by the outbreak in 2009, positive reactions were obtained in 2 cases considerably sooner after clinical onset with MP than with WB. The lecture describes the outbreak in the county of Békés this year and comments on a cluster of cases reported from the county of Baranya in 2008.
Human trichinellosis still occurs in Hungary, as it does in other South and East European countries. Apart from a small family outbreak, only sporadic cases of trichinellosis occurred in Hungary from 1999 to 2007. The results show that the date of clinical onset of trichinellosis may still be important to obtain a correct laboratory result. Commercial ELISA was found to be useful for screening, but the results show that the positive results have to be confirmed (except in clinically clear-cut cases). Also given are some possible reasons for the high incidence of doubtful results with commercial ELISA and discrepant positive samples showing negative results by confirmatory methods.

MICROBIAL DIVERSITY IN FIELDS OF CONVENTIONAL AND ORGANIC AGRICULTURE: RESULTS OF TWO YEARS LONG INVESTIGATIONS

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Components of agricultural management regime (crop rotation, tillage, compost, manure, herbicide and fertilizer application) and water regime, are key determinants of microbial community structure in soil. Plant type is also important factor since they are providing microorganisms with specific carbon sources. Several studies show that organic farming leads to higher soil quality with higher microbiological activity than conventional farming, due to regular crop rotations, reduced application of synthetic nutrients, and the absence of pesticides. Higher actinomycete abundance and diversity is reported in organic fields than conventional ones. Populations of fungi and thermophile microorganisms have been recorded to be in significantly higher numbers in soils from organic and

sustainable than conventional fields. The diversity of bacterial functional communities is greater in soils from organic farms, while species diversity is similar. The propagule numbers of *Trichoderma* has been shown to be higher in soils from conventional farms. It is assumed that *Trichoderma* sp. may be affected to a lesser extent than other soil fungi following a soil disturbance (after the use of herbicides and pesticides), and are able to quickly colonize niches left by other organisms in conventional fields. Other investigations clearly indicate that the potential fungal community (including spores), investigated by the cultivation-independent approach, is almost entirely uninfluenced by the season, soil type and farming management practices, whereas active population, investigated by the isolation of hyphae using a soil-washing technique, show a clear response to environmental changes. Objectives of the investigation were to determine the microbial diversity in the soil of four conventional and three organic agriculture fields during two year period. The amount and diversity of MO was analyzed by conventional plating. The amount of bacterial-K-strategists and bacterial-r-strategists was estimated. The genetic diversity of fungal communities was detected by ARDRA. The quantity of *Trichoderma* spp. DNA was determined by RT-PCR. Soil samples were obtained two times in each growing season (on June and August). Organic agriculture fields are established in year 2003. They are fertilized only with green manure. Depending from the crop conventional fields receive different pesticides and fertilization.
Conclusions are that all seven analyzed fields show diverse results with tendency for organic fields to have higher diversity of cultivable microorganisms, higher fungal diversity estimated with ARDRA and higher amounts of Trichoderma spp. DNA. Such tendency can be explained with yearly usage of pesticides in conventional fields that can cause long term impact on soil microbial populations that dominate over seasonal changes and impact of the crop. In order to prove this statement investigations will be continued in year 2010.

REGULATION OF D-GALACTOSE METABOLISM IN *ASPERGILLUS NIDULANS*

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D-Galactose is present in hemicelluloses and pectin which are constituents of the plant cell wall. In pectin, α-1,4-linked D-galactose residues are present in galactan or in arabinogalactan side chains. In hemicelluloses, D-galactose residues are present in the form of side residues which are α-linked in to xyloglucan and xylan, but β-linked in to galactoglucomannan. For the degradation of these structures by filamentous fungi, several enzyme classes are required depending on the linkage. These classes are α- and β-galactosidases and endo- and exogalactanases. *Aspergillus nidulans*, a saprobic filamentous fungus, is able to use D-galactose efficiently as a carbon source. *A. nidulans* can convert D-galactose through two pathways: the common Leloir pathway as well as the recently described alternative D-galactose utilization pathway. Recently two regulators, GalR and GalX were identified that control D-galactose metabolism in *A. nidulans*. The interaction of these regulators, their control of the various genes of the two D-galactose utilization pathways as well as genes encoding extracellular galactose releasing enzymes will be discussed.

ANTIGENEERING OF PLASMID DNA VACCINES

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Antigen engineering (ANTIGENeering) is a critical part of our nanomedicine vaccine technology to design and to test plasmid DNA (pDNA) encoded antigens mimicking the gene expression and structure of a target pathogen. ANTIGENeering consists of three steps: (1) the identification of the target pathogen and establishment of a computerized knowledgebase; (2) the design of a single pDNA using the knowledgebase and connected bioinformatics tools developed in-house, considering the expression of many antigens, formation of virus-like particles (VLP+), safety and immunogenicity; (3) the characterization of the pDNA with antigens and VLP+ expression, safety and immunogenicity. ANTIGENeering was first utilized in the design of DermaVir, a candidate nanomedicine presently in Phase II clinical development stage as immunotherapeutics for HIV/AIDS. DermaVir contains a single pDNA authentically expressing most HIV-derived antigens forming a VLP+ to induce potent immune responses with broad specificity. To ensure the safety several HIV genes have mutations and deletions resulting a VLP+ that cannot replicate, reverse transcribe or integrate. To obtain the nanomedicine vaccine the pDNA is formulated to pathogen-like nanoparticles with a polymer (PEIm) and delivered transdermally to the lymph node dendritic cells with DermaPrep, which is our new CE-marked medical device. Dendritic cells express the VLP+, process it according to the HLA type of the patient and present the HIV antigens to naive $CD4^+$ and $CD8^+$ T cells that leads to the polyclonal amplification of HIV-specific T cells capable to kill infected cells. We have previously proven *in vitro*, in mice, in monkeys and in human subjects that DermaVir is capable of inducing Th1 type immune response required for the elimination of virus infected cells. To optimize the DermaVir-induced immune responses in people infected with different viruses we have been ANTIGENeering several pDNA constructs to make subtype or CRF (circulating recombinant form) optimized DermaVir products (CRF_C, CRF_BC, CRF_BF, CRF_AB). Subtype-optimized DermaVir products will support personalized treatment and distribution of specific products in different geographical areas of the world: these subtypes are responsible for epidemics in Africa, China, South-America and Ukraine respectively. ANTIGENeering technology has been utilized to develop an immunotherapeutic nanomedicine for the treatment of human papillomavirus-associated diseases like cervical neoplasia. We have ANTIGENeered a single pDNA for the expression of several viral antigens of HPV16 and for the production of VLP+. To induce immune responses with broad specificity the pDNA encodes for both capsids and other viral proteins to produce *in vivo* VLP+. Safety of the pDNA is ensured by eliminating oncogenic, replicative and integrative functions of the relevant genes. ANTIGENeering of several pathogen specific pDNAs can create strikingly broad range of pipeline nanomedicine vaccine products while the regulatory, development and manufacturing pathway stay similar or identical. This supports the rapid and cost effective development of new immunotherapeutic nanomedicines against viral and neoplastic diseases.

ANTIBIOTIC RESISTANT BACTERIA IN THE FOOD CHAIN AND ENVIRONMENT

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Antibiotics, as tool for epidemic disease control, brought the great economy benefit of safe food production in the last few decades. However, this success story now seems to turn to a false way, for some reasons as follows. 1. Sterile areas became degraded, by wishing to create too many of them; while too little attention was paid to create the high-graded infrastructural background of the high-grade technology we used. Easy-to-cleanse surfaces, appropriate user education, waste recycling

regulations and wastewater treatment - all conditions needed for a sustainable use - remained far behind the development performed by chemical industry. 2. Target-specific use remained a wish only, while some users even do not know, that disinfectants may be used only over clean surfaces - and operate with more and more wide-spectrum, more and more stable chemicals to break down the antibiotic resistance resulting from inadequate environmental circumstances of use. Resistant flora emerged in treated patients, hospitals and city communities; animals, processing plants, farm environments and food chain. Antibiotic residues released together with masses of resistant bugs, formed widespread, not too diverse but very active resistance reservoirs in all these environments, also spread worldwide by food trade and tourism. Pathogens entering such an environment may get resistance genes easily - often bearing also co-resistance with disinfectants - and once resistant, they will prefer residue-contaminated ("well disinfected") areas to colonize them. 3. People were taught to look at bacteria as our enemies. Edaphic, coastal and water flora may react by losing their biodiversity and functions, keeping Earth living and habitable, when suffer antibacterial effects. Water capacity and plant diversity of residue contaminated soils may suffer severe destruction. The thousands of tons of antibacterials manufactured may result in desert formation, releasing equal carbon dioxide from living soils as oil industry produces! Moreover, desert formation affects the balancing system of global carbon dioxide household, and losing a buffer capacity will not obviously result in linear dose-response answers? The risk of inhibition of freshwater circulation also could result in worse climate balance - less cloud cover and more solar energy intake - and worse agricultural harvest locally and globally. To present how far resistance phenomenon developed, we present the 2008. year antimicrobial resistance statistics of Salmonella strains, isolated from broiler and other fowls in the food chain. To control the process, keep antibiotics work, and save the integrity of Environment, we propose as follows. All professions using antibiotic and disinfectant chemicals must be managed in a regulated manner, under complex chemical safety precautions. Enhancement of effective, biological waste remediation is needed in all residue sources: factories, hospitals and farms. These compounds must be regarded as toxic - on the base of their real environmental toxicity and not only their human effects - and the education of sustainable use must be done for all the users: farmers, medical staff and people running their household as well.

EFFECT OF HUMAN PAPILLOMAVIRUS 16 E6 AND E7 ONCOGENES ON THE EXPRESSION OF INVOLUCRIN IN HUMAN KERATINOCYTES

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Involucrin is a component of the keratinocyte crosslinked envelope. It is found in the cytoplasm and crosslinked to membrane proteins by transglutaminase. During keratinocyte differentiation, the level of involucrin increases due to rising extracellular and intracellular calcium. So, it can be used as a marker for keratinocyte differentiation. The productive phase of the human papillomavirus (HPV) life cycle is closely linked to keratinocyte differentiaton. HPVs initially infect proliferating basal cells of the epidermis, while virus production is associated with terminally differentiated layers. The dependence of viral reproduction on keratinocyte differentiation is paradoxical, because differentiated keratinocytes are normally unable to reenter the cell cycle. The purpose of the present study was to investigate the effects of HPV16 E6 and E7 oncogenes on the expression of involucrin in primary keratinocytes. Primary human foreskin keratinocytes were maintained in serum free, low calcium medium and transduced by LXSN (control) retrovirus or virus vectors expressing HPV16 E6,

HPV16 E7 or HPV16 E6/E7 genes. These cells were induced to differentiate by culture in high calcium and serum containing medium for 24h. Total RNA isolated from differentiating and non-differentiating infected cells was reverse transcribed, and the expression level of involucrin was estimated using real-time PCR. The involucrin protein was detected by western blotting using monoclonal anti-human involucrin antibody. Primary human keratinocytes were co-transfected by a luciferase reporter plasmid carrying involucrin promoter and vectors expressing HPV 16 E6 or E7 genes. Luciferase assay was used to measure the effect of the HPV oncogenes on involucrin promoter activity. The differentiation of keratinocytes by serum and calcium highly increased the transcription level of involucrin. The E6 and E7 oncogenes of HPV16 together caused downregulation of the involucrin mRNA both in differentiating and non-differentiating cells. In order to verify the effect of HPV oncogenes on the involucrin promoter, we made transient transfection assays and found that the HPV E6 and E7 repressed involucrin promoter activity. The effect of HPV16 E6 and E7 on cellular involucrin protein level was similar to that found for mRNA level. High calcium and serum stimulated the level of involucrin protein and the presence of the two oncogenes reduced it. These results suggest that, in human keratinocytes the HPV16 oncogenes decrease mRNA and protein levels of involucrin through down-regulating the activity of the involucrin promoter.

CHARACTERIZATION OF THE MURINE HERPESVIRUS ISOLATE 4556 (MHV4556): SEQUENCE AND ANALYSIS OF GENES ENCODING VIRION ASSOCIATED PROTEINS

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Murid herpesvirus 4 infection in mice is a suitable animal model for studying gammaherpesvirus pathogenesis, but also virion structure, assembly and composition, since MHV efficiently completes the lytic phase and productively infects cultured cells. We found that strain MHV-72 and the MHV4556 isolate coming from the same/different free-living murid species (*Clethrionomys glareolus/Apodemus flavicollis*) as strain MHV-68 and differ also in their virulence and oncogenicity. Here, we report the identification of five out of thirteen MHV4556 genes coding for virion associated proteins which are different in sequence from that of the prototype MHV-68 strain. Together seven mutations were identified in the ORFs encoding the virion envelope-associated glycoprotein B (ORF8), the triplex-2/capsid protein homologue (ORF26), and two virion associated proteins (ORF48 and ORF52). One mutation was found in ORF11, a gammaherpesvirus conserved gene, encoding p43, currently suggested being a virion component distributed in perinuclear space of infected cells. Two mutations were identified in M4, in one from set of MHV unique genes localised at left end of MHV genome that are thought to be important in virus-host interactions and latent infection. All mutations found cause changes in hydrophilicity and surface exposure of encoded proteins and may have influence upon at least virulence of strain MHV4556. The mutations detected are MHV4556 strain specific (with exception of one in ORF52 which is common with MHV-72 strain). Via a rise of new restriction site (BpmI in ORF11) or disappearance of restriction site detected in MHV-68 genome (RsaI in ORF8) two mutations may be used for quick identification of the MHV4556 isolate.

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CHANGES IN THE SOIL PARAMETERS IN PREVIOUSLY CULTIVATED AND UNCULTIVATED LANDS

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As a result of human activities, previously cultivated and uncultivated lands can often get contaminated and easily become illegal dumpling sites, e.g. communal wastewater can be deposited on them. We measured the soil parameters (pH, TOC% and enzyme activities) and compared the fatty acid profile of *Bacillus* species of some uncultivated soil samples. The aim of our examination was to reveal the changes of soil parameters, which occurred under the influence of contamination. The soil parameters in 7 different sites: pH changed between 4,33-4,81; TOC% 5,58-9,42. We studied the dehydrogenase (139-247,5 µg INTF), cellulase (20,9-52%); invertase (0,73-7,25mg glucose/10g/24h) and phosphatase (0,78-0,98 P_2O_5/g/2h) enzymes activities and our results show that the dehydrogenase the most important factor the soil homeostasis and show the micro-organisms activities. Seven species of Bacillus genus were investigated: *Bacillus brevis*, *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Bacillus megaterium*. With the help of Cluster Analysis, we compared the fatty acid profiles of certain bacteria species of the samples with fatty acid profiles of the reference cultures (Collection of Agricultural and Industrial Microorganisms). Within a given species, there are significant differences among the stocks of different sampling sites ($p < 0.05$). Considering the differences, a wide range of fatty acids (2-OH 10:0, C12:0, 2-OH 12:0, 3-OH 12:0, C14:1, C14:0, i-15:0, a-15:0, C15:1, C15:1, C17:1, C18:2n6t, C20:3n6, C20:2, C20:1) could be observed both qualitatively and quantitatively. Presence of a given fatty acid depends on the given species as well as the site. Stocks of a given species show relationship on the basis of fatty acid composition; however fatty acid profiles change under the influence of environmental factors. By the help of these modifications, bacteria can adapt to the changes of the environment. When the areas become cultivated again, the modified microflora of polluted areas may have an influence on the state of the soil. The diversity of micro-organisms in soil is a critical factor, regarding the maintenance of good soil health. Micro-organisms are involved in many important soil functions such as carbon and nitrogen cycling, and organic-matter transformation.

TRANSCRIPTIONAL RESPONSE OF J774.2 MACROPHAGE LIKE CELLS INFECTED WITH *CANDIDA PARAPSILOSIS*

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C. parapsilosis is typically a commensal of human skin, and its pathogenicity is limited by intact integument. *C. parapsilosis* is notorious for its capacity to grow in total parenteral nutrition and to form biofilms on catheters and other implanted devices, for nosocomial spread by hand carriage, and for persistence in the hospital environment. *C. parapsilosis* is of special concern in critically ill neonates, causing more than one-quarter of all invasive fungal infections in low-birth-weight infants in the United Kingdom and up to one-third of neonatal *Candida* bloodstream infections in North America. Additionally, it is the predominant fungal organism isolated in many neonatal intensive care units (NICUs), where it is often associated with neonatal mortality. Since the 1980s, there has been a

marked increase in blood- stream infections due to non-albicans *Candida* species, especially *C. glabrata* in the United States and *C. parapsilosis* and *C. tropicalis* in Europe, Canada, and Latin America (Trofa et al 2008). Although *C. parapsilosis* is often considered less virulent than *C. albicans*, it is the *Candida* species with the largest increase in incidence since 1990.
Despite the increasing clinical importance, little is known about the host response upon *Candida parapsilosis* infection. Thus, in this study we have analyzed the transcriptional responses of murine macrophage-like cell line J774.2 cells to infection with *C. parapsilosis* at 3h and 8h.
The complex response of murine macrophages to infection with *C. parapsilosis* was investigated at the level of gene expression using an Agilent mouse microarray. More than 500 genes were identified as being differentially regulated. Many of the upregulated genes encoded molecules were involved in immune response and inflammation, transcription, signalling, apoptosis, cell cycle, electron transport and cell adhesion. Of particular interest was the upregulation of proinflammatory cytokines, typical of the classically activated macrophages such as TNF, IL-1 and IL-15, and also the upregulation of TNF-receptor family members such as *TNFRSF9* associated with Th1 T-helper cell responses. Understanding how macrophages respond to *C. parapsilosis* at the molecular level may facilitate the development of new therapeutic paradigms. Additionally, the microarray data suggest significant differences between the response against *C. parapsilosis* infection and that of *C. albicans*.

ON THE ORIGIN OF (VIRUS) SPECIES BY MEANS OF NATURAL SELECTION

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Virus evolution is based on the same principles as that of other organisms. However, as viruses can multiply only in cells, the host itself is one of the (or the) main selective environmental factor. In general, viruses and their hosts co-evolve, and as the host separates slowly into different species, so do their viruses. One would expect to find different viruses in the different hosts, and the difference between viruses should reflect the evolutionary distance of their hosts. The majority of accumulating data support this scenario. However, looking only at a single characteristic of a virus (the present day host) may be misleading since host switches can also occur. Molecular approaches help in recognition of such events that seem to be the cause of the uneven distribution of certain virus families among vertebrate hosts. Our knowledge is still very limited to understand all these ancient host switches and this is well represented in the virus taxonomy. Some obviously related viruses have not been combined yet into common taxons, while other ones were combined prematurely without any serious (molecular) proofs. Successful host switches must have occurred rarely because such a virus has to survive in a "hostile" new environment and natural selection works effectively against this. The virus in the new host may have to use new cellular receptors and compete with new viruses or new defence systems. A further fatal problem in host switching could be too high pathogenicity in the novel host. If the virus kills the host rapidly then the virus lineage might go extinct. Nonetheless, elevated pathogenicity can frequently be observed in a new host before serious adaptation occurs. Viruses evolve with a constant gradual changing through point mutations and selective adaptations to an ever changing environment. The most common and most effective hostile environmental factor is the immune defence of the host, which induces relatively quick changes in the neutralization epitopes of the virus so that it can escape. This is reflected in the hypervariable regions, which can be well demonstrated by full genome comparisons. However, changes that counteract with the immune system may also occur in more radical evolutionary steps, i.e. by homologous recombination when a

new variant can effectively escape the immune attack and overgrow the earlier variant. Further one-step evolutionary happening is the pick up of possibly useful new genes by the viruses from their environment, or duplication of an important gene to increase capacities. Viruses might also loose genes, which become non-essential in an altered cellular environment. When trying to estimate the evolutionary distance and phylogeny of certain viruses, we should bear in mind all these evolutionary possibilities that happen with different speed. Otherwise we cannot avoid false conclusions. A recent practical contribution to the better understanding of virus evolution comes from the study of viruses of exotic and wild animals. The discovery and characterization of such viruses will provide important clues to uncover significant evolutionary happenings of the past. Consensus (family specific) PCRs are available for the examination of viruses that cannot be isolated. Non-specific virus detection methods might facilitate the discovery of yet unknown viruses.

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EXTRACELLULAR ENZYME SYSTEMS INVOLVED IN THE PATHOGENESIS OF *TRICHODERMA PLEUROTUM* TOWARDS OYSTER MUSHROOM

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The Trichoderma-caused green mould disease of cultivated oyster mushroom (Pleurotus *ostreatus*) has been reported to result in significant crop losses in more and more countries throughout the world. The causal agents have been described as the new species *T. pleurotum* and *T. pleuroticola*. The involvement of extracellular enzymes in the mycoparasitic activity of biocontrol Trichoderma strains is well-known, and certain enzymes are also supposed to act as potential virulence factors of the Pleurotus-pathogenic *Trichoderma* species, therefore the objective of this study was to examine the involvement of extracellular enzyme systems in the pathogenesis of *T. pleurotum* towards *P. ostreatus*. A wild-type isolate of *T. pleurotum* was subjected to UV-mutagenesis and 150 surviving colonies were tested in plate assays for their ability of producing several extracellular enzyme systems. The antagonistic potential of the parental strain and its derivatives with altered pectinase, cellulase, amylase, lipase, protease, chitinase and glucanase production was checked in dual in plate assays with *P. ostreatus*. Numerous derivatives with altered enzyme producing ability were isolated. In the dual plate assays the parental strain could overgrow and conidiate on the colony of *P. ostreatus,* which is considered to be a sign of mycoparasitism. No strains exhibited higher mycoparasitic ability towards oyster mushroom than the wild-type strain but several deficient mutants showed significantly lowered mycoparasitic potential. Based on our results it is suggested that among the enzyme systems tested, deficiencies in the protease, lipase, chitinase and glucanase systems can mostly reduce the mycoparasitic potential of *T. pleurotum* towards *P. ostreatus*. However, since UV mutagenesis produces random mutations, further studies are required for a better understanding of the role of these enzymatic functions in mycoparasitism.

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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PREDATORY BACTERIAL STRAINS

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Chemical pesticides used in agriculture against plant pathogenic bacteria can be harmful also for other living creatures of the treated area, which effect however might be softened by the application of predatory bacterial strains within the frames of integrated pest management. The objective of our work was the isolation, identification and characterization of BALO (Bdellovibrio-and-like organism) strains that can be used to control plant pathogenic *Pseudomonas syringae* and *Xanthomonas campestris* strains. Four putative BALO strains were isolated from soil samples derived from the rhizosphere of tomato plants, which were identified as *Peredibacter starrii* by the aid of polymerase chain reaction carried out with BALO-specific primers and subsequent sequence analysis of the PCR products obtained. In our experiments carried out both on plates and in shaken cultures, the isolates proved to be effective against all tested *X. campestris* isolates, while a Bdellovibrio bacteriovorus strain derived from culture collection DSMZ, was used successfully against numerous *P. syringae* and *Pectobacterium carotovorum* isolates. The results of experiments performed with this strain and *P. syringae* in the presence of copper sulfate demonstrated that it might be used in integrated pest management strategies against *P. syringae* in combination with copper-containing pesticides.

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INDUCIBILITY OF GALACTOSIDASE ENZYMES FROM *LACTOBACILLUS ACIDOPHILUS* LA-5

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Lactic acid bacteria (LAB) are widely used in food industry including preservation of foods and application as probiotic, functional food components. Probiotics are live bacteria, which are consumed as a food components or supplements, and upon digestion, they exert beneficial effects to health status and well-being. Lactobacilli and Bifidobacteria are the most frequently used microorganisms in probiotic products. *Lactobacillus* species have been found in large numbers as part of the intestinal microbiota of humans and animals, where they are thought to increase resistance to common intestinal disorders, especially those with a microbial pathogenesis. They can achieve this by fortifying the normal microflora either through their fermentation products or by the production of glycosidases, which degrade carbohydrates, thereby supplying energy for the growth of other bacteria. Other functional food component is prebiotic, which selectively supports the growth of probiotic bacteria. Most commercially available prebiotics such as fructo-oligosaccharides, soy-oligosaccharides and xylo-oligosaccharides are produced from vegetables. Nowadays a new approach has come to light in the synthesis of prebiotic. According to this concept the application of hydrolytic enzymes from the probiotic bacteria could be a powerful mean in production of tailor-made prebiotic. In this research work α-and β-galactosidase activities of a probiotic *Lactobacillus acidophilus* La-5, strain which has a long history in the food processes were studied. LAB was cultivated in MRS media containing carbon sources with different chemical structures at 37°C for 24 hours. Samples were taken at a given time. Fermentation was followed by the measurements of pH, determination of living

cell number by plating methods and enzyme activity assay. The enzyme activities were determined in both extracellular and intracellular fractions. The harvested biomass was disrupted by French Press homogenizer. Based on our results we may conclude: - *Lactobacillus acidophilus* La-5 strain synthesises a- and b-galactosidase enzymes intracellulary. No galactosidase activity was detected in extracellular fraction. - Both a- and b-galactosidase enzymes of *L. acidophilus* La-5 strain are inducible. - The α-galactosidase activity was observed when the probiotic bacterium was cultivated in media containing carbohydrates with α-galactosidic linkages such as melibiose or raffinose. The highest α-galactosidase activity was gained at 2 % raffinose and in this case the activity of the biomass recovered from 100 ml ferment broth were 13,51 U. - La-5 strain showed the maximum α-galactosidase activity in the late exponential or early stacioner growth phase in medium contain 2 (w/v) % lactose, the activity of the biomass recovered from 100 ml ferment broth was 7,11 U. After the optimization of the fermentation and down stream processes our future goal is the implementation of these enzymes in the synthesis of prebiotic oligosaccharides.

INCREASED RATE OF DIVERSIFICATION IN THE GENUS *COPRINELLUS*?

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The genus *Coprinellus* represents the most species rich assemblage of coprinoid mushrooms, classified on the basis of the structure of cap covering and being deliquescent (autodigesting). Still, species of this genus are among the smallest of all mushroom species, some approaching only 1 mm of cap diameter on maturity. Accompanied by a number of other peculiar morphological features evolved, one would raise the question whether such a combination of unique features offers adaptive or selective benefits to the taxa. To test this, we reconstructed phylogenetic relationships of the genus including most of the described taxa, based on ITS and beta-tubulin gene sequences. Using Bayesian MCMC and Maximum Likelihood methods, we indentified species limits in the genus and obtained significant statistical support for most morphologically recognized taxa. In addition, we sequenced several hitherto undescribed species available in our herbarium. All analyses recovered clades corresponding to section *Setulosi* and *Domestici+Micacei* with strong support. Basal relationships in the *Setulosi* clade were difficult to resolve, due to very short waiting times between putative speciation events. Therefore, we tested whether the rate of diversification (speciation rate minus extinction rate) is increased in the Setulosi clade. Lineage through time plots show an increase in the rate of diversification in the early branchings of the Setulosi clade. This suggests that *Setulosi* taxa have undergone rapid diversification in the early phase of their phylogeny. Whether this represents an increase in the rate of speciation or a decrease in the rate of extinction, however remains to be tested, but the results suggest that the peculiar morphological features these taxa possess might imply an improved fitness, which has led to rapid net diversification.

LONG TERM CHANGES IN THE INTRACELLULAR BACTERIUM SPECTRA OF *ARCELLA* SPP. (LOBOSEA, ARCELLINIDA)

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Free-living amoebae are important members of microbial communities, and may occur in different kinds of wet environment (e.g. soil, natural water, tapwater, swimming pools etc.). Being predators, they play a substantial role in regulating bacterial communities in biofilms. There are, however, several kinds of bacteria which may survive a phagocytosis by an amoeba and thus grow and reproduce within the host cell. The majority of the described endobiont bacteria are medically harmless, but there are some potential pathogenes (*Legionella pneumophila, Pseudomonas aeruginosa*) which can avoid the digestion by the host. Intracellular bacteria have long been known to occur in amoeboid organisms, but so far most of the studied amoeboid hosts were pathogenes. In this experiment we examined the total bacterial spectra of different species of Arcella, a free-living, lobose testate amoeba. Our samples derive from natural water, from different parts of Hungary. After establishing axenic clonal cultures of various species, the endocytobiotic composition of the cultures were examined every year in a three-year period. Besides clonal cultures we also examined Arcella cells newly isolated from natural water samples. The host cells were fed with *Enterobacter aerogenes* to avoid contamination, and were washed in sterile water before molecular work. The total bacterial DNA extraction was followed by the amplification of the 16s rRNA gene. Denaturing gradient gel electrophoresis (DGGE) analyses were performed to identify and compare the members of the host cells's intracellular bacterium spectra, and follow the changes during the years. The most remarkable bands were cut out from the gel, DNA of the band were extracted and sequenced. Apart from DGGE we also performed taxon specific PCRs to detect the members of the Legionella and Pseudomonas genera. In the first year the bacterial diversity of the recently established clonal cultures were much more diverse than the following years. Diversity of bacterial spectra seemed to decrease with time. The bacterial spectra of the different Arcella clones were various, but similarities were also detected. Legionella and Pseudomonas were more frequent in natural samples than in older clonal cultures.

HUMAN CATHELICIDIN PEPTIDE LL37 INHIBITS BOTH ATTACHMENT CAPABILITY AND BIOFILM FORMATION OF *STAPHYLOCOCCUS EPIDERMIDIS*

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The coagulase-negative *Staphylococcus epidermidis* are commensals of the human skin and mucous membranes, but they are also a major cause of nosocomial and health-care related infections. They cause over 50% of all late onset sepsis (after 3 days of age) in preterm infants and re the main cause of device-related infections, particularly when venous catheters are being used. The main known virulence factor of these bacteria is their ability to attach and colonise the polymer surface by the formation of a thick, multilayered biofilm. The aim of this study was to investigate the possible effect of the human cathelicidin antimicrobial peptide LL37 on the biofilm formation process of these bacteria, a peptide produced by phagocytic leucocytes, cells of the mucosal epithelium, and keratinocytes. The following methods were used: initial attachment assay and biofilm formation solid surface assay in microtiter plates, as well as growth experiment in liquid medium, using the laboratory strain *Staphylococcus epidermidis* RP62A (ATCC35984). We found, that already a low concentration of the peptide LL37 (1 $mg\mu l^{-1}$) significantly decreased both attachment of the bacteria to the surface and also the biofilm mass. The peptide did not inhibit the planctonic growth of *S.*

epidermidis at 8 and 16 mgμl^{-1}.The present study reports a new finding, that the human cathelicidin peptide LL37 inhibits the attachment and the biofilm formation of *S. epidermidis*. Since the formation of biofilm protects bacteria during infections and allows survival in a hostile environment, the inhibition of biofilm formation by this peptide may have a key role to prevent bacterial colonisation on indwelling devices. Our results also indicate, that LL37 can be a potential aspirant of therapeutic experiments, such as stimulating the endogenous production of natural peptide or using these peptides as a template to develop artificial analogues with optimized biological activities.

ENZYMATIC ONE-POT PRODUCTION OF SPECIFIC STRUCTURED LIPIDS AND PLANT STEROL ESTERS

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Structured lipids refers to those oils and fats containing polyunsaturated fatty acids (long chain C14 <, mainly essential fatty acids) and medium- (C6-C12) or short chain (C1-C4) fatty acids located randomly on the glycerol backbone. While in the case of specific structured lipids each group is located specifically at the sn-2 or sn-1,3 positions of the glycerol backbone. Structured lipids are often produced by interesterification either chemically or enzymatically. Enzymatic modification of oils and fats for the production of specific structured lipids and their positive effects on health have been of a great interest for a long time. Plant sterols and plant sterol esters help decrease the cholesterol level in blood and have other health benefits as well. Practical applications of free sterols in foods are limited due to their low solubility, therefore fatty esters of sterols are generally preferred in the food industry. Publications related to health, nutrition and metabolism suggest the usefulness of phytosterols in oil based composition as well. Structured lipids combined with phytosterol esters in so-called healthy oil compositions have the advantage of both. Such blends having substantial edible oil content can function as „value-added" additives in food systems improving health, nutrition and metabolism., especially by individuals exhibiting overweight. Structured lipids combined with plant sterol esters can provide an improved quality of lipid intake and excellent composition for the edible oils having good physical properties and low trans-isomer levels as well. Nowadays consumer demands are more and more tend towards natural products produced by eco-friendly processes and/or with the utilization of by-products which even possess health promoting and illness preventive effects as well. In the light of these the purpose of this study was to find integrated enzymatic methods resulting in a special stuctured lipids from natural vegetable oil and plant sterol ester useful as a beneficial food additive. A special emphasis was taken using starting materials from natural sources. The enzymatic one-pot production of mixtures of specific structured lipids and plant sterol esters were carried out in organic solvent free medium starting from a mixture of plant sterol, caprylic acid and sunflower oil. Nine biocatalysts - seven commercially available lipases and two *Aspergillus solid* state fermentation biocatalysts - were screened for lipase activity and for sterol esterase activity. As chemical transesterification has been avoided, this process resulted in products with close to native oils and metabolic functions without any increase in the initial trans-fatty acid content.

EPSTEIN-BARR VIRUS AND HUMAN CYTOMEGALOVIRUS IN PULP/PERIAPICAL INFLAMMATION

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Pulpitis and apical periodontitis are dental inflammatory diseases caused by opportunistic bacteria, but it has been shown recently that affected tissues often contain herpesviruses, particularly Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV). In periapical inflammation, herpesviruses may cause an immune impairment by inducing the release of tissue destructive cytokines (IL-1β, IL-6, TNFα, INF-γ) that help the local pathogenic bacteria to overgrow. Symptomatic and large-size periapical lesions are often associated with EBV/HCMV active infections. The present study was initiated to evaluate the role of these viruses in the pathogenesis of apical periodontitis. 80 samples were included in the study: 40 periapical lesions (17 asymptomatic, 23 symptomatic) collected from surgical apico-ectomy and 40 controls from impacted third molars. RNA was isolated from tissues by using TRISOL LS Reagent and for DNA isolation viral nuclear acid kit was used. Primary or nested polymerase chain reaction (PCR) and reverse transcription-PCR were used to identify the presence of herpesviruses. On the basis of preliminary data analysis we suppose that EBV/HCMV DNA and RNA are present in periodontitis in significally higher percentages compared with healthy pulp controls.

LACTIC ACID FERMENTATION ON WHEAT FLOUR VIA SHF AND SSF TECHNOLOGY

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Lactic acid is a low-value, higher-volume chemical and its derivates might be feasible substitutes of the petrol chemistry produced conventional raw materials of the plastic and solvent industries (PLA, ethyl-lactate, butyl-lactate etc.). The use of excess biomass or agricultural and industrial wastes to produce lactic acid via fermentation is not a novelty, however in Hungary has just shown up a claim to use surplus grain capacity (corn, wheat) in white-industrial technologies [1]. Wheat is an appropriate raw material for bacterial lactic acid fermentation because the starch content can cover carbon source need of lactic acid bacteria, while protein content serves as nitrogen source for their growth. Using starch as carbon source can be handled in different ways: some lactic acid producing strains can convert starch to lactic acid without previous hydrolysis (direct fermentation); in some cases producing strain needs a starch liquefaction step before fermentation but its own gluco-amylase enzyme converts dextrins to glucose for lactic acid fermentation; most lactic acid bacteria need complete hydrolysis to glucose which can be performed separately from (separate hydrolysis and fermentation - SHF) or in line with fermentation (simultaneous saccharification and fermentation - SSF) [2]. Normally lactic acid bacteria are mesophiles (20-40°C) and normal hydrolysis of starch is done at elevated temperature (>55°C). These problems could be solved by either applying thermophilic bacteria or fungi, or using semi-simultaneous saccharification and fermentation. Linko and Javainainen [3] applied different hydrolysis duration at the optimal temperature of the enzyme (60°C), then inoculated the hydrolysate with mesophilic bacterium and examined SSF process at 37°C. Targets of our experiments are to produce lactic acid from wheat flour as a renewable raw material, with a minimal supplementation beside starch and protein content of the plant; comparing

SHF and SSF technology to reduce hydrolysis and fermentation time, hereby enhancing lactic acid productivity and depressing production cost of this low-value chemical.

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HIGH-RESOLUTION MELTING ASSAY FOR THE IDENTIFICATION OF *FUSARIUM* SPECIES

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Fusarium head blight (FHB) is a disease complex of cereals in which several fungal species may cause symptoms. One of the species, known as the major cause of head blight of wheat, is the *F. graminearum*. Less frequently isolated species are *F. acuminatum, F. avenaceum, F. poae and F. sporotrichioides*. FHB can significantly reduce grain yield and quality. Identification of *Fusarium* species is of high importance in relation to FHB. The Real-time polymerase chain reaction (Real-time PCR) is a handy and fast technique for the identification and differentiation of *Fusarium* species. The melting curve analysis, the so-called High-resolution Melting (HRM) assay, is used to characterize nucleic acid samples based on their dissociation (melting) behaviour. The samples can be discriminated according to their sequences; therefore it is a useful tool to distinguish closely related species from each other.Our aim was to identify five *Fusarium* species, isolated from Hungarian wheat grains, by the use of the elongation factor gene EF-1/EF-22 primers. After the Real-time PCR the products were submitted to HRM analysis and the melting curves were compared. In our investigations we found that the melting curve analysis can be used to distinguish the five *Fusarium* species. The *F. acuminatum* differs the most from the other *Fusarium* species in its dissociation behaviour, whereas the melting curve of the other species are much more similar to each other. As a summery we can conclude that the HRM assay is an efficient, reliable and novel method for the identification of the examinated Fusarium species. In the future we plan to investigate further *Fusarium* species with HRM method.

BACTERIAL COMMUNITIES AND FACTORS INFLUENCING THEIR STRUCTURES IN DRINKING WATER NETWORK OF BUDAPEST

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Drinking water systems (DWSs) represent distinct microbiological habitats where due to low organic carbon concentration, different types of tube surfaces, flow velocity , antimicrobial treatments etc. special microbial, especially bacterial communities can exist. In spite of frequent microbial investigations, characterisation of these communities in DWSs is poorly documented. Potable water of Budapest is originated from riverbank filtration wells located at the two side of the islands of River Danube, and much of it treated only by chlorination before supplying.
In the recent study diversity of bacterial communities was investigated in 10 sampling points (3

wells, 3 points of unchlorinated, 4 points of chlorinated part) of the drinking water network of Budapest in 6 sampling time for half year based on T-RFLP fingerprinting. From the 60 samples, 10-14 litres of water were filtered, respectively. DNA was isolated from the filter, and after partial 16S rDNA amplification T-RFLP analysis was performed. To identify TRF peaks, clone libraries were constructed. Clones were grouped by ARDRA, sequence and TRF lengths of representatives were determined. Bacterial communities were compared by statistical analyses (e.g. PCA) based on size and relative quantity of TRFs. Bacterial cell count was determined by fluorescent microscopy. Abiotic parameters were measured using standard protocols.
According to fingerprints, unchlorinated water samples were characterised by very diverse communities with oligo-heterotrophs (e.g. *Sphingomonas* spp., *Bradyrhizobium* spp.) and chemolithotrophs (e.g. *Gallionella* sp., *Nitrospira* sp.). Chlorination treatment resulted decrease of the quantity and diversity of bacteria. Communities of chlorinated water were less diverse and dominated by members of the non harmful genera *Methylocella* and *Mycobacterium* with cell counts 10^3-10^4 cell/ml. Correlations were found among the changes of communities and abiotic background (chemical and physical variables). In addition changes on time could be observed and explained with the seasonal changes of raw water (ion concentration influenced by the active biomass of River Danube), and significant shifts occurred after flood or draught. Certain wells and collecting system points changed seriously in the case of bacterial communities as well as abiotic parameters (e.g. water temperature) during the sampling period, which indicates that these wells are influenced more significantly by external factors, than others. On the other hand chlorinated water samples had rather uniform chemical characteristic, because the supplying system contains the mixture of water from different wells. In spite of the chemical similarity, bacterial communities of chlorinated water samples showed serious differences and fluctuations. It indicates that efficiency of antimicrobial treatment decreases in the distal part of the system, and after recolonisation of water different communities exist in different part of the system due to unique circumstances and stochastic effects.

THE INFLUENCE OF PATULIN ON *SCHIZOSACCHAROMYCES POMBE* PLASMA MEMBRANE

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The fission yeast *Schizosaccharomyces pombe* ura4D-18, h^- strain was used to examine the interaction of patulin with the plasma membrane. The growth inhibitory activity of patulin was measured in shaked cultures of supplemented minimal broth at 30 °C. In 15 hours old cultures, 5 μM patulin did not induce any inhibitory effect. The growth inhibition at 50 μM, at 500 μM, and at 1000 μM, was 32%, 82.5% and 92.5%, respectively. Survival rate was 5% after 60 minutes treatment with 500 μM patulin in HEPES (pH 5.5) buffer. The minimal inhibitory concentration (MIC) of patulin was 3.08 μM: it was determined with microdilution method (M27A) according to the NCCLS standards. Spheroplasts were used for the determination of the dynamic properties of lipid bilayers. Spheroplasts incubated in 0.5 M KCl, at 30 °C, with the applied patulin concentrations, did not show lyses during the 120 minutes long experiment.To determine the effect of patulin on plasma membrane electron spin resonance (ESR) experiments were carried out using the 5-(4',4-dimetyloxazolidine-N-oxyl)stearic acid (5-SASL) fatty acid spin probe. According to the ESR measurements, 500 μM

patulin fluidizes the plasma membrane and increases the phase-transition temperature. These results indicated significant plasma membrane disorganization. It was found that 500 μM patulin induced an efflux of substances absorbing at λ=260 nm from the cell. 60% decrease of these intracellular substances was detected. All these indicate a substantial alteration in the barrier function of plasma membrane. Our results suggest that the plasma membrane is the first target of patulin in fission yeast: it induces changes in the structure and function of the membrane on such extent that 500 μM patulin treatment could induce cell-death within 60 minutes.

DEMONSTRATION OF A PROTEIN WITH ENHANCED RESISTANCE TO PROTEINASE-K IN TRANSMISSIBLE CYTOPATHIC CONDITION ELICITED BY CELL-FREE LYSATE OF FREE-LIVING AMEBA *NAEGLERIA GRUBERI*

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The cell-free lysate of free-living amebae *Naegleria gruberi* was reported to elicit cytopathic effect in various cell lines that could be indefinitely transmitted by the culture media. The causative agent showed sensitivity to treatments detrimental to proteins while resisted exposures damaging to nucleic acids. We previously demonstrated the presence of a protein with enhanced resistance to proteinase K in the cytopathic cell line in an SDS PAGE experiment. Now we confirm the accumulation of this partially proteinse K resistant protein with the more advanced technique of capillary gel electrophoresis. It is proposed that the protein showing enhanced resistance to proteinse K is a conformationally altered host protein converted by adhering protein(s) in the ameba lysate and could act in a prion-like fashion as a proteinaceous pathogen.

IMPACT OF CULTURE MEDIA ON SOME PROPERTIES OF *VIBRIO CHOLERAE* NON O1 STRAINS

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Vibrio cholerae is a gram-negative, noninvasive enteric bacterium that naturally inhabits the aquatic ecosystem. Of the 200 O-antigen serogroups so far identified among *V. cholerae* isolates, only two serogroups O1 and O139 are known to cause epidemic and pandemic cholera. *V. cholerae* non O1 serotypes possess very similar biochemical and morphological properties to choleraic (serogroup O1) but not agglutinate with polyvalent O1 antiserum. These strains are associated with gastroenteritis, sporadic diarrhoea, acute septicemia, skin infections, etc. The effect of six culture media (CM) (5-complex, 1- mineral) on hydrophobicity, biofilm formation, motility, sensitivity to oxidative stress, enzymatic activity (lipase, proteinase) in three *V. cholerae* non O1 strains were evaluated. In vitro tests for hydrophobicity (adherence to xylene), for quantitative biofilm formation (crystal violet), motility (0.35 % agar), response to oxidative stress (hydrogen peroxide), lipase (Tween 20) and proteinase (azocasein) were applied. Composition of CM substantially did not affect adherence vibrios to xylene (with the exception of mineral medium (MM) in D/04 1 strain). Individual strains adhered to xylene in the range of 50.5-61.1 % (strain 84/233), from 33.0-37.6 % (10/116) and from 11.0-31.1 % (D/ 04 1). Biofilm formation by the strains was the highest after cultivation in tryptone

soya broth enriched by 8 % glucose (TSM+GL) (absorbance from 0.347-0.411) and in tryptone soya broth (TSM) (from 0.316-0.417 and being the lowest in MM (from 0.098-0.177). Significant differences were not found among tested media concerning bacterial motility, this was dependent mainly on the strain. The highest motility manifested strain 84/233 in TSB+GL, 10/116 in TSB and D/O4 1 in peptone water (PW). Similarly, vibrios did not manifest significant differences in response to oxidative stress in dependence on culture media (with the exception of 10/116 in TSB and MM). Higher sensitivity have shown bacteria growing in PW (inhibition of bacterial growth 25.2 mm), more resistant were vibrios after cultivation in MM (21.3 mm). CM affected lipase production, with the highest one in PW. No lipase activity was found in TSB+GL as well as in MM. As the most suitable media for proteinase production were shown Mueller-Hinton broth (MHB) and PW, the lowest quantity of enzyme was found in TSB+GL and/or TSB. Mainly the production of biofilm and enzymatic activity of vibrios were affected by composition of CM.

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MURINE GAMMAHERPESHERPESVIRUS AS A MODEL FOR STUDY OF CORRELATION BETWEEN PERSISTENCE OF VIRAL GENOME AND TUMOR DEVELOPMENT

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Murid herpesvirus 4 (MuHV 4) is a useful model for studying human herpesviruses, such as Epstein-Barr virus (EBV) and Kaposi sarcoma-associated virus (KSHV), which can cause severe diseases and induce various types of malignancies. The most frequently investigated strain MHV-68 is lymphotropic, but it also infects epithelial cells and fibroblasts. It is capable to persist in infected cells causing malignant transformation. However, several strains and or isolates of MuHV 4 differ in their molecular-biological, pathogenetic and immunological parameters. Absence of 9,538 kbp sequence in the left part of genome is characteristic for the MHV-76 strain. Also MHV-78 differs in its polypeptide profile. In this study, we deal with the potential of persistently MuHV-infected cells to induce tumors in mice. We used the tumor cell line Nb.78 which contains persistent MHV DNA. This cell line was derived from a mouse infected with the isolate MHV-78. In addition, we used HeLa cells latently infected with MHV76 (μHeLa) and control HeLa cells. MHV-78 was reported to form tumors in 7% of infected mice, while no proof about formation of tumors after infection with MHV-76 had been reported. We inoculated 105 cells pre mouse to the cervical area. The animals were divided into three groups. Four tumors formed in the group of 17 mice (23%) inoculated with Nb.78 cells, but none was formed in the groups inoculated with μHeLa and HeLa cells. We investigated the presence of viral DNA in tumors as well as in selected organs of inoculated mice. DNA of MHV-78 was detected in all tumors and in spleens and lungs of tumors bearing mice. On the contrary, there was no viral DNA in the organs of mice inoculated with μHeLa or HeLa cells. We also looked for the viral antigen by indirect immunofluorescence; the results corresponded with those mentioned above as well as with those obtained from organ titration on permissive VERO cells, which also showed positive results in tumors.

INTEGRATED APPROACH TO MATHEMATICAL MODELLING OF ATRAZINE BIODEGRADATION IN DIFFERENT REACTION SYSTEMS

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Based on known approaches and published mathematical models, as well as on theoretical consideration using our experimental data, the integrated approach to mathematical modelling of atrazine biodegradation processes was applied and sophisticated mathematical models for different reaction systems were developed. The applicability of these mathematical models which are based on the physical, chemical, biochemical and biological complexity of atrazine biodegradation was further investigated in comparison to mathematical models describing simple consecutive reaction systems by first order kinetics and mathematical models taking into account the biocatalytic role of mixed microbial populations. Kinetics of atrazine degradation was asessed and compared in liquid media and soil contaminated with atrazine. Biodegradation experiments in liquid media were conducted at atrazine concentrations ranging from 0.14 mmole/L to 25.00 mmole/L and temperatures of 10°C, 20°C and 30°C, while the experiments in soil were conducted at atrazine concentration of approximately 0.44 μmole/g and temperatures 10°C and 30°C. For explaining experimental results and to test the adequacy of mathematical models a series of computer simulations were performed. Detailed analysis of computer simulation data showed that developed integrated mathematical models are generally the most convenient, although even the simplest mathematical models are suitable for explaining some experimental results, especially when evaluating the effects of temperature on biodegradation efficacy of the applied mixed bacterial cultures.

THE EFFECT OF PREBIOTIC INGREDIENTS OF BAKERY BASIC MATERIALS ON THE GROWTH OF BACTERIA

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A number of factors compromise the health of modern people: stressful lifestyle, unbalanced nourishment, excessive consumption of refined foods with a big measure, admission of different chemical agents into the human body. These factors harm directly or indirectly the intestinal activity, that forms a considerable part of the immune system, including the production of essential substances that have beneficial effects on the human body. The role of the so-called prebiotics (e.g. inulin, various oligosaccharides, raffinose, resistant starch and cyclodextrin) is to prevent and reduce the damage of useful microbes, which are termed as probiotics, as well. These substances selectively facilitate the propagation of probiotic bacteria (e.g. *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Enterococcus faecium*, *Lactobacillus acidophilus*), therefore increase the rate of the synthesis of vitamin B and of beneficial short chain fatty acids, improve the absorption of minerals, decrease the level of cholesterol, triglycerides, insulin, glucose, ammonia and uric acid and improve the functioning of the immune system. The majority of the examination results about prebiotics is based on clinical dietary and animal experiments. In contrast to this we simulated the process of digestion and the effect of prebiotics on probiotic and non-probiotic bacteria selected by us in an artificial digestion model. In this digestion model the different prebiotic substances get through the simulated oral cavity, stomach and intestine and may be modified in the course of this travel. The

probiotic and non-probiotic bacterial strains were added to the model in the process of digestion in the simulated colon, then the digestion continued on the appropriate temperature (37°C), pH and under anaerobic atmosphere. The pH decreasing effect of lactic acid producing bacteria was compensated by pH buffer. The first sampling was carried out 24 hours after inoculation, whereas the second one was performed 36 hour after inoculation. At the same time preparation of dilution series and inoculation of the bacterial strains onto the adequate selective plates were performed. Based on the number of colonies counted on the selective plates we could see whether the prebiotics did help the propagation of probiotic strains against the non-probiotic strains. In another experiment we digested dairy products containing various probiotic strains together with different baking industrial products that were supplemented by various simple sugars and prebiotics, respectively. In the course of the examination we determined the germ number of the various dairy products at the start and at the end of the experiments. In addition we determined the rate of the decrease of germ number due to the digestion on low pH in the stomach. In the course of the digestion process in the colon we surveyed the effect of baking products containing prebiotic materials on the survival and propagation rate of bacteria. To perform this survey dilution series were prepared after 24 and 48 hours cultivation then the bacteria were inoculated onto selective media and the growing colonies were counted.

CYTOTOXICITY OF SOME FUROFURAN PRECURSORS OF AFLATOXIN BIOSYNTHESIS

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Many studies have been carried out to elucidate cytotoxic, mutagenic and carcinogenic effects of aflatoxins. Some aspergilli that produce aflatoxins may contain some of the precursors of aflatoxin biosynthesis in their spores depending on the stage of aflatoxin production. A limited number of reasearch has been carried out on biological effects of aflatoxin percursors that contain furofuran ring, which is incriminated for toxic effects of aflatoxins.

Therefore, the purpose of our study was to determine cytotoxicity of some dihydrofurofurans (veriscolorin A or VER A, 6-deoxyversicolorin A or 6-DEOXY-VER A and 5-methoxysterigmatocystin or 5-MET-ST) and tetrahydrofurofurans (versicolorin B or VER B). Cytotoxicity of these compounds was detemined for human adenocarcinoma cell line A549 using colorimetric MTT assay (at 595 nm). Cells were exposed to various concentrations of VER A and 6-DEOXY-VER A (from 0.5 to 150 μM) as well as VER B and 5-MET-ST (from 0.5 to 250 μM) for 24 hours. Cytotoxic concentrations of tested compounds that inhibited 50% of cell viability (IC_{50}) were: IC_{50}=78 ± 12 μM (6-DEOXY-VER A); 109 ± 3,5 μM (VER A); 172 ± 4 μM (VER B) i 181 ± 2,6 μM (5-MET-ST). VER B and 5-MET-ST applied at 0.5 μM exerted proliferative effect. Diferences in cytotoxic potential of tested compounds are probably the result of some changes in their structure which might affect lipophility/hydrophility ratio and therefore affect their transfer through cell membrane as well as their biotransformation. To our knowledge this is the first report on cytotoxic potential of furofuran precursors in aflatoxin biosynthesis.

YEAST *SACCHAROMYCES CEREVISIAE* AS A MODEL ORGANISM FOR INVESTIGATING ACTION OF OXIDATIVE/ANTIOXIDATIVE COMPOUNDS

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Investigating of action of oxidative/antioxidative compounds in the cells is currently not possible in human systems. In drug research, where the major challenge is also to study their effect on metabolic and cellular pathways affected by a drug, several different approaches are being developed in the yeast *Saccharomyces cerevisiae* [1]. Yeast *Saccharomyces cerevisiae* is an excellent model for investigating fundamental cellular processes, stress responses and metabolic pathways of the human [2, 3]. Comparative genomics studies have shown that 40 % of yeast proteins share amino acid sequence similarity with at least one human protein [4] and 30 % of genes with with a recognized involvement in human disease have ortholog in yeast [5].
Yeast presents many technical advantages over human cells. It is well suited to highthroughput methods because of its life cycle is rapid and it is inexpensive to maintain and grow. It is highly amenable to genetic modifications such as gene disruption, deletion and replacement [6]. Experiments with yeast will always be technically easier, more rapid and much less costly than experiments with human cells and it is anticipated that yeast will remain a good first line of attack in the hunt of drugs (1) as well as other compounds. To show experimental accessibility and value of yeast as model organism, different cases using proteomic and cellular approach to study action of oxidative and antioxidative compounds will be presented.

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COMPARATIVE EXAMINATION OF METABOLIC FINGERPRINT OF *HISTOPHILUS SOMNI* STRAINS ISOLATED FORM CATTLE

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Histophilus somni (former name: *Haemophilus somnus*) is a Gram-negative, fastidious, facultative pathogenic bacterium, that mainly occurs on the mucous membranes of the respiratory and genital tract of cattle and sheep. It can cause thromboembolic meningoencephalitis, pneumonia, reproductive problems and septicaemia in cattle. In sheep it is reported as a cause of orchitis and epidydimitis of rams, pneumonia, mastitis and septicaemia as well. Asymptomatic carriers can also occur in both animal species. *H. somni* was also described in goat, bighorn sheep and American bison. The authors collected vaginal swabs from 5 and lung samples from dead calves in 12 cattle stocks of Hungary. Using the adequate culturing methods, they isolated 45 bacterial strains identified as *H. somni* on the basis of morphological, cultural and biochemical characteristics and they selected *H. somni* strains

out of the 34 *H. somni* strains formerly isolated and stored in the culture collection of the Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University. The comparative study of the metabolic fingerprint of 62 different H. somni strains was carried out using the Biolog Microstation ID System (Biolog, Ca). The system analyses the ability of the utilization of 95 single carbon sources simultaneously thus allows of detecting slight differences among the strains. A dendrogram was made on the basis of carbon source metabolism and the relationship was evaluated of the 62 *H. somni* strains. Studying the dendrogram based on the carbon source utilisation of 62 *H. somni* strains, several highly similar strains as well as different ones were found in certain cattle flocks. There were two highly similar *H. somni* strains of respiratory origin that could be isolated in the same cattle stock sampling it in different years.

GROWTH INHIBITION AND THERMAL SENSIBILIZATION OF *ALICYCLOBACILLUS* SPP. AND *BACILLUS CEREUS* SPORES WITH COMMERCIAL ROSEMARY EXTRACT FORMULATIONS

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Gram-positive, spore-forming bacteria have been recognized as the causative agents of spoilage and also human infection or intoxication with various foods. Thermoacidophilic *Alicyclobacillus* spp. have contributed to beverage spoilage during the last two decades, due to the survival of *Alicyclobacillus* spores and, possibly, vegetative cells, and their subsequent growth in acidic fruit juices. *Bacillus cereus* can cause food spoilage as well as foodborne illness, either an emetic or a diarrheal syndrome. Consumer demand of natural, fresh, chemical-additive free and safe food products is increasing, so there is growing interest for development of novel combinations of natural antimicrobial systems in conjunction with reduced levels of traditional physical and chemical food preservation processes to improve the quality and safety of foods.

We have studied bacteristatic and bactericidal activity of commercial rosemary extract formulations containing different levels of carnosic acid against different food-related bacteria [1,2], but in this study we present the results for food isolates of *A. acidoterrestris*, *A. acidocaldaruis*, *A. acidiphilus*, *A. hesperidum, A. cycloheptanicus* and *B. cereus*. Disc diffusion and agar dilution method as screening tests and broth dilution assay for MIC and MBC determination were employed for assessing bacterial growth inhibition. Kinetic studies were conducted with MICs in different matrices (i.e. in TSBYE and AM as laboratory media and in chicken boujon concentrate as a food model system) at different temperatures (8°C, 37°C) and inocula (10^2-10^5 CFU/ml).

Rosemary extracts inhibited the growth of all tested strains in correlation with external conditions, but the endospore-forming organisms can not be eliminated from food with these antimicrobial additives. So we tested also the impact of selected rosemary extract on bacterial spore sensitivity to heat treatment. In selected conditions we confirmed the reduction of D-values in the ranges 90-100°C after plant extract addition at MIC concentrations of rosemary extracts for all tested strains of *B. cereus* and *A. acidoterrestris*.

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FERMENTED MILKS, BASED ON LACTOSE-HYDROLYSED MILK, SUPPLEMENTED WITH HONEY

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Nowadays the role of probiotics is well known. Production of reform yogurts, kefires based on special milks and supplements give several possibilities for a healthy way of life. For example, lactose sensitive persons have to always eat dietetic, functional foods with low lactose content. There are probiotic yogurts on the market in Hungary, based on lactose hydrolysed milk (0.1%lactose content), fermented by *Bifidobacterium* species and *Lactobacillus acidophilus* but these fermented milks are too sour for children and old people. Both the flavour/aroma compounds and the therapeutic properties of these probiotics may be important for consumers who suffered from lactose intolerance. For example, a supplementation with honey is able to stimulate the probiotic starters in the lactose hydrolysed milk. The taste of these fermented milks may be better too. For this reason new species, *Bifidobacterium breve* and *Lactobacillus paracasei*, were used in our experiments. Another aim of our work was to try a new supplement: Hungarian forest honey.

The fermentations were run by *Bifidobacterium breve* (3%) and *Lactobacillus paracasei* (3%), in 2 duplicate, at 37 and 43°C, until the complete coagulation. Before the inoculation 10% forest honey was added into the lactose-hydrolysed milk. During the fermentation the titritable acidity was measured. The number of Bifidobacterium breve and *Lactobacillus paracasei* was controlled by microscopic method (Breed strain). The organoleptic characters were obtained by 10 panelists (Kramer method) after cooling and ripening (24 hours/4°C.) An electric tongue was used to measure the difference of tenfold whey of fermented milks. After, a mathematical statistical method (CDA) was given to compare these data with each other and commercial yogurts and kefires.

The fermentations were faster at higher temperature and in the case of supplementation with forest honey. The samples were different by the sensory evaluation: the probiotic yoghurts seems to be better in case of supplementation with 10% forest honey. The samples were different by sensors of electric tongue, too. The significant differences were estimated by computer program (SPSS-14) at high level, depending on the temperature and supplementation.

SENSITIVITY OF GENETICALLY MODIFIED *MUCOR CIRCINELLOIDES* TO VARIOUS STRESS RESPONSES

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Carotenoids are one of the most diverse and widely distributed groups of natural pigments. Recently Mucor circinelloides has been used to investigate the biosynthesis of carotenoids and the regulation of the pathway. *M. circinelloides* accumulate β-carotene as the main carotenoid and several other compounds can also be found. In an earlier study (*Agrobacterium aurantiacum* astaxanthin biosynthesis genes were expressed in *M. circinelloides* [1]. Four transformants produced zeaxanthin, β-cryptoxanthin, canthaxanthin, echinenon and astaxanthin in different rates. The role of carotenoids

as antioxidants, light protective substances and as signalling compounds is discussed. In this study we present data about sensitivity of genetically modified *M. circinelloides* to various stress responses. Minimal inhibition concentrations of different oxidative and heavy metal stressors (H_2O_2, menadione, t-BOOH, Cd, Cu and Cr) have been measured. Although, significant differences were not observed in the sensitivity to H_2O_2 and Cu, xanthophyll producing transformants turned out to be less sensitive to menadione and t-BOOH than the β-carotene producing wild type strain. Transformants showed higher tolerance to Cd and Cr(IV) also. In the mutants catalase activity was also determined.

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ANTIRETROVIRAL EFFECT OF POLY-THYOLATED COMPOUNDS AS HIV ENTRY INHIBITORS

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The replication efficiency of HIV is multifactorial, but the receptor and coreceptor binding properties of the virus, determined by gp120 and gp41 surface glycoproteins, are with primary significance for HIV replication. Replication levels of recombinant HIV constructs (pseudovirions) that carried the glycoproteins of wild-type HIV isolates on a generic viral background correlated with replication of their corresponding natural isolates indicating that the glycoproteins contribute to viral fitness. The aim of this study was the analysis of coreceptor binding properties of different HIV isolates, and evaluating the anti-HIV effect in vitro of 3 chemically modified pyrimidine nucleotides such as UD29,UD30 and UD31. To produce pseudovirions two plasmid types were used. Different HIV-1 glycoprotein genes were amplified and cloned into the eukaryotic expression vector pCI resulting in a set of env expression plasmids (pEnv, like pEnv-HXB2, pEnv-M-ad, pEnv-HXB2/M-ad). The other type of proviral plasmids carry HIV gag-pol-(other) genes and firefly luciferase (pGJ3-luci) or eGFP (pGJ3-eGFP) as reporter genes. To amplify plasmids competent DH5-*E. coli* were transformed. Pseudovirions were produced by transfecting both pEnv and pGJ3-luci/pGJ3-eGFP plasmids into HEK293T cells by the calcium phosphate method. In transfected cells phenotypical mixtures of constructs (pseudoviruses) form. To examine transfection efficiency at the time of pseudovirion harvest, expression of the reporter genes was observed by measuring the luciferase activity in cell lysates with luminometer or detecting eGFP expression in cells with UV-microscopy. 3T3.T4.CCR5; 3T3.T4.CXCR4 and P4-CCR5 cell lines, expressing CD4 and CCR5 or CXCR4 selectively or both, have been used for the determination of pseudovirion tropism. 48 hours after infecting the cells with pseudovirions the viral infectivity can be quantified by measuring the luciferase activity with luciferase assay. UD compounds in concentrations 2.5-5-10-20-40 microgram/ml were added prior and at the time of infection of cells with HIV pseudovirions (moi: 2). Low and high entry fitness were observed with glycoproteins from viruses of the same genetic clade, R5- and X4-tropic strains, and in different target cell lines. UD31 showed the most prominent antivral effect in the concentration of 5 microgram/ml. As UDs inhibits the glyceraldehydes-3-phosphate dehydrogenase (GAPDH), results suggest that this thiolated nucleotide may interfere with the function of the essential -SH groups of CD4 molecule (the primary receptor of HIV), and may function as an entry inhibitor for HIV. The wide range of entry fitness suggests that the glycoproteins play a significant role in viral replication. This should be taken into consideration to produce an effective HIV vaccine.

INEVESTIGATION OF ROLE OF CHROMATIN ASSOCIATED HMG-BOX PROTEINS IN *ASPERGILLUS NIDULANS*

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HMGB proteins are ubiquitous nuclear proteins, highly conserved across several species including mammals, plants, yeast, *C. elegans*, *D. melanogaster* and *P. falciparum*. Previous studies showed that they regulate and facilitate various DNA-related activities such as transcription, replication, recombination, repair and chromatin assembly. We have identified two HMGB type proteins (AnHMGB-A: AN2885.2 and AnHMGB-B: AN1267.2) in *Aspergillus nidulans* genome by in silico analysis and subjected them to functional analysis. We carried out developmental Northern analysis to find out the transcription activity of these genes during the development. Expressions of HMG-A and B genes show a low basic level in the conidia cultivated for 1 hour and keep this low level of expression during early germination. At the later germination stage the expression increases and reaches a high level at the fifth hour when the germination stage turns to the mycelia stage. The high level of expression is maintained in the later periods. We deleted AnHMGB-A and AnHMGB-B genes from the genome via substitution of the coding sequences with selection marker containing cassettes constructed by the double-joining PCR method. Deleted strains were subjected to growth tests on different carbon sources, nitrogen sources, pH, temperatures, osmotic and oxidative stress compounds. The sexual and asexual reproduction processes were tested, too. We found no obvious phenotype for the deletions. We observed that both of deletions result a delay of germination and additionally AnHMGB-A deleted strain shows atypical germination tubes and altered osmotic tolerance when they are kept on media with pH2. Therefore, we carried out quantitative Real Time PCR experiments to study the expression level of genes involved in cell wall synthesis and osmotic tolerance. On the basis of the results we propose that elevated level of trehalose synthesis might be the reason of the experienced phenotype of AnHMGB-A. We generated a ΔAnHMGB-A/ΔAnHMGB-B double mutant strain and tested them similarly to those of single mutants. Double mutant dies at 42 C indicating that both HMG-box proteins have role in the maintenance of normal cell functioning. Transcriptome analysis of wild type versus double deleted strain is under progress after growing of mycelia at 37 C for 16 hours and 37 C for 15.5 hours then shifted to 42 C for 30 minutes. We expect that transcriptome analysis will give us new insights into the role and importance of these chromatin-associated HMG-box proteins.

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STUDIES OF THE MICROBIAL COMMUNITIES OF THE WATER PURIFICATION SYSTEM IN A HUNGARIAN POWER PLANT

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Ultra pure water used in the industrial environment examined during this work is very oligotrophic with low nutrient content so it represents an extreme environment for most of the microorganisms. In such habitats a common way to survive is to constitute special communities like biofilms. In the

present work the microbial communities of the water purification system of a Hungarian power plant was examined. The aim of the work was to determine the „critical points" which are extremely contaminated by microbes in the water purification system. The examined parts were: the crude water tank, lime softening and gravel filtration system, the scavenger, cation- and anion-exchange and the mixed bed ion-exchange resins. In the first step samples were taken from three points of the plant: anion- and mixed bed ion-exchange resins and the crude water tank. The cell numbers (DAPI, epifluorescent microscopy-Nikon80i) were determined and a comparative analysis of microbial communities was made with DGGE method. Later, based on these previous results the following sampling strategy was applied: The cation- and anion-exchange resins were sampled in every 4 hours during one cycle, (takes 16 hours). From 4 points (crude water tank, lime softening, gravel filtration system, scavenger resins) samples were taken in every day for a week and 2 more points (the mixed bed ion-exchange resins and its washing water) were sampled in every week for a month. CFU was counted (on medium R2A) in case of three samples (crude water tank, lime softening and bed filtration system) and viable cell counts were determined by epifluorescent microscopy (DAPI, Nikon80i) in each samples. Comparative analysis was made by DGGE in case of 25 samples. Nevertheless the structure of biofilms evolved on the surface of each ion-exchange resins were studied by Scanning Electron Microscopy. Based on our results serious microbial contamination could be observed on 2 points of the water purification system: gravel filtration system, mixed bed ion-exchange resin. Contrarily, in case of other parts of the water purification system, like lime softening and anion-exchange resin, the plate counts and cell numbers showed remarkable lower values. It can be caused by the high pH values measured in these parts of the system. Based on the results of the DGGE it is evident that the same sampling points grouped together and characteristically differed from other points of the system. It shows that the different parts of the water purification system has own, unique microbial communities. It was confirmed even by SEM analysis in case of ion-exchange resins: on the surface of the scavenger resin many organic contamination could be observed. The cation- and anion-exchange resins seemed to be less polluted at the same time the mixed bed ion-exchange resin was the most covered by microbes. In addition all resin types were more or less broken.

PREVALENCE AND SEASONALITY OF HUMAN VIRUSES IN HUNGARIAN RIVERS

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Human pathogen - mainly enteric - viruses in surface waters may cause human infection in case of contact, i.e. bathing or consuming inefficiently treated drinking water. Infection by a few virus particles can already cause illness, especially viral gastroenteritis. Some human enteric viruses (e.g. Noroviruses) have a distinct seasonal epidemic pattern (more cases in winter months). As the surface waters are usually contaminated by the sewage of the infected population, seasonality is expected to be reflected in the prevalence of the viruses in the water. The concentration of viruses is lower in aquatic environments, than in clinical samples, therefore it is essential to concentrate large volume (up to several 10 L) of water. Traditionally the first step is a negative or a positive filtration, followed by eluation and flocculation. In this study, two types of negatively charged adsorbents were used: glass-wool and a mixed cellulose-ester membrane with glass-wool prefilter. From 10 sampling sites 16 surface water samples (2 x 10 L) were filtered using compressed air. The adsorbed virus particles were eluted with alkaline beef extract. The nucleic acid was extracted from the flocculated

concentrated organic matter. The human adenoviruses and noroviruses were detected with taxon-specific polymerase chain reactions. The viral nucleic acid from the PCR positive samples was identified and typed with sequence analyses. In parallel with the human pathogen virus detection, colony counts of the fecal indicator bacteria and the somatic coliphage titer were also determined. Three sites along the Danube river in Budapest were sampled repeatedly (autumn, winter, spring) to assess the spatial and seasonal variability of the viral prevalence. The presence of adenoviruses and/or noroviruses was confirmed in six Hungarian rivers. With glass-wool filtration, 12/16 samples were positive for adenoviruses and 9 for noroviruses. The concentration with membrane filtration was less efficient (9 and 7 positive samples for adeno- and norovirus, respectively). The difference in efficiency between the applied adsorbents is probably due to the larger active surface of the glass-wool. The sequence analyses showed that the samples contained human enteric adenoviruses (Ad40, Ad41). The detected noroviruses mainly belonged to the genogroup II. The three Danube sampling sites were located upstream, near downstream and further downstream from the main sewage inflow. Almost all samples were positive for both adenovirus and norovirus, thus spatial differences could not be discerned. The concentration of the microbial indicators reflected the expected flux due to the sewage contamination. The virus concentration method is sufficiently sensitive to detect viruses even in the less contaminated samples; quantitative analysis is necessary to assess the spatial heterogeneity. The observed seasonal changes were also slight due to the high ratio of positives. Spring samples were the most contaminated (6/6 positive samples for both viruses). This is potentially due to the frequent rainfalls and the high water level, which may facilitate the spread of the viruses. The recovery of the viruses was lowest in the winter. The future aim of the study is the quantitative determination of the human pathogen viruses to get a more detailed picture on their spatial and temporal heterogeneity.

PROTEOMIC INVESTIGATION OF BETA-LACTAMASES IN HUMAN AND BOVINE ISOLATES OF BORDERLINE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS

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Staphylococcus aureus is one of the most widespread pathogenic bacteria and because of the frequent occurrence of multiresistant strains it is among the most dangerous ones causing nosocomial infection. Borderline methicillin resistant *S. aureus* (BORSA) strains have MIC values for methicillin between the values of susceptible and resistant strains; the main reason of their resistance is the production of beta-lactamases. Methicillin and oxacillin hydrolyzing enzymes of 7 borderline methicillin-resistant *S. aureus* strains isolated from human clinical samples and 4 other borderline methicillin-resistant *S. aureus* strains isolated from bovine mastitis cases were investigated.

Previous studies revealed that those strains produced „A" type inducible beta-lactamases that were able to degrade methicillin and of which membrane-bound forms were also present in the cells. We isolated membrane fractions and prepared concentrated supernatants from all strains, and applied the unsalted samples to pH 3-10 strips for isoelectric focusing (IEF) and then to SDS-PAGE for a second dimension. After regenerating the enzymes and applying nitrocefin, a chromogenic beta-lactam substance beta-lactamases were localized on the 2-D gels; corresponding spots from parallel gels were cut, digested and analyzed by mass spectrometry. The analysis showed that BlaZ was

responsible for the hydrolysis of both methicillin and oxacillin. All supernatant and membrane fractions contained the same enzyme with slight sequence variations. The size and pI of the proteins were also variable probably due to spontaneous hydrolysis and/or post-translational modifications. We found cytotoxins and virulence factors in some nitrocefin hydrolyzing dots, suggesting that those proteins might have a role in the reduction of local antibiotic concentration.

BARTONELLA ENDOCARDITIS: SEROLOGICAL CROSS-REACTION WITH *COXIELLA BURNETII*

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Infective endocarditis (IE) is a serious, life threatening disease. The condition is traditionally diagnosed microbiologically, based on blood culture positivity, but in about 2-30% of all cases routine blood culture is negative. Both *Bartonella* and *Coxiella* are known causative agents of blood culture negative endocarditis. Because the organisms are difficult to culture, the diagnosis usually depends on serology. A titer of ³1:800 for IgG antibodies to either *Bartonella henselae* or *B. quintana* is required for diagnosis of endocarditis due to *Bartonella* sp. An immunoglobulin (IgG) titer of ³1:1600 supports the diagnosis of *Coxiella endocarditis*. However previous studies have showed that in serological tests cross-reactivity might occur. While the clinical presentation may be similar, distinguishing between these organisms is of utmost importance, because they need different management. To overcome these limitations, alternative diagnostic approaches are needed, like nucleic acid detection by PCR technology. Here we present a case of a 39 years old homeless male patient with persistent fever, and with a history of alcohol abuse. He was admitted to the „Budai Irgalmasrendi" hospital because of FUO. Standard aerobic and anaerobic cultures showed no growth. Transesophageal echocardiography demonstrated the presence of aortic valve vegetation, consistent with the diagnosis of bacterial endocarditis. Based on the positivity of the microimmunfluorescence test, a presumptive diagnosis of Coxiella endocarditis was made, and an antibiotic treatment with Doxycycline was started. Based on the positivity of *Bartonella* MIF test, as a further possible diagnosis *Bartonella endocarditis* has emerged, and as a further medication Ceftriaxone was given. Mycoplasma pneumoniae serology (ELISA) also gave positive result. Because of the size of the vegetation and the risk of embolisation, cardiac surgery and valve replacement was indicated. During the operation, the condition of the patient deteriorated, and he died.

The vegetation was resected, and processed in the laboratory. PCR was performed for *Mycoplasma pneumoniae*, *Coxiella burnetii* and *Bartonella*. Only the Bartonella PCR resulted in a positive signal, based on the amplification of a 414-bp fragment of htrA gene, encoding a 60-kDa heat shock-like protein. In a second reaction we used a primer set designed to amplify a fragment of the 16S-23S rRNA intergenic region. The serological cross-reaction between Bartonella and Coxiella have been described. The case demonstrates the difficulties in distinguishing between Coxiella or Bartonella endocarditis by using serology alone. In the presence of predisposing conditions, such as homelessness, alcoholism and exposure to ectoparasites, one should consider the possibility of Bartonella infection. We demonstrate the utility of culture-independent polymerase chain reaction (PCR) in the diagnosis of IE.

THE LINK BETWEEN SURVIVAL AND INFECTIVITY OF *CAMPYLOBACTER JEJUNI* CELLS UNDER CHANGING ENVIRONMENTAL CONDITIONS

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Bacteria are exposed to different stress conditions in the environment, or during food production as well as in the host. Temperature changes, availability of nutrients or the presence of toxic substances and antimicrobials are some of them. Despite the lack of many stress response mechanisms that other food-borne bacteria possess, campylobacters display a distinct survival potential in the extraintestinal environment and consequently also high incidence as a cause of human infections. This is at least partially the consequence of microbial adaptation to environmental stresses [1,2]. Consequently, discovering the link between survival and infectivity is vital for risk assessment purposes and for establishing the dynamics of potential food-borne infection. Since there are not many publications in this field we investigated *C. jejuni* following heat shock, starvation and oxidative stress. The environmental impact on physiology, survival and virulence properties were studied by culturability, viability as well as the ability of adhesion, invasion and intracellular survival within in vitro cell culture model using Caco-2 intestinal cells and murine macrophages J774 [3]. With additional in vivo experiments in the murine model we investigated the influence of *Campylobacter* stress response on its virulence potential. At different time points after infection bacterial spreading and tissue invasion of infected BALB/c mice was determined [2,4].

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A GENOMIC ENCYCLOPEDIA FOR BACTERIA AND ARCHAEA

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A recent discussion on reconciling microbial systematics and genomics at an American Academy of Microbiology workshop, resulted in the recommendation to coordinate an international effort to construct genome sequences of each of the roughly 9000 known type strains of the Bacteria and Archaea. Following this recommendation the Joint Genome Institute (JGI, USA) and the German Collection of Microorganisms and Cell Cultures (DSMZ) entered a large scale collaboration to generate a Genomic Encyclopedia for Bacteria and Archaea (GEBA), with the aim to systematically fill the gaps in the genomic tree of life by sequencing along the archaeal and bacterial branches of the tree. Though the wide variety of microbial sequencing projects undertaken throughout the world has created a rich, diverse collection of microbial genomes, strong biases in what has been sequenced thus far are evident. The here described project represents the first systematic attempt to use the tree of life itself as a guide to sequencing target selection. This phylogenomic approach will be of great value for: (I) improved identification of protein families, which will improve annotation of other microbial genomes; (II) improved phylogenetic anchoring of metagenomic data; (III) improved gene discovery; (IV) a better understanding of the processes underlying the evolutionary diversification of

microbes; (V) a better understanding of the evolutionary history of microbial species; and (VI) improved correlations of phenotype and genotype in microbes. To test the feasibility of the GEBA approach we have meanwhile started about 250 genome sequencing projects, with over 50 of them already finished, and the first set of analysed genomes recently published in Standards in Genomics Sciences (SIGS), a novel Open Access Journal that will host the publication of the Genomic Encyclopedia in a dedicated monthly section to make it available to the scientific community.

STUDIES ON PLANKTONIC BACTERIAL COMMUNITIES OF PONDS LOCATED ON DIFFERENT EPIKARSTIC SYSTEMS OF HUNGARY

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Karsts are especially sensitive areas and can be characterized by different aboveground and underground processes. The damage and pollution of karsts take place through the karst's epikarstic systems that have connection with the surface. It gives a special significance to the cognition of natural processes taking place in epikarstic systems and the analysis of changes due to human impacts. Two Hungarian sample areas that are different in many ways had been chosen to study. Both locations have many karstic pits with thick soil and deposit filling, at some places with periodical water cover. Surface water are extremely sensitive to external impacts, therefore the aim of our research was the comparative physico-chemical and microbiological analysis of the water of karstic ponds. The aim of the microbial investigation was to gain information about the presence, distribution and diversity of bacterial communities by using cultivation-based and cultivation independent molecular methods. Water samples were taken from the small lakes of Derenk, Aggtelek and Vörös-tó (Aggtelek National Park) as well as Alsó-Csertó and Pokol-tó (Bakony) in spring 2009. Serially diluted samples were plated onto 0.1% nutrient agar to determine the numbers of CFU. Total cell counts were detected by epifluorescent microscopy. MPN method was applied to determine the amount of coliforms. Following membrane filtration DNA was isolated from each water samples, as well. The diversity of planktonic bacterial communities was investigated by DGGE. According to the results of CFU numbers and the analysis of the DGGE profiles, the three ponds of Aggtelek NP proved to be the most similar to each other. The ponds found in Bakony were characterized with higher numbers of CFU-s and coliforms. The highest diversity in the water samples of Aggtelek and Vörös-tó was detected on the basis of the DGGE patterns. The dominant bands excised from the gels were subsequently sequenced. Due to the short sequences obtained only the approximate phylogenetic affiliation was processed. The sequences of the bands showed the highest 87-95% similarities to hitherto uncultured environmental clones of phyla Proteobacteria and Bacteroidetes.

STUDY OF ROOT COLONIZING DARK SEPTATE ENDOPHYTIC FUNGI OF INVASIVE AND INDIGENOUS PLANTS OF SEMIARID SANDY AREAS

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The majority of the terrestrial vascular plants forms mutualistic symbiosis with different fungi. Besides the well-known mycorrhizae the root colonization by endophytic fungi is also an important

type of the plant-fungus interactions. The so-called „dark septate endophytes" (DSE) is a form-group of root endophytic fungi. Important characteristics of DSE fungi are the darkly pigmented intraradical hyphae and the intracellular microsclerotia. The aims of our study were (i) to gain data on structural diversity of DSE fungi, and (ii) to test if there are DSE fungi specific to invasive or indigenous plants of semiarid sandy areas on the Great Hungarian Plain. Samples were collected from three sandy areas near Bugac, Fülöpháza and Tatárszentgyörgy. Endophytic fungi from the roots of invasive (*Ambrosia artemisiifolia, Asclepias syriaca, Prunus serotina, Ailanthus altissima*), and indigenous plant species (*Juniperus communis, Helianthemum ovatum, Fumana procumbens, Medicago minima*) were isolated and kept on MMN medium. After DNA extraction, the ITS region of nrDNA was amplified, sequenced and compared with sequences deposited in public databases. In vitro tests with *Allium porum* plants were established to test whether a strain was endophytic and formed microsclerotia. Root colonization, both in vitro and natural samples, was studied by light microscopy. More than 300 strains were isolated from more than 100 root samples. The ITS of approximately 200 strains were sequenced. This presentation shows the results and the comparison of the DSE fungal communities of different plants and areas.

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IN VITRO ANTICANDIDAL ACTIVITY OF THERMAL WATER FROM MAKÓ ON VARIOUS *CANDIDA* SPECIES

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Candida infections are widespread throughout the world. Although *C. albicans* is the most frequent causative agent of human infections attributable this genus, the number of infections caused by non-albicans strains has increased during the past decade. The purpose of this study was to examine the antifungal activity of thermal spring water from the Makó pleasure resort on 12 pathogenic *Candida* species. The spa has two separate wells established in 1955 and 2007. The wells differ from each other in deepness and chemical properties. Water from the both sources have been tested by standard methods for microbial contamination, and found to be devoid of microorganisms that can be harmful to humans. *Candida* strains were incubated in the thermal water and colony forming units were determined after different incubation periods. Survival rate in physiological salt solution was used as control. Out of the 12 *Candida* species examined, 7 were found to be sensitive to the thermal water. The antifungal effect of the two thermal spring waters were identical, only some minor differences have been revealed. Based on these results, the water from these thermal wells in Makó may influence cutaneous infections caused by certain *Candida* species in a beneficial way.

EXAMINATION OF THE DIVERSITY OF INDOOR MOULDS IN A STUDENT HOSTEL

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Moulds are widely distributed in indoor and outdoor environments. They are common in household dust and can cause allergic symptoms or invasive infections in humans with weak immune system.

They can also be harmful through their toxin producing abilities. Our aim was to examine the diversity of indoor moulds in a student hostel. Isolation of the samples were carried out by using dichloran glycerol (DG18) media. After purification, the isolates were identified by ITS sequence analysis and morphological traits. Samples were collected from different places of the kitchen, the bathroom and the living room during the summer (in August), and during late autumn (in November). The most frequently identified genera were *Aspergillus, Alternaria, Cladosporium, Penicillium* and *Aureobasidium*. A high diversity was observed at the species level based both on the sampling location and the season. In general, more species were recovered during the summer period than during November. A similar trend was observed when the number of *Aspergillus* species were compared: more species were recovered during summer than in November. High numbers of *Eurotium* and *Aureobasidium* isolates were detectable in the summer sample set, whereas the samples collected in November did not contain any of these species. On the contrary, the frequency of Alternaria, Cladosporium and Penicillium isolates did not differ significantly between the two sampling periods. Further studies are in progress to examine the diversity of indoor moulds in other seasons and in other locations.

A PCR BASED APPROACH FOR IDENTIFYING *MICROSPORUM CANIS* AND *TRICHOPHYTON TONSURANS*

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Dermatophytes can cause skin, hair and nail infections due to their ability of utilizing keratin. The identification of these fungi can be difficult because of the high morphological and physiological similarities. Our aim was to develop a PCR-based species specific technique for the identification of two important pathogens *M. canis* and *T. tonsurans*, responsible for dermatophytosis with various clinical appearances. Reactions were designed to use a common reverse primer and two specific forward primers and were optimized to be efficient under the same PCR conditions allowing the multiplex detection of these two fungi in a single reaction volume. The specific reactions were tested using both collection strains of other dermatophytes and clinical samples. In connection with these methods, four DNA extraction techniques were also tested to assist for an efficient detection.

PCR-BASED IDENTIFICATION OF *FUSARIUM* SPECIES OF WHEAT GRAIN ORIGIN

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Fusarium head blight (FHB) is a disease complex of cereals in which several fungal species may cause symptoms. The species, found as the major cause of head blight of wheat are *F. graminearum* and *F. culmorum*. Less frequently isolated species are *F. acuminatum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides*. FHB can significantly reduce grain yield and quality. *Fusarium* species are known as mycotoxin producers. The majority of mycotoxins produced by Fusaria are the trichothecenes, which can be formed in preharvest infected plants still standing in the fields, or in stored grain, as well. Mycotoxins in feed and food may cause chronic or acute mycotoxicoses in livestock and in humans. Species identification of mycotoxin-producing *Fusarium* species is of high importance in

relation to FHB. The polymerase chain reaction (PCR) is a handy technique for the identification and differentiation of *Fusarium* species. Species-specific primers have been used for PCR detection or identification of several *Fusarium* species. The aim of this study was to apply species-specific PCR-based assay for the identification of *Fusarium* species from Hungarian wheat grains. In addition to the species identification we performed PCR reactions to reveal the presence/absence of genes responsible for the production of several toxins (DON, 3-ADON, 15-ADON, NIV, ENs) in the Fusarium isolates. PCR identification of *F. graminearum* isolates is already described, but species-specific PCR primers for the identification of other *Fusarium* spp. from wheat grain of Hungarian origin has not been reported yet. After processing 30 wheat samples of different geographical origin we found 59 Fusarium isolates. Identification with species-specific PCR primers resulted the following distribution of species: *F. acuminatum* 5 %, *F. avenaceum* 30.5 %, *F. graminearum* 37.5 %, *F. poae* 22 %, *F. sporotrichioides* 5 %. The results were confirmed by morphological identification after culturing the isolates in Petri-dishes.

MODELLING MOULD GROWTH

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Predictive microbiology has included the development of models capable of describing the growth of pathogenic and spoilage bacteria. Predictive microbiology also can be employed to predict food product shelf-life. Sigmoidal equations, such as logistic, Baranyi and modified Gompertz models, have been widely applied to describe the growth of bacteria. However, predictive modelling of filamentous fungal growth has not received the same attention. Two of the most important environmental parameters that determine the ability of moulds to grow on foods are water activity and temperature. Our aim was to use bacterial growth models to describe mould growth. The growth of two mould species (*Penicillium vermiculatum*, *Aspergillus niger*) were investigated on solid medium (MEA), incubated at ambient and 35°C temperature in a humidity chamber (a_w=0,99). The colony diameter was measured in every twelve hour. Baranyi's bi-phase bacterial growth model was used to describe the mould growth, and this way the lag phase, the maximum growth rate (μ_m) and the maximum colony diameter (D_{max}) were calculated. The sigmoid curves fitted well with the Baranyi's model. The maximum growth rate of *A. niger* was two times higher, the maximum growth rates of *P. vermiculatum* and that of the mixed culture were three times higher at 35°C than at room temperature. At room temperature the lag phase for *A. niger* could not be determined, however, for the mixed culture incubated at 35°C it was possible. Further experiments are in progress to determine the lag phase and the maximum growth rate for the moulds, as a function of temperature and water activity.

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IN VITRO ACTIVITY OF DIFFERENT STATINS ON THE PERMEABILITY OF YEAST PLASMA MEMBRANE

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Statins originally identified as fungal metabolites (lovastatin, simvastatin, pravastatin) but synthetic

compounds are also available (e.g., atorvastatin, fluvastatin, rosuvastatin). They act as selective inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase. This enzyme is responsible for the conversion of the hydroxy-methylglutaryl-coenzyme A into mevalonic acid. The conversion is the rate-limiting step in biosynthesis of isoprenoid compounds e.a. sterols, therefore statins are frequently used for reduction of the cholesterol level in human cells.
In this study the in vitro inhibitory activities of different statins on *Saccharomyces cerevisiae* were investigated. Lovastatin, simvastatin, pravastatin and rosuvastatin had no effect on *S. cerevisiae* in the applied concentrations but atorvastatin and fluvastatin inhibited the growth. The synergistic effect of atorvastatin and fluvastatin was demonstrated as they lowered the MIC applying together. As statins inhibit the ergosterol biosynthesis in fungal cells the permeability of the membrane increases. The increased membrane permeability was checked by staining the cells with Calcein-AM and detecting the fluorescence by flow cytometry. Statin-treated cells showed increased fluorescence in each case. These results suggest that even if they do not have therapeutic application, statins can be useful compounds to increase the susceptibility of the cells to other antifungal drugs.

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SCREENING OF *DEBARYOMYCES OCCIDENTALIS* STRAINS FOR AMYLOLYTIC ACTIVITY AND ETHANOL PRODUCTION

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Starch containing raw materials or agricultural by-products are important sources of bioethanol production. Fermentation processes apply different strains of *Saccharomyces cerevisiae* and closely related species, which are considered as far the most ethanol tolerant yeasts. However, because *Saccharomyces* strains are not able to utilize raw starch as a carbon-source its hydrolysis by different amylolytic enzymes prior to the ethanolic fermentation is required. Several yeast species are known that harbour very efficient starch hydrolysing enzyme systems and some of them are being fermentative. Application of these species for direct fermentation of starch to ethanol is hampered by their week ethanol tolerance. We performed a screening process when different strains of the starch fermenting yeast *Debaryomyces occidentalis* were tested for the propagation ability on raw starch, production of gluco- and alpha-amylases as well as their ethanol production and tolerance. According to our results all the screened strains were able to propagate on starch as the sole carbon-source under both aerated (aerobic) and static (semi-anaerobic) conditions but considerable differences were found among the strains in their amylolytic enzyme activities, ethanol production and tolerance. Based on the results of the screening process two strains of the best performance activities were selected for determination of the optimal conditions of starch-based ethanol production.

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IDENTIFICATION AND PARTIAL CHARACTERISATION OF A GENE CODING FOR AN ANTIMICROBIAL PEPTIDE IN *NEOSARTORYA FISCHERI*

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The number of microbial infections has increased over the past years. Infections caused by fungi are especially problematic, because the most widely applied antifungal drugs are quite toxic and have serious side effects. Therefore, there is a substantial demand for new compounds with extensive antimicrobial activity. The proteins with similar structure like defensins secreted by some filamentous fungi are interesting from this respect: they have effective inhibitory potential against bacteria and fungi. The features of these proteins are a low molecular mass (5.1-6.6 kDa), a basic character, and the presence of 6-8 cysteine residues and several disulfide bonds. *In silico* investigation of genomic databases has been revealed a putative defensin-like protein in *Neosartorya fischeri*. Started from this data, experiments have been carried for the molecular characterization of *N. fischeri* antimicrobial protein (NFAP) and for the investigation of its antimicrobial activity.

A selected *N. fischeri* strain has been screened via PCR experiment to identify and isolate the antimicrobial protein NFAP encoding gene, as well as its promoter and terminal region. The specifically amplified DNA revealed high homology with the nucleic acid sequence of the *in silico* determined gene. Several putative regulatory elements that might be involved in the transcriptional regulation of *nfap* in response to environmental signals and stress were observed in the promoter region. The production of NFAP has been optimized. The biological activity of the partially purified protein on the growth of bacterial and fungal isolates was investigated with an agar diffusion technique and a broth microdilution method. Inhibitory potential of the partially purified NFAP was exerted against some bacteria and fungi. Analysis revealed the presence of a small protein (approximately 6.6 kDa) in the partially purified ferment broth. Further *in silico* examination revealed the physical and chemical properties of the mature NFAP: it is small molecular mass (6625.5 Da), basic (pI=8.93) protein consisting of 57 amino acids, which contains two domains and is stabilized by three disulfide bridges. The tertiary and quaternary structure of the protein (based on the presumed 3D model of NFAP) is very similar the defensin-like molecules: 2 α-helixes connected by one β-sheet. NFAP is a new, small antifungal protein. Further experiments are in progress to clarify the antifungal spectrum of the purified protein, and its mode of action.

AN IMAGE ANALYSIS-BASED METHOD FOR THE EVALUATION OF THE AGGRESSIVITY OF *TRICHODERMA* STRAINS TOWARDS *PLEUROTUS OSTREATUS*

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The significance of *Trichoderma* species in mushroom production is that some of them are capable of causing the so-called green mould disease, which results in serious crop losses world wide. Besides the green mould disease of Agaricus bisporus caused by *T. aggressivum*, serious economic losses due to Trichoderma were reported also in the cultivation of Pleurotus ostreatus, commonly known as the

oyster mushroom. The *Trichoderma* species responsible for the disease on *P. ostreatus* proved to be different from *T. aggressivum*, recently they were described as the new species *T. pleurotum* and *T. pleuroticola*. In vitro assays on agar plates are the generally used methods for the evaluation of antagonistic abilities. The aim of the present study was to develop an accurate, image analysis-based method for the evaluation of the aggressivity of *Trichoderma* species towards Pleurotus ostreatus. *Trichoderma* isolates belonging to the species *T. aggressivum f. aggressivum*, *T. aggressivum f. europaeum*, *T. pleurotum*, *T. pleuroticola* and *T. harzianum* - all of them belonging to the Lixii/Catoptron clade of the genus - were tested against *Pleurotus ostreatus*. In the currently developed method, the areas of the fungal colonies were calculated on Petri dishes by measuring the occupied surface of the medium on digital images. The inhibition effect was recorded as the value of aggressivity index (AI), which was calculated from the ratio of the area of the Trichoderma colony and the total area occupied by the colonies of Trichoderma and the oyster mushroom. The proposed method was tested for numerous parameters, and the results revealed that AI proves to be capable for the accurate measurement and scale of the aggressivity of *Trichoderma* strains towards *P. ostreatus*.

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ADAPTATION OF BACTERIAL BIOTESTS FOR MYCOTOXIN MONITORING

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Mycotoxins are very complex micropollutants that can be found in every variety of grain and forage produced for food or feed. These substances are secondary fungal metabolites that have mutagenic, carcinogenic, teratogenic, immunomodulant and cytotoxic effects, thus biomonitoring of mycotoxins have an increasing importance nowadays. Our aim is to monitor mycotoxins by bacterial biotests. Besides expensive chemical analytical methods, these biotests can be alternative screening methods for selecting mycotoxin degrading microbes (eg. screening transposon mutagenesis libraries containing thousands of clones), as they provide prompt information, they are reliable and cost effective methods. Five mycotoxins, Aflatoxin B1 (AFB1), Zearalenon, T2-toxin, Deoxinivalenol (DON) and Ochratoxin were analysed by two biotests. Effects of these toxins were analysed by detecting the luminescence intensity of *Aliivibrio fischeri* and genotoxicity was analysed by SOS-Chromotest using *Escherichia coli*. The *A. fischeri* bioluminescence assay is one of the most sensitive bacterial toxicity assays across a wide spectrum of toxicants. However, effects of mycotoxins have been hardly examined. We carried out our examination in liquid cultures of *A. fischeri* with 2–20 μg/ml toxin concentration and inhibition was determined after 3.5, 10, 15, 25 hour contact time. We found that *A. fischeri* shows great sensitivity at 20 μg/ml toxin concentration in case of all five toxins. Mycotoxins at 10 μg/ml concentration had diverse effects for the test microbe. According to our results the most toxic substance was AFB1, as significant inhibition was detected at every contact time. In case of Zearalenon, Ochratoxin, T2-toxin and DON we detected decreased inhibition after 10h incubation. Moreover, DON increased significantly the luminescence after 15h.
Genotoxic effect of mycotoxins was analysed by *E. coli* PQ37 tester strain. The principle of the assay is the SOS response that is induced by DNA damaging agents. The test took advantage of an operon fusion placing structural gene of β-galactosidase under the control of *sfiA* gene responsible (with 17 other genes) for SOS error-prone. Thus, β-galactosidase activity highly depends on *sfiA* expression.

We have examined AFB1 0,078–10 μg/ml concentration by two-fold serial dilutions. We found that genotoxic effect of AFB1 is still detectable at 0,078 μg/ml concentration.
We have adopted two bacterial biotests to examine mycotoxins. Sensitivity of these tests is above the mycotoxin treshold limit of food and feed, thus these methods are not suitable for direct examination of toxins, but can be appropriate for screening mycotoxin degrading microbes. We could successfully use SOS-Chromotest to select microorganisms that has the best AFB1 degrading potential and can degrade AFB1 without genotoxic by-products. Our results highly correlated with data of parallel ELISA tests made by Soft Flow Biotechnology Ltd.

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EFFECT OF FRUIT EXTRACTS ON FOOD BORNE PATHOGENS

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Bacteria of the genus *Campylobacter* and *Salmonella* are important human pathogens causing foodborne infections worldwide. In spite of the use of traditional and modern methods in food safety techniques as many as 30% of the people in industrialized countries suffer from a food borne disease each year. Therefore, the need for new methods of reducing or eliminating food born pathogens remains. Raw and processed fruits, as well as waste products remaining after processing) are good sources of compounds with antimicrobial activity (e.g. peptides, lectins, phenolic compounds, terpenoids, essential oils). The antibacterial effect of fruit juices and pomace extracts from 13 wild and cultivated fruits (*Prunus avium*, *Prunus avium* var. *Gold*, *P. cerasus*, *P. armeniaca*, *Crataegus monogyna*, *Morus alba*, *M. nigra*, *Ribes nigrum*, *R. rubrum*, *R. uva-crispa*, *R.* x *nidigrolaria*, *Rubus idaeus* and *R. fruticosus*) against two foodborne enteric pathogens (*Salmonella* ser. Typhimurium, *Campylobacter jejuni*) was evaluated by broth microdilution assays. Juices and/or extracts of *P. cerasus*, *P. armeniaca*, *R. idaeus*, *R. nigrum*, *R. rubrum*, *R. uva-crispa* and *R.* x *nidigrolaria* efficiently inhibited the growth of both investigated bacteria (growth ≤ 25%). Juices and extracts from *P. avium* (red and yellow cultivars), *C. monogyna*, *R. fruticosus* and pomace extracts from *M. alba* and *M. nigra* had a similar strong inhibitory effect on the growth of *C. jejuni*, but had weak or no effect on *S.* ser. Typhimurium. *P. cerasus*, *R.* x *nidigrolaria* and *Rubus idaeus* pomace extracts revealed a substantial antibacterial effect at both acidic and neutral pH. The observed inhibitory potential of the investigated fruit juices and pomace extracts on bacterial growth may be utilized in the development of functional foods and natural food preservatives. The by-products of juice industry may represent an economically interesting source for the extraction of active compounds.

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EFFECT OF SOLID STATE FERMENTATION ON THE ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF SOUR CHERRY POMACE

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The utilization of food industry byproducts (pomace, canning waste, whey) in the production of health protective food additives has been investigated more and more intensively. The amount of extractable useful components (antioxidants etc.) can be enhanced by solid phase fermentation. In our experiments, *Rhizomucor miehei* was grown on liofilized sour cherry pomace, and the time course effect of solid state fermentation on the antioxidant and antimicrobial properties of pomace extracts was investigated. In contrast to the literature data on cranberry and pineapple pomace, our results showed decreasing amount of soluble phenolics during the fermentation followed by the decrease in the antioxidant capacity of pomace extracts. There was no antifungal activity against the investigated molds and yeasts: *Aspergillus nidulans, Penicillium frequentans, Saccharomyces cerevisiae* and *Hansenula anomala.* Pomace extracts also showed no antibacterial activity against *E. coli* and *Lactobacillus casei.* Untreated pomace and pomace on the second day of fermentation showed growth inhibition on *Bacillus subtilis, B. cereus* and *Serratia marcescens.*

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EFFECT OF SPICES ON THE GERM COUNT OF GROUND PORK

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Herbs and spices can act *in vitro* as good antimicrobial agents due to active ingredients such as organo-sulphur compounds in onion and garlic; or essential oils like carvacrol in oregano or thymol in thyme. The complex matrix of foods can protect microbes from the growth inhibitory effect of spices. In our experiments fresh garlic (*Allium sativum),* onion (*Allium cepa)*, thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare)* were mixed alone or in combination, with and without 1% salt, in 1 % concentration to ground, refrigerated pork. After 24, 48 and 72 h, total and coliform cfu (colony forming unit) was evaluated by the Hoskins method determining MPN (most probably number). The antibacterial effect of spice aqueous extracts against *E. coli* was investigated by the agar diffusion method. In general, aqueous extract of fresh spices had strong inhibitory effect on the growth of *E. coli* in culture but showed weak or no inhibition on the total and coli-form cell count of ground pork. Combination of spices did not exert any influence on the antimicrobial activity of them.

CHARACTERISATION OF MULTIRESISTANT BACTERIAL STRAINS IN THIS YEAR

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The aim of this study was to determine the prevalence of multidrug-resistance of bacteria isolated from in-patients and out-patients in our laboratory during a one-year prospective study. The collected strains were identified by VITEK (bioMérieux). Antimicrobial susceptibility testing was performed by both diffusion and dilution and interpreted according to the latest CLSI documents. MRSA isolates were screened for reduced susceptibility to glycopeptides on Mueller-Hinton agar and 5 mg/l

teicoplanin as recommended by CLSI. The macromethod Etest was performed as secondary screening for all isolates which grew on this medium, PAP profile analysis were determined in the case of suspected hVISA/VISA. ESBL production among Enterobacteriaceae was screened phenotipically and confirmed by PCR techniques by accepted methods. The presence of carbapenemase was screened by means of the MBL-Etest, and further investigated by following carbapenem hydrolysis and its inhibition by EDTA. PCR and sequencing were carried out by standard procedures. 17 % among isolated S. aureus strains were oxacillin resistant and 33% of MRSA strains were evaluated with higher or equal than 2 mg/L vancomycin MIC level. The diversity of type of ESBLs produced by a wide range of species among Enterobacteriaceae has been proved in our institution, the emergence of CTX-M group beta-lactamases and the rising proportion of ESBL-producing *E.coli* strains. 6,5% of all the member of the Enterobacteriaceae family isolates, were ESBL positive (*E. coli* 47%, *Enterobacter* spp. 25%, *Klebsiella* spp. 16%, *Proteus* spp. 7%, *Citrobacter* spp. 4% and *Serratia* spp. 1% of them), respectively). 3 imipenem resistant and 5 ertapenem resistant strains were characterized. Among *P. aeruginosa* isolates17 % was resistant to imipenem and 16% to meropenem. Three non-related carbapenem resistant *Acinetobacter* strains were isolated. The MBL production should be studied with appropriate therapeutic protocols and a regular screening /monitoring system when the strain showed a profile of resistance to cefotaxim, ceftazidime and cefepime but sensitive to aztreonam and imipenem. The changing spectrum in the incidence and epidemiology of microbial pathogens has resulted in an increase in resistance to many antibiotic compounds emphasizing the need to monitor the prevalence of resistance in these strains.

KILLER PROPERTIES OF THE YEAST BIOTA IN A WINERY OF THE VILLÁNY REGION

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Wine production has been traditionally based on spontaneous fermentation, however application of foreign commercial starters has predominated in recent years. To save the specific regional characteristics of wines, the use of indigenous starter cultures would be more suitable. When the starters are killer yeasts they can produce mycocins which are able to counteract the activities of undesired microorganisms during wine fermentation. For such potential killer starters were searched in the Villány region, Hungary. Killer behaviour of the indigenous yeast biota was studied in two kinds of red wines (Oporto and Cabernet Franc) from a winery of Villány. Samples were collected from musts in different states of fermentations. Yeast colonies were isolated and altogether 300 were selected for further studies. All isolates were analysed in bioassay for their killer activity at pH 4.2 against four *Saccharomyces* species belonging to both the sensu stricto and sensu lato groups. K1, K2 and K28 toxin producing *Saccharomyces cerevisiae* strains were used as killer references.

Eighty-four percent of the selected isolates were able to produce killer toxin. Biotyping revealed that the toxins were either of a broad scale (i.e. active against all the testers) or a narrow scale (i.e. active against only one of the testers). The activity was also highly effected by the environmental temperature (20-30°C). According to the above mentioned

Genetic background of the mycocin production was examined in two of each representative strains of the groups by analysis of the total nucleic acids. Two extranuclear RNA bands were found in each cases. The size of both the larger (LRNA) and smaller RNA (MRNA) were determined. RNase protection assay revealed that these dsRNA molecules were covered by proteins, so the killer phenotypes were determined by killer viruses. According to the results of the RNA analysis three

among the seven types were similar to the reference K2 virus, but the others were different.

CHARACTERIZATION OF *BIPOLARIS* ISOLATES USING MOLECULAR AND BIOCHEMICAL MARKERS

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Members of the genus *Bipolaris* (Ascomycota, Euascomycetes, Pleosporales, Pleosporaceae) are dematiaceous, filamentous fungi; their teleomorphic states are placed in the genus *Cochliobolus*. They can be isolated from plant debris and soil, but the best known representatives of the genus are plant pathogens causing diseases in a wide range of gramineaceous hosts. Three closely related species, *B. australiensis*, *B. hawaiiensis* and *B. spicifera*, are frequently isolated from human and animal phaeohyphomycoses. They have been reported to infect both immunocompetent and immunocompromised hosts; the clinical spectrum is diverse and includes invasive sinusitis, keratitis, endophthalmitis, endocarditis, osteomyelitis, meningoencephalitis, peritonitis, otitis media (in agricultural workers), fungemia and cutaneous and pulmonary infections. Moreover, these *Bipolaris* species together with the members of the closely related *Curvularia* genus are the third most frequently reported causative agents of mycotic keratitis. Currently, species separation is principally based on conidial morphology and culturing characteristics but inconsistency and variability among the isolates often make morphological identification difficult.

In our study, about 40 *B. australiensis*, *B. hawaiiensis* and *B. spicifera* isolates were examined including several strains isolated from fungal keratitis. For comparison, further *Bipolaris* and *Curvularia* isolates representing the *Cochliobolus* group I and II were also involved. Molecular phylogenetic comparisons based on the sequences of the ITS region and different protein coding genes have been performed in order to study the possibilities of the species delimitation in this group. Useful markers for species and strain identification could be established.

Very limited data are available on the antifungal susceptibility of *Bipolaris* species. Therefore, sensitivity of the involved isolates to several antifungals, including amphotericin B and different azoles, was also tested. In general, all drugs were effective against the tested isolates, except fluconazole. The tested species proved distinguishable on the basis of their sensitivity to the drugs.

ANTIFUNGAL EFFECT OF OPHIOBOLINS

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Ophiobolin A is a secondary metabolite characteristic to the phytopathogenic fungal genus *Bipolaris* (Ascomycota, Euascomycetes, Pleosporales, Pleosporaceae). These dematiaceous fungi are cosmopolitan in nature associated primarily with grasses, but they can be isolated from plant debris and soil also. Ophiobolins are a group of sesterterpenoids with a common basic structure. All ophiobolin analogs are characterized by a tricyclic 5-8-5 ring system; the skeleton of these substances

is derived from a head to tail linkage of five isoprene units. Various biological actions have been attributed to them, such as phytotoxic, cytotoxic, nematocidal, antimicrobial and antiviral effects. However, there are only a few reports on the antifungal effect of ophiobolins and their activity against Zygomycetes has never been studied. Zygomycetes fungi have practical importance as postharvest pathogens of different agricultural products and some species are also known as opportunistic pathogens of humans and animals. In the present study, the *in vitro* antifungal activity of ophiobolin A was tested against several different fungi known to be opportunistic pathogens of humans and animals. Strains of *Candida albicans*, *Aspergillus fumigatus*, *Trichoderma harzianum* and several Zygomycetes species were involved. The tested Zygomycetes strains represented the genera *Micromucor*, *Mortierella*, *Mucor*, *Rhizomucor*, *Rhizopus* and *Gilbertella*; these fungi generally have a substantial intrinsic resistance to the most widely used antifungal drugs. Susceptibility tests were performed in a 96-well microtiter plate bioassay by measuring the absorbance of the fungal cultures. Ophiobolin A proved to be active against the majority of the tested Zygomycetes strains; it completely blocked the germination of sporangiospores at the concentrations of 3.2-12.5 μg/ml. *Candida*, *Aspergillus* and *Trichoderma* were also found to be highly sensitive to the metabolite. To clarify the mode of the antifungal action of ophiobolin A, the morphology of *M. circinelloides* and *R. niveus* growing on ophiobolin A containing medium was examined with microscopy. Germinating spores formed degenerated cells containing septa; cytoplasm effusions were also observed.

FURTHER DATA ABOUT HUMAN DIROFILARIOSIS IN HUNGARY

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At the Department of Parasitology, National Center for Epidemiology in the period 2001-08 we diagnosed 44 cases of dirofilariosis caused by *Dirofilaria repens* in 23 male and 21 female patients. The mean age of the patients was 55 years. *D. repens* was identified on the basiss of the morphological characteristics and measured microscopic parameters of the intact worm and in the histopathological section. Twenty-three cases had ocular localozation, 21 were subcutaneous and one case was diagnosed in a histopathological section of removed axillary lymph node in patient with lyphoid leukemia. Eosinophilia was found in one case. We used the Knott concentration technique for detection of microfilariae in 26 cases, with 1 positive result! Most of the patients were living in close or general proximity to dogs and/or cats. In their history in terms of dirofilariosis no significant trips abroad had been recorded. Analyses of the territorial distribution of these 44 cases showed that they were localized on the watershed of the Danube and Tisza River, and in one case in close proximity to Lake Balaton. The differential diagnosis from the other filaria species which occur in humans is not required since the patients' histories contain no data that refer to rhat possibility. Based on the available epidemiological data it can be deduces that most of these cases are autochthon infections. In one case we detected microfilariae. Because of this rare finding and because 5 worms were surgically removed from the patient, this case deservs special attention. During the clinical examinations the immune status of the patient seems to be normal. During next year and subsequent clinical examinations Non-Hodgkin lymphoma was diagnosed. Supposedly, the immunodeficiency could be in the background of the presence of multiple *D. repend* and microfilariemia. CONCLUSION: Visiting or living near riverbanks where mosquitoes are abundant appears to be a significant risk factor in contracting the infection. The increasing number of diagnosed cases suggest that direct attention must be paid to this zoonosis, since its incidence may rise with the improvement of clinical diagnosis. Furthermore, the diagnosed immunodeficiency, presence of multiple *D. repens*

and microfilariemia propose the possibility of opportunistic nature of human dirofilariosis.

THE EFFECT OF A ZINC- AND IRON-CHELATES ON THE VIABILITY OF THE FUNGUS *THERMOMYCES LANUGINOSUS* AND THE STABILITY OF THE PRODUCED ENDO-1,4-BETA-XYLANASE

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Xylan-degrading microbial enzymes are important in various industrial processes, such as biobleaching and DGS/DDGS utilization via the production of xylose and xylo-oligosaccharides for bioethanol production. The xylanase enzyme-producing capacity of the Rumino-Zyme ® (produced by Dr. Bata Rt, strain is deposited in the NCAIM(P) 1288 *Thermomyces lanuginosus*, Budapest, Hungary) fungus was studied under experimental SSF (solid state fermentation) conditions. The aim of our experiments was to study the viability of *T. lanuginosus* in SSF and submerse cultures and the stability of endo-1,4-beta-xylanase when added zinc- and iron-glycinate chelates in different concentrations. We found that the combined use of zinc- and iron-glycinate chelates in 25 ppm Zn and 125 ppm Fe concentration has a synergic effect on the growth and enzyme production of the fungus, having detected 1000-1200 FXU activity.In liquid cultures (submerse fermentation) fungal cultures tolerated the presence of metallic ions in a concentration up to 8 times higher than the above mentioned values.Under SSF culturing conditions, at trace element concentrations 10-12 times higher than the estimated beneficial, synergical concentrations, the growth of the fungus *T. lanuginosus* is inhibited and the enzyme activity is blocked, having measured only 300-500 FXU.

INHIBITION OF RISC ACTIVITY BY THE P1 SILENCING SUPPRESSOR OF SWEET POTATO MILD MOTTLE VIRUS CORRELATES WITH ARGONAUTE BINDING THROUGH WG/GW MOTIFS

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The type member of the Ipomovirus genus, Sweet potato mild mottle virus (SPMMV), differs from other viruses within the family Potyviridae by presenting a large P1 serine protease while retaining a typical HC-Pro region. The silencing suppression activity in SPMMV is mainly associated with the first product, and mechanistically the P1 suppressor seems to exert its function by inhibition of programmed RISC throughout targeting Argonaute (Ago). Molecular dissection mapped suppression activity in the N-terminal part of P1, a region containing WG/GW motifs essential both for Ago binding and for suppression, as proved by side directed mutagenesis analysis. This mechanism represents a novel mode of RNA silencing suppression which might take place by outcompeting cellular components with similar motifs, and that is radically different from other mechanisms previously described for viral proteins, including the HC-Pro of potyviruses and the P1b of other ipomoviruses. Insights into the SPMMV infection process in its natural host that could derive from this peculiar mechanism of silencing suppression are also discussed.

ROTAVIRUS STRAINS IN HUNGARY, 2007-2008

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Vaccination is the main strategy to control severe dehydrating gastroenteritis caused by rotaviruses in early childhood. The availability of new generation rotavirus vaccines has led to an intensification of strain surveillance worldwide, in part, to gauge the impact of the possible vaccine driven immune selection of wild-type rotavirus strains Rotavirus positive stool samples were collected from patients, mostly children <5 years of age with gastroenteritis in different regions of Hungary in 2007-2008. The extracted genomic RNA was subjected to genotyping using multiplex RT-PCR assay. Type-specific primers included in our assay targeted G1 to G4, G6, G8 to G10, and G12 VP7 specificities, and P[4], P[6] and P[8] to P[11] VP4 specificities.

A total of 1035 rotavirus positive specimens was collected from 1007 patients and 968 of these were successfully G typed. The prevalence data showed the predominance of G1 (42%) strains followed by G4 (26.1%), G2 (18.6%) and G9 (10.4%) rotaviruses. Minority G types included G3 (0.8%), G12 (0.6%), G6 (0.2%), G8 (0.1%) and G10 (0.1%). Mixed G types were identified in 1.1% of samples and 3.8 % of strains remained G non-typeable. The P type could be determined for 988 strains. Genotype P[8] (79.9%) rotaviruses were the most prevalent followed by P[4] (18%) strains. Genotypes P[6] and P[9] were identified at low prevalence (1.3% and 0.4%, respectively). Four strains were positive for more than one P type and 1.9% of the strains were P non-typeable. A total of 953 strains were fully G and P typed showing the circulation of the globally common antigen combinations (G1P[8], G2P[4], G4P[8], and G9P[8]) and minority strains endemic in Hungary (G1P[4], G2P[8], G3P[9], G4P[6], G6P[9], and G12P[8]), however, we identified some rare strains (i.e., G2P[6], G8P[8], G9P[6], G9P[4] and G10P[6]) for the first time in our country. Our data indicates that the antigen specificities of medically important rotavirus strains identified in this 2-year study are represented in the vaccines available in the pharmaceutical private market in Hungary. Depending on the vaccination coverage achievable in the forthcoming years, the post-vaccination rotavirus strain surveillance may allow us to gain comprehensive information on the impact of rotavirus vaccines on the prevalence of circulating rotavirus strains.

MICROBIOLOGICAL CHARACTERIZATION OF FOREST SAMPLES CONTAMINATED BY DE-ICING FLUIDS

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Soil samples arrived from the Oslo International Airport at Gardemoen, Norway have been characterized microbiologically for the presence of total countable- and pollutant-decomposing microbial components in vitro. The airport is a relatively homogenous flat sandy area, with weakly podsolized soils. Norway spruce is the dominant tree species besides some Scots pine. The surface near the airport and the runways is covered by forest, mainly spruce with some open areas with pioneering vegetation, such as grass, bushes, young birch. De-icing fluids (DF) containing propylene-glycol and other constituents are used at the airport on aeroplanes in winter between October and April. During mechanical removal of snow from the runways and by the airborne drift of DF from the aeroplanes during take-off, the DFs are mixed with snow. The chemicals infiltrate at the soil surface along the runways when the melting of snow begins around April. It is important to ascertain that these chemicals do not contaminate the groundwater. More than 50% of the groundwater recharge occurs during the snowmelt period 3-5 weeks in April-May. Microbiological characterisation was carried at RISSAC of soil samples obtained from the lysimeters installed at the Gardermoen site near the runways. These experiments form a part of the biogeochemical characterisation and monitoring of the sites in investigation and included determining the abundance of total culturable heterotrophic bacteria, culturable anaerobic bacteria and the culturable microfungi, determining total catabolic microbial activity by fluorescein diacetate hydrolysis (FDA). For all 5 lysimeters highest microbial activity was demonstrated in the top 20-30 cm segment of the soil and a substantial drop was observed in all microbial activities below this segment. Culturable aerobic heterotrophic bacteria were about 2 orders of magnitude more prevalent in the soil samples than anaerobic bacteria and microfungi. Further experiments using the most probable number (MPN) method demonstrated that a key limiting factor in the biodegradation of DF during the 3-5 weeks of snowmelt infiltration is the low soil temperatures (0,2-9 C), however, bacterial strains biodegrading DFs at 4°C could be cultured and isolated from the soil samples from the Gardermoen site.

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ISOLATION AND CHARACTERIZATION OF FOUR CATALASE GENES FROM *RHIZOPUS ORYZAE*

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Zygomycosis is a diverse group of opportunistic infections caused by members of the fungal class Zygomycetes. These frequently fatal infections can be associated with diabetic ketoacidosis, deferoxamine treatment, cancer and its therapy, solid organ or bone marrow transplantations, extreme malnutrition and neutropenia. Thermophilic members of the genus *Rhizopus*, especially *R. oryzae*, are considered to the main causative agents. During the past decades, zygomycosis has emerged in an increasing number due to the widespread use of immunosuppressive therapy, intensive cancer chemotherapy and application of broad-spectrum antimicrobial agents. High mortality rates, difficulties in the diagnosis and resistance to the most widely used antifungal drugs urge the development of new strategies to prevent and treat these infections.

Generation of oxidative products by the phagocytic cells is known to be one of the important host defence mechanisms directed towards the killing of invading microorganisms. Catalases may provide protection against the reactive oxygen species produced by neutrophile granulocytes of the human

immune system. Neutropenia is a considerable risk factor of zygomycoses. In this study, catalase encoding genes of *R. oryzae* have been isolated, and their functional analysis has been started.
Four possible catalase genes were found in the *R. oryzae* genome database [1] by similarity searches using sequences of known fungal catalases. The proposed genes and their adjacent regions were amplified by PCR from the genomic DNA of *R. oryzae* and cloned into the vector pBluescriptII SK+ (Stratagene). To reveal the function of the isolated genes and to investigate their possible role in the pathogenicity, four vectors suitable to create deletions in the appropriate genes were constructed. In each vector, the *pyrG* gene of *R. oryzae* encoding the orotidine-5'-monophosphate decarboxylase was placed between the 5' and 3' flanking regions of the different catalase genes. To ensure the gene replacement by double crossing over, linear fragments containing the deletion cassettes were cut from the plasmids and used to transform protoplasts of a uracil auxotrophic *R. oryzae* strain.
Integration of the transferred DNA into the host genome and deletion of the corresponding catalase genes was proven by PCR and Southern blot analysis. Catalase activity of the recipient strain and the four mutants constructed were *in vitro* tested. Effect of hydrogen peroxide on the fungal growth was examined on agar plates and in a microtiter plate assay. Each of the four catalase genes proved to be functional. In all types of mutants, deletion of a catalase gene increased markedly the sensitivity of the transformants to hydrogen peroxide.

[1] Rhizopus oryzae Sequencing Project, Broad Institute of Harvard and MIT 2004, http://www.broad.mit.edu

HUMAN METAPNEUMOVIRUS INFECTION IN CROATIA

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Acute respiratory tract infections (ARTIs) are the major cause of morbidity and mortality in children worldwide. Many viruses are responsible for ARTIs in children, including human metapneumovirus (HMPV), the virus that was first isolated by van den Hoogen et al. in 2001. The signs and symptoms of HMPV infection in infants resemble those induced by respiratory syncytial virus (RSV) infection. HMPV infection has been mainly associated with bronchiolitis, pneumonitis, asthma exacerbation and upper respiratory tract infections including acute otitis media. Several seroprevalence surveys indicated that virtually all children are infected by 5-10 years of age. In order to asses seroprevalence of HMPV in Croatia, a total of 137 serum specimens from Croatian people aged from 6 days to 51 years without respiratory symptoms were collected. Sera were examined using an indirect immunofluorescent assay. HMPV overall seropositivity rate of the studied samples was 77.4% (106/137). The seropositivity rate increased from 18.7% in children aged 6 months to 1 year to 100% in people over 20 years of age. Moreover, to determine the incidence of HMPV infection in hospitalized children with ARTI in the winter season 2005/2006 nasopharyngeal secretions (NPSs) were collected from 402 inpatients up to 5 years of age. NPSs were tested by real time RT-PCR assay targeting the nucleoprotein (N) gene of HMPV. NPSs were also tested for RSV, influenza viruses, parainfluenza viruses (types1-3) and adenoviruses by direct immunofluorescence assay. HMPV was detected in 33 (8.2%), RSV in 101 (25.1%), adenoviruses in 41 (10.2%), parainfluenza viruses types1-3 in 24 patients (6.0%) and influenza viruses in 3 (0.7%) patients. In 3 HMPV positive patients other viral pathogen (1- parainfluenza virus type 3, 1- RSV, 1- adenovirus) was also

detected. The peak incidence of HMPV infection was detected in January (18/33). No difference was noted between HMPV infected children and children infected with other viruses regarding to the sex. Children infected with adenovirus were significantly older than children infected with other viruses (HMPV, RSV and parainfluenca viruses). The majority of the infections caused by HMPV (69.7%) children presented with lower respiratory tract infections (LRTI). Comparative analysis of the clinical presentation of HMPV and RSV infected children revealed no significant differences between those two groups, although pneumonia tended to be more frequent in HMPV infected children. To perform the phylogenetic study, partial nucleotide sequences were obtained for HMPV fusion (F) gene of 30 HMPV positive samples. Phylogenetic analysis showed the circulation of two main genetic lineages (A and B), with B lineages being prevalent. It also showed the existence of two sublineages within the group B (B1 and B2) and three subclusters within lineage A (A1, A2a and A2b).

EFFECT OF DIFFERENT GAS MIXTURES ON THE PROLIFERATION OF RESIDENT FLORA MEMBERS OF FOODSTUFFS

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A specially designed jar system was used to determine the effect of five different gas mixtures on the change of facultative microbiota of different raw food stuffs during storage. Chicken-, fish-, pork-, and beef were stored at 8°C degrees for 3 days under modified gas atmospheres (MGAs) where five different gas compositions were tested. In the case of all investigated specimens microbial tenability could be gained by applying 25%CO_2+75%N_2, 50%CO_2+50%N_2, and 25%CO_2+50%N_2+25%O_2. The restrictive effect of the different gas mixtures differed depending on the individual foodstuffs. The mixture of 75%N_2+25%O_2 proved to be un-effective for preservation practices in the jar system we used since no effect on the growth of facultative microbiota could be detected. Only the three most effective gas mixtures (25%CO_2+75%N_2, 50%CO_2+50%N_2, 25%CO_2+50%N_2+25%O_2) were tested in more detailed experiments, where CFU changes of the microbiota in minced pork were tracked for 3, 5, 7, 10, 14 and 21 days. Kinetically 50%CO_2+50%N_2 proved to be the most effective, however by the end of the 3rd week differences in the restrictive effects on the bacterial proliferation could not be demonstrated among the different gasses. The aim of this study was not only to compare the effect of different gas mixtures on the proliferation of the contaminating bacteria, but we also tested a specially designed user friendly jar system where MGA can be used to preserve foodstuffs in households.

CELLULOLYTIC ENZYMES ON AGRICULTURAL WASTE IN SOLID STATE FERMENTATION BY ZYGOMYCETES

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Zygomycetes fungi are known to be good producers of different extracellular enzymes. Due to this feature, numbers of them have been assumed to play an important role in the decomposition of plant

and other organic materials. Corn is a major crop in the eastern European countries and therefore large amount of corn stalk arise as agricultural waste. Utilization of this resource requires hydrolysis of cellulose to fermentable reducing sugars in the first step. Cellulases, responsible for the hydrolysis of cellulose, are complex mixture of enzyme proteins with different specificities to hydrolyze the β-1,4-glycosidic linkages bonds. Three major enzyme activity classes in the cellulose enzyme complex are endoglucanases, cellobiohydrolases (1,4-β-D-glucan cellobiohydrolase) and beta-glucosidases (β-D-glucoside glucohydrolase). Currently, the rapidly evolving biotechnological applications require the isolation and characterization of new cellulose-degrading microorganisms. The aim of the present study has been to evaluate the production of fungal cellulolytic enzymes on a cheap, freely available agricultural waste. Therefore, isolates of *Mucor corticolus* (syn.: *M. circinelloides f. corticolus*) and Gilbertella persicaria have been screened for their secreted cellobiohydrolase and beta-glucosidase activities. To investigate the production of cellobiohydrolase and beta-glucosidase, solid state fermentations were performed by using chopped corn-stalks and corn leaves as carbon sources. The cultures were incubated at 25°C for 12 days and enzyme activities were determined from the crude water extracts obtained every second day. Isolates of both species showed intensive growth on these substrates, and high activities of the investigated enzymes were observed during the fermentation period. Extracellular beta-glucosidase activities of these fungi were found to be higher than their cellobiohydrolase activities. The potential application of these fungi for biodegradation and enzyme production is discussed.

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BIOFILM FORMATION AND ELIMINATION FROM SURFACES OF INDUSTRIAL FACILITIES

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Many industrial applications suffer from the microbial contamination of ultra pure water that they use e.g. as cooling water. The microbes occur not only in the water but also form biofilms on surfaces and induce corrosion processes. In a Hungarian power plant corrosion processes were observed which was impossible to explain by physical or chemical factors. Therefore microbiological investigation has started to reveal biofilms on the surfaces of the industrial facilities. The aim of the present work was to study microbial communities in the biofilms of pipelines and on the surfaces of different ion-exchange resins (scavenger, cation- and anion-exchange as well as mixed bed ion-exchange resins). The overall structure of biofilms was studied by Scanning Electron Microscopy. Isolation of bacteria was evaluated using oligotrophic media, identification of the strains was revealed by 16S rRNA gene sequencing. T-RFLP was used as molecular fingerprint method, peaks were identified based on a previously constructed clone library. To study the possible elimination of biofilm bacteria biocide Kathon WT was used against the isolated bacteria as well as complete biofilms. For this purpose first a preliminary essay was done and later a laboratory model system was also created. Based on our results complex microbial communities could be observed by SEM (coccoid cells, rods, filamentous organisms, budding bacteria, eukaryotic microorganisms) on all studied surfaces. Many differences could be revealed on the surfaces of different ion exchange resins, mixed bed resin was the most covered by microorganisms. Results of cultivation of biofilm bacteria showed that Gram positives dominated the microbial communities (*Kocuria* sp., *Microbacterium* sp., *Mycobacterium* sp., *Brevibacterium* sp., *Bacillus* spp., *Lactococcus* sp., *Staphylococcus* sp.). Besides them high number of Proteobacteria could be observed. T-RFLP showed also the appearance of diverse communities

with the dominance of Proteobacteria. Many of the identified taxa can contribute to biofilm formation and corrosion on the pipelines' surfaces: many are able to produce EPS layer, heterotrophs or facultatively chemolitotrophs, some are H_2 autotrophic and even able to degrade hardly degradable compounds. The studied biocide was effective already in low concentration against many bacteria but Mycobacterium species and *Bacillus horikoshii* showed high resistance. Total elimination of complex biofilms from the surfaces with biocide is hard but 25ppm concentration of Kathon WT killed many bacteria and blocked their multiplication. Already 8hrs treatment with biocid was effective, remultiplication of bacteria was not observed in any cases after it.

EXPLOITATION OF SOLID NEXT GENERATION SEQUENCING PLATFORM IN MICROBIOLOGY

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The world around us is largely inhabited and maintained by a vast crowd of unseen microorganisms. Until the advent of genomics tools we were unaware of just how prevalent and important these organisms were to life. Though much work has been done in trying to understand this microbial world, the surface has only been scratched. Appearance of Next-Generation Sequencing technologies may speed up this process. The potential applications are as numerous as the samples to be analyzed. Each next-generation platform is optimized for specific sequencing applications. SOLiD platform can generate the highest number of reads, produces millions of short tags of 35-75 nucleotides in each run. Repeated cycles allow for multi-colour-encoding of each base in the DNA sequence, thereby reducing errors in the sequence and allowing for detection of complicated genomic variations, such as single-nucleotide polymorphisms (SNPs). Due to its enormous throughput, the SOLiD platform is an excellent choice for whole genome resequencing projects and for gene expression analyses, either for whole transcriptome analyses (RNA-Seq) or SAGE applications (serial analyis of gene expression). RNA-Seq is a serious competitor of traditional whole transcriptome analysis methods (microarray based methods). SOLiD performs whole transcriptome analysis in digital way (digital gene expression analysis), this method can be used quantitatively over five orders of magnitude and is not dependent on previous knowledge of transcribed sequences. Moreover the platform is suitable for metagenomics projects as well (analyses of environmental and food samples), dynamic changes, metabolic activities of microbial populations can be monitored using SOLiD. Our own experiences regarding the above mentioned applications in microbiology projects will be discussed.

A MULTIFUNCTIONAL BIOSENSOR BASED ON SURFACE PLASMON RESONANCE FOR MYCOTOXIN DETECTION IN WATER SAMPLES

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Mycotoxins are a group of highly toxic fungal secondary metabolites that may contaminate water and foodstuffs. Their early detection is therefore very important to protect human and animal health. In this contribution we present research and development results of a new multifunctional biosensor based on surface plasmon resonance (SPR) for mycotoxins detection in water samples. The

developed biosensor was designed for simultaneous measurement of up to 10 different molecular interaction sites (analytes) per one sample run, and has a lower limit of detection (LOD) 10-5 refractive index unit. A simple methodology and design of polydimetylsiloxane (PDMS) based microfluidic system was developed for controlled sample introduction and complex imobilization of biomolecules on the sensor detection surface, to form biochips with up to 100 interaction sites. Immobilization of biomolecules could be either conduced in the detector and measured in real-time or performed manually outside. For this purpose an inhibition immunoassay test for aflatoxin B1 was optimized on a commercial SPR detector, and transferred on the developed prototype. Based on preliminary measurements the LOD for aflatoxin B1 in spiked water samples resulted ~25ng/ml using 10µL sample volumes and requesting approx. 2 min/sample analysis time. In addition specific and non-specific interactions could be measured to control the functionality of biochips.

CHARACTERISATION OF EXTENDED-SPECTRUM-BETA-LACTAMASE (ESBL) PRODUCING *KLEBSIELLA PNEUMONIAE* STRAINS

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Endemic and epidemic nosocomial infections caused by extended-spectrum-ß-lactamase (ESBL)-producing *Klebsiella pneumoniae* cells represent a persistent problem in many parts of the world. Since 1983, nosocomial outbreaks of ESBL-producing *K. pneumoniae* infections in Europe, the United States, and South America were described. The aims of our present study were to determine the (i) incidence and origin of ESBL producing *K. pneumoniae* strains isolated from the University Hospitals of the University of Pécs medical School, (ii) to reveal the clonal diversity among the strains with random amplified polymorphic DNA (RAPD) - PCR and (iii) to characterise the produced beta lactamases by isoelectric focusing. Altogether 103 ESBL *K. pneumoniae* strains were collected from routine clinical specimens between 2004-2009. The isolates originate from the following diverse sources: 58 urine samples, 23 blood cultures, 8 respiratory tract cultures, 7 wound discharges, 2 abdominal fluids, 3 samples from central venal catheters, 1 vaginal swab and 1 abdominal drain. Clonal investigation of the strains by using RAPD-PCR revealed that these strains could be divided into 7 well distinguishable groups. 71.8% of the strains belonged to one major group while 26 strains belonged to three smaller and 3 strains to three minor groups. Isoelectric focusing revealed the presence of different type ß-lactamases.

A NOVEL MULTIPLEX CATABOLIC-GENE SPECIFIC SINGLE NUCLEOTIDE PRIMER EXTENSION ASSAY FOR THE DETECTION OF PUTATIVE *DEHALOGENASE* EXPRESSION

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Halogenated compounds are common groundwater contaminants all over the world. These chemicals can be naturally degraded by various microorganisms. The members of the genus *Dehalococcoides* have the capacity to degrade several halogenated compounds by reductive dehalogenation. Reductive

dehalogenases are the key enzymes responsible for these processes. A new molecular approach based on single-nucleotide primer extension (SNuPE) was designed to detect the expression of different groups of putative *dehalogenases* in an enrichment culture dominated by *Dehalococcoides*. This culture originated from contaminated groundwater and degrades (among others) chlorinated ethenes to ethene. Reductive *dehalogenase* homologous genes were amplified with primers targeting conserved regions within the *rdh* genes. The amplicons were cloned, reamplified from the plasmids and screened by restriction fragment length polymorphism analyses. The clones were grouped according to their restriction pattern and one insert per restriction pattern group was sequenced. Seven different putative *dehalogenases* were identified from the sequences of the clones and five group-specific SNuPE probes were designed targeting the major groups.
Anaerobic microcosms were set up with different electron acceptors (PCE, TCE, c-DCE and VC) inoculated with the highly enriched *Dehalococcoides* culture. RNA was isolated during growth and reductive dechlorination. By means of the newly designed SNuPE probes, we could detect differences in the *dehalogenase* expression between the microcosms fed with different electron acceptors.
One type of *dehalogenase* could be detected only in the culture fed with TCE and one other sequentially in every type of microcosms. We established that the method is useful for the analysis of catabolic genes expression. In the future this approach could be used to analyze the gene expression in the presence of other substrates, i. e. DCA, halogenated aromatic compounds. We also would like to design other specific SNuPE probes to decipher the role of other putative *dehalogenases* in dehalorespiration processes.

ARABINAN AND L-ARABINOSE METABOLISM IN *TRICHODERMA REESEI* (*HYPOCREA JECORINA*): COMPONENTS, REGULATION AND OTHER SURPRISES.

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The use of plant material as a carbon source for biofuel production makes it essential to improve transformation of the different plant cell wall constituents cellulose, hemicelluloses and pectins. The saprotrophic fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*) has been well established for the biotechnological production of cellulases and xylanases for biofuel production. But the degradation of other plant carbohydrate polymers such as L-arabinose polymer arabinan still needs improvement. An inspection of the genome of *H. jecorina* reveals four genes capable of degrading arabinan to L-arabinose, i.e. the α-L-arabinofuranosidase encoding genes *afb1*, *afb2*, *afb3* and *bxl1*, which encodes a α-xylosidase with a separate α-L-arabinofuranosidase domain and activity. Following its uptake, L-arabinose it is further degraded by the following sequence of enzymes: the aldose reductase XYL1, the L-arabinitol dehydrogenase LAD1, the L-xylulose reductase LXR1, the xylitol dehydrogenase XDH1 and the xylulokinase XKI1. Transcriptional analysis reveals that *abf1-3* and *bxl1* are induced by L-arabinose and to a lesser extent by L-arabinitol. Growth on arabinan and L-arabinose is severely affected in a strain deficient in the general cellulase and hemicellulase regulator XYR1 and XYL1. This impairment can be overcome by constitutive expression of the aldose reductase *xyl1*. Induction by L-arabinitol, however, is strongly enhanced in a *Δlad*1 strain lacking L-arabinitol dehydrogenase activity, and impaired in an aldose reductase (*Δxyl1*) strain, suggesting a cross-talk between L-arabinitol and the aldose reductase XYL1 in α-L-arabinofuranosidase gene expression. Strains bearing a knock-out in the cellulase regulator *xyr1* do not show any induction of *abf2* and *bxl1*.

Cloning of an LXR1 enzyme responsible for NADPH dependant reduction of L-xylulose to xylitol was previously claimed. Our analysis revealed that *lxr1* is not induced by L-arabinose and that its deletion does not affect growth on L-arabinose. LXR1 belongs phylogenetically to the group of D-mannitol 2-dehydrogenases and its deletion results in reduced D-mannitol 2-dehydrogenase activities. We conclude therefore that the real L-xylulose reductase is still unknown and that the *lxr1* gene encodes a D-mannitol 2-dehydrogenase.

THE HOMOLOGUES OF BACTERIAL ALKB PROTEIN IN CYANOBACTERIA AND *ARABIDOPSIS THALIANA*

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Cyanobacteria and higher plants belong to evolutionary distant groups of photosynthesizing organisms. Despite of this fact all of them share homology of *alkB* gene primary discovered and studied in *Escherichia coli*. AlkB protein is a non-heme iron (II) and α-ketoglutarate-dependent dioxygenase that oxidatively demethylates 1meA and 3meC lesions in DNA, with recovery of A and C building RNA or DNA. The aim of this study was to describe the activity of AlkB homologues in both groups of organisms: Cyanobacteria and higher plants – *Arabidopsis*. We have chosen ten cyanobacterial strains (*Acaryochloris marina* MBIC11017, *Arthrospira maxima* CS-328, *Cyanothece sp.* PCC7425, *Microcoleus chthonoplastes* PCC7420, *Prochlorococcus marinus* MIT9313, *Synechococcus* sp. BL107, *Synechococcus sp.* CC9311, *Synechococcus* sp. RS9916, *Synechocystis* sp. PCC6803) which genomes have been sequenced and revealed existence of at least one *alkB* homologue. Most of the coded enzymes contain whole AlkB domain. On the other hand the genome of *Arabidopsis thaliana*, a plant model organism, shows at least ten *alkB* homologues that produce proteins with whole or part of the AlkB domain, according to *in silico* analysis. Cyanobacterial and *Arabidopsis* cDNA of *alkB* homologues were inserted into high copy number plasmid pET28a in the frame with His-tag in order to overexpress them in *E. coli* strain BL21. The proteins were purified on metal affinity columns (Ni-Sepharose) and used in enzyme activity assay (demethylation activity on oligonucleotide containing 3-methylcytosine residue). Simultaneously, cDNA of *alkB* homologues were inserted into low copy number vector (pVB1x); the resulting plasmid constructs were used in complementation assays in *E. coli* strain deprived of its own *alkB* gene.

ANTIMICROBIAL ACTIVITY OF *SATUREJA HORTENSIS* L. ESSENTIAL OIL

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Due to the broad topic of biological activity the essential oils of higher plants are used for food preservation and potential applications for medicinal purposes including antimicrobial, antiparasitic, anticancer activities etc. Our work refers results about the antimicrobial activity of essential oil from *Satureja hortensis* against clinical isolates from wound and its chemical composition. The aerial parts of *Satureja hortensis* L., cultivated in Serbia were collected in the full-flowering stage. After the

period of drying, essential oil was isolated by hydro-distillation in the Clevenger-type apparatus. A broth micro-well dilution method was employed to determine the essential oil activity against pathogenic bacteria, multiresistant clinical isolates from 10 different genera: *Klebsiella*, *Escherichia*, *Proteus*, *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Enterococcus*, *Enterobacter*, *Citrobacter* and *Acinetobacter*. The essential oil showed activity against all tested strains. MIC/MBC values were in the range from 0.78 - 25 µl/ml, with the exception of the strain *P. aeruginosa*. Microbicidal concentration for this particular strain was the highest for tested concentration of 50 µl/ml. Except for three tested strains, essential oil showed inhibitory and bactericidal effect at the same concentration (MIC=MBC) against all residual strains. Qualitative and quantitative analysis of the isolated essential oil was performed by GC and GC/MS. From 29 identified compounds representing 99.49 % of the oil, carvacrol (67.00%), γ-terpinene (15.3%) and p-cymene (6.73%) identified as the main oil components. Essential oil of the species *S. hortensis* is very effective natural antimicrobial agent which could find application in the treatment of the wound infections.

INTERACTION BETWEEN *LISTERIA MONOCYTOGENES* AND *ACANTHAMOEBA CASTELLANI*

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Listeria monocytogenes, gram positive, facultative intracellular rod, is found ubiquitously in the environment and is capable to grow and survive in a wide range of environmental conditions. However, the current knowledge of its ecology and in particular, the mode of its environmental survival and transmission remains limited. It has been suggested that free-living protozoa serve as an environmental reservoirs and vehicles for the transmission of several obligate and facultative intracellular pathogens, but the ability for intracellular multiplication and survival of *L. monocytogenes* in *Acanthamoeba* spp. is still unclear. The aim of this study was to investigate the interaction between the protozoan *Acanthamoeba castellanii* (ATCC 30234) and EGD strain of *Listeria monocytogenes*. The results indicate that *L. monocytogenes* does not reside within *A. castellanii*, as we were unable to recover any intracellular bacteria after 4 hours of co-cultivation. However, extracellular bacteria benefit from co-cultivation with amoeba showing increased growth and multiplication. In conclusion, *Listeria monocytogenes* is not able to survive inside *Acanthamoeba castellani*, but is quickly degraded under the laboratory conditions tested. *A. castellani* is not able to act as environmental reservoir for *L. monocytogenes* but may provide a survival advantage for extracellular listeria during co-cultivation of the two organisms.

LACTOSE INDUCTION OF CELLULASE GENE EXPRESSION IN *HYPOCREA JECORINA*: A ROLE FOR GALACTOSYL-TRANSFERASES?

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Hypocrea jecorina (=*Trichoderma reesei*) is the main industrial source of cellulases and hemicellulases used to depolymerize plant biomass to simple sugars that are converted to chemical

intermediates and biofuels, such as ethanol. Cellulases and hemicellulase formation can be induced by several mono- and disaccharides, e.g. sophorose, xylobiose, lactose, D-xylose, L-sorbose, most of which are too expensive for industrial fermentations. Thus the range of technically applicable carbon sources is limited. Lactose (1,4-0-β-D-galactopyranosyl-D-glucose) is produced to around 1.2 million tons worldwide, primary as a by-product from cheese manufacture or from whey processing industries, but utilized only to a low degree and mostly not very profitable. Thus, lactose is one of the largest renewable substrates available today. The obvious advantage of lactose over cellulose is that it is soluble, and therefore provides a preferred carbon source for the production of recombinant proteins driven by cellulase (e.g. cel7a) promoters. *H. jecorina* hydrolyzes lactose to D-glucose and D-galactose extracellularly instead of taking it up first and hydrolyzing it intracellularly. Further metabolism of D-galactose occurs in part by the Leloir pathway, which consists of galactokinase (GAL1), UDP-galactose uridyltransferase (GAL7) and UDP-galactose epimerase (GAL10). However, growth on lactose also induces an alternative second D-galactose degrading pathway initiated by the aldose reductase XYL1. Single and double knock out strains in gal1 as well as xyl1 are impaired in the utilization of lactose and in the formation of cellulases. Our hypothesis is that both pathways are required to form the inducer from lactose. To this end, we analyzed the intracellular glycome in the parent strain and the pathway mutants. Cellulase formation during growth on lactose indeed strongly correlated with the intracellular accumulation of galactosyl-tetrasaccharides putatively also containing hexitols. In order to identify the enzymes forming these oligosaccharides, we screened the *H. jecorina* genome, which retrieved 10 putative candidate β-galactosyltransferases, which are members of GT-families 1, 17 and 31. To identify which of these GTs could be involved in cellulase induction we tested the expression of these genes in strains which are defective in cellulase formation. Most of these GT are expressed during growth on lactose but we could identify three GTs were not expressed in a double gal1/xyl1 strain impaired in cellulase formation on lactose. The affect of these GTs on cellulase expression during growth on lactose is currently investigated by the use of knock-out mutants in *H. jecorina*. Four genes have already been identified whose deletion results in significantly reduced, albeit not eliminated cellulase formation, suggesting redundancy of inducer forming GTs.

INVESTIGATION OF AEROBIC AND ANAEROBIC MICROBIAL DEGRADATION OF IBUPROFEN USING MOLECULAR AND CULTIVATION BASED METHODS

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The increasing drug consumption habits of the households lead to the appearance of detectable amounts of pharmaceutical residues in natural surface water bodies because the present sewage treatment plant technologies can not remove effectively these micropollutants. The ecosystems of natural water sources are exposed to several pharmaceutically active compounds (PhACs) like antibiotics, hormone contraceptives, different anti-inflammatory drugs. The latter includes ibuprofen, which is the world's third-largest drug substance. To investigate the effect of ibuprofen on bacterial communities we set up two different experimental systems: activated sludge and compost samples served as inocula for aerated model systems and in the anaerobic experiments digested sludge and Danube sediment were used as microbial inocula. Changes in the ibuprofen concentration were monitored by HPLC-MS analysis, and experimental setups that showed significant concentration

decrease were further examined by molecular fingerprinting techniques (Terminal Restriction Fragment Length Polimorphism, T-RFLP) as well as standard aerobic and anaerobic cultivation methods. Four different anaerobic enrichment cultures (nitrate-, sulfate-, ironreducer and methanogenic enrichment media) were established where samples from the Danube setup were used as inocula and ibuprofen as the only carbon and energy source. Determination of the degradation products of ibuprofen is under progress.

We set up and optimized an experimental model system, which allowed to test the biodegradation potential of pharmaceutical residues, and their influence on bacterial communities. Our primary studies showed that ibuprofen concentration decreased significantly in the Danube sediment and the activated sludge systems but did not changed in the compost and digested sludge experiments. In the case of the aerobic activated sludge the degradation process started after a short 5-day lag period and the complete removal took only two days after initiation. Elimination in the anaerobic Danube sediment started following a longer two-month lag phase and the process was slower due to its anaerobic property. Community structure analysis (T-RFLP) of the activated sludge and the Danube sediment showed significant reduction of the microbial diversity, which is likely – confirmed by the chemical analysis – the result of the growth of ibuprofen utilizing microorganisms in this selective environment. Low diversity was also observed during the aerobic cultivation procedure. We could detect bacterial growth even after the third transfer of the anaerobic enrichment cultures, which indicates the probability of ibuprofen being the sole energy and carbon source.

STATISTICAL ANALYSIS OF THE TIME-PROFILE OF CELL LENGTH GROWTH IN FISSION YEAST

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In the first half of the 20th century yeasts have become model organisms in cell cycle studies. Since the late 1950s *Schizosaccharomyces pombe* (fission yeast) has been spotlighted through its favourable physiological features, for example, its symmetrical division enables good synchronisation techniques. The cylindrically shaped fission yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. As a consequence, cell length is an easily measurable parameter, which characterizes cell age. Length growth patterns may therefore indicate connections between volume changes and cell cycle events. The classical method to study the growth of individual cells is time-lapse microphotography; cells are growing on the surface of an agar pad in a thermostated photomicroscope, and later on one can study the growth pattern of cell length simply by a projector.

In different cell types, there is considerable controversy concerning the exact growth profile of size parameters during the cell cycle. Linear, exponential and bilinear (i.e., two linear segments with a rate change point) models are commonly considered, and the same model may not apply for all species. Selection of the most adequate model to describe a given data-set requires the use of quantitative model selection criteria, which are suitable for comparing differently parameterised models. Recently, length increase data from individual fission yeast cells, measured on time-lapse films have been reanalysed using these model selection criteria. A linearised biexponential model (called bilinear) was developed, which makes possible a smooth, continuously differentiable transition between two linear segments. Essentially all the quantitative selection criteria considered here indicated that the bilinear model was somewhat more adequate than the exponential one for fitting the fission yeast cell data.

Since this method was developed by using only two old cells' length growth patterns, we have extended it for 182 newly measured fission yeast cells, namely 60 wild type, 62 *wee1*Δ mutant and 60 *cdc2-3w cdc25*Δ double mutant ones. The above mentioned model selection criteria were used for discriminating among linear, exponential and bilinear models and selecting the most adequate one in the case of all these cells' length growth patterns. Although relatively small differences were found in several cases, essentially all the quantitative selection criteria considered here indicated that the bilinear model was generally more adequate than either the exponential or the linear ones. In the case of wild type and *wee1*Δ mutant cultures, more than 2/3 of the cells had a bilinear pattern, while in the case of *cdc2-3w cdc25*Δ double mutant, the ratio of bilinear cells was slightly above 50%. "Average cells" were also constructed from all the individual cells' data for all three strains, whose patterns were definitely found to be bilinear by any criterion used. This method is also planned to be applied for further cell cycle mutants of fission yeast.

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HUMAN BOCAVIRUS IN RESPIRATORY SAMPLES

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Human bocavirus (HBoV) is a newly described human virus closely related to bovine parvovirus and canine minute virus. It is currently classified in the genus Bocavirus within the family Parvoviridae. This virus was first identified in respiratory tract specimens from lower respiratory tract infections (RTI). However, the pathogenic role of HBoV is uncertain because other viruses have been frequently detected in HBoV-positive children with lower RTIs. The objective of this study is to assess the impact of HBoV infections on respiratory tract illnesses and to describe the clinical manifestations of these infections in children. Nasal or throat swabs from children with acute upper and lower respiratory illnesses were collected from June 2009 and tested for viral pathogens (HBoV, rhinovirus, adenovirus, respiratory syncytial virus, parainfluenza virus) by PCR method. The specimens were collected from hospitalized patients younger than 17 years old. HBoV was detected by PCR using primers specific for two different regions of the genome and phylogenetic analysis was performed on a part of the VP1/VP2 genes of HBoV-positive samples.

SIMULATING THE ORIGIN OF HIV-1 GROUP M IN KINSHASA: WHY DID THE EPIDEMIC EMERGE WHEN IT DID?

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Humans have probably long been exposed to Simian Immunodeficiency Viruses via bushmeat hunting, yet the origin of all epidemic HIV lineages dates back to a period of just a few decades. Coincidence suggests that specific conditions might have facilitated the human-to-human transmission of zoonotic SIV infections within this time window. While a number of theories have been put forward to explain the opening of the time window, the relative contribution of the different factors to the risk of HIV emergence has not been investigated quantitatively. Furthermore, all

current theories fail to explain why no major new epidemic HIV groups have emerged since the end of this period. We developed computer simulations to test whether the period around the origin of epidemic HIVs was particularly permissive for the emergence of HIV epidemics, and to identify key factors that might have facilitated the spread of the virus. As a case study, we implemented historical scenarios for Kinshasa (the likely epicentre of HIV-1 group M) before, during and after the estimated origin of the epidemic. The models were parameterized on the basis of detailed population, sociological and medical data (compiled through original archival research). The stochastic, individual-based computer simulations followed the early spread of the virus over a dynamic network of heterosexual contacts composed of several groups of varying promiscuity (married and single men and women, 'femmes libres' and commercial sex workers). Transmission parameters were set according to current HIV data, adopting the estimated relative effects of modifiers (e.g. genital ulcer diseases and male circumcision, which were also tracked in the simulations), but assuming a lower baseline transmission probability for ancestral HIV.The comparison of simulated historical scenarios confirmed that the period around the origin of HIV-1 group M was uniquely permissive for the heterosexual spread of the virus. This result was robust with respect to varying uncertain parameters in the model. A systematic analysis of potential facilitating factors revealed that the strongest direct effect was associated with the extremely high levels of genital ulcer diseases (GUD) at the time, which promoted HIV transmission. Remarkably, the direct effects of population size and circumcision frequency proved relatively small. According to our results, the window of opportunity for epidemic HIV emergence was probably closed by the aggressive treatment campaigns against GUDs from the mid thirties. We thus propose that GUD-facilitated heterosexual transmission provides a parsimonious explanation for the emergence of epidemic HIVs in a limited time window. Our results can be generalized to the origin of all other epidemic HIV lineages.

GENOME ANALYSIS OF THE *THERMOBIFIDA* GENUS USING HIGH-THROUGHPUT SEQUENCING

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Thermobifidas are aerobic thermophilic lignocellulose decomposing actinomycetes. The genus containing four species: *T. fusca*, *T. alba*, *T. cellulosilytica* and *T. halotolerans*. *T. fusca* YX is far more the best characterized strain from this taxon. Several cellulases and hemicellulases were cloned from this strain in this decade for industrial purposes, like paper industry, biofuel and feed applications. Unfortunately sequence data of such enzymes are almost exclusively restricted to this single species; however we demonstrated earlier by zymography that other *T. alba* and *T. cellulosilytica* strains encode the same enzyme set. Recently the advances in whole genome sequencing by the use of next generation genomics platforms allows of cloning valuable hydrolases from uncharacterized bacterial species on the basis of sequence data compared to the time and cost consuming conventional methods (e.g. expression library construction, protein purification and sequencing). Currently, there are more than 900 completed genome-sequence projects most of which are microbial. However, each of these genome sequences provides only a glimpse of the real genetic make-up of microbial populations. In many bacterial species, as well as the 'core' set of genes found in all individuals, there are large numbers of 'dispensable' genes that can vary enormously between

closely related species, pathovars and strains. The complete genome-sequence of *T. fusca* YX strain was published in 2007. Because understanding of the genetic basis of species- as well as strain-specific differences requires additional sequence data, we sequenced *T. cellulosilytica* TB100 and *T. alba* strains using the second generation sequencing technology. In addition, we used two different template preparation (fragment and mate-paired) techniques, which allowed stringent and accurate mapping of the obtained reads. Our analysis revealed that even though *Thermobifida* strains form a single genus, the species within exert significant genetic diversity, with number of SNPs and deletions occurring both in coding as well as in intergenic regions. We found that different *Thermobifida* species harbour the same but highly altered cellulase, xylanase and mannanase enzymes in accordance with the results of previous zymogram analysis.

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CLONING AND CHARACTERIZATION OF THREE HMG-COA REDUCTASE GENES IN *MUCOR CIRCINELLOIDES*

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Mucor circinelloides is a carotene producing Zygomycetes fungus used as a model organism in several fields of the biological research. Carotenoids are terpenoid-type chemical compounds and first part of their biosynthesis occurs via the mevalonate pathway in fungi. A rate-limiting step of this pathway, the conversion of the HMG-CoA to mevalonate is catalysed by the enzyme HMG-CoA reductase. As the *M. circinelloides* genome contains three HMG-CoA reductase genes (*hmgR*1, *hmgR*2 and *hmgR*3) [1], the aim of our study was to clone these genes and to analyse their expression.

Accordingly, the three genes were isolated and built in different expression vectors. The constructed plasmids carried the *Mucor pyrG* gene as selection marker and a uracil-leucine double auxotrophic *M. circinelloides* strain was used in the transformation experiments, which were carried out by the polyethylene-glycol mediated method. The transformation was verified by PCR; all transformants maintained the introduced plasmids in an autoreplicative way. Thus they contained the appropriate *hmgR* gene in extra copies. In previous studies, it has been shown, that over-expression of the HMG-CoA reductase gene lead to increased carotene production [2]. Carotene content of the transformants was determined and it was found that copy number of the *hmgR* genes affected the carotene production. It is also known that statins inhibit the HMG-CoA reductase and may induce apoptosis-like process in *Mucor* [3]. Accordingly, sensitivity of the recipient strain and the transformants to fluvastatin were also measured by a broth microdilution method. Tranformants highly resistant to fluvastatin were found and characterized.

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QUINONE BODIES OF *THERMOPLASMA ACIDOPHILUM*

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Cryo-electron tomography of frozen *Thermoplasma acidophilum* cells revealed the existence of cytosolic globular particles of an average diameter of 50 nm. Guided by the physical parameters obtained by cryo-EM we purified and investigated these particles for their nature. We found that they contain more than 200 small compounds (the greatest fractions being menaquinones) encapsulated by a protein layer almost exclusively formed by protein Ta0547. This protein was expressed at similar level at the investigated growth conditions (aerobic, anaerobic, elevated pH), however its expression level was increasing linearly during the growth phase. The production of soluble quinones in the microbial world is not unprecedented, and their occurrence is probably much more frequent as it has been explored and the biogeochemical significance of soluble quinone and/or quinone body producing microorganisms can be high. On one hand naphtoquinone ring of menaquinones, and the benzothiophene ring of sulfolobusquinones might be the key compounds of naphtalins and benzothiophens of petrols, on the other hand excreted quinones play crucial role in extracellular electron transfers shuttling electrons to possible oxidants including poorly soluble minerals.

MONITORING OF DRUG RESISTANT HIV IN HUNGARY

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The high error rate of reverse transcriptase (RT) and recombination events contribute to the expansion of the genetic heterogeneity of HIV-1 *in vivo* and resulting emergence of drug resistant HIV variants. In our nationwide program since 2003 we are monitoring and determining the mutations associated with resistance to antiretroviral drugs in primary HIV infected individuals. Previously we reported mutations in RT genes leading to resistance to NRT/NNRT in average of 15%,and in PR gene in<3% in therapy naïve white Hungarian MSMs primarily infected with HIV (Nagy K. et al 2006,2008*). Here we present results of genotyping of drug resistant HIV strains transmitted as primary infection between Hungarian and foreign partners from African (60.5%), Asian (3.5%) and other European countries (36%). Drug resistance genotyping of HIV RT and PR was done by in situ DNA hybridization using a Line Probe Assay (Inno-LiPA) ,direct sequencing using the Stanford AIDS database, and by Truegene HIV-1 Genotyping kit and OpenGene Sequencing System (Siemens). HIV-1 subtypes, non-B clades and circulating recombinant forms (CRF) were also determined. Mode of transmission: heterosexual (67.8%), homosexual (21.4%), IDU (7.2%), and nosocomial (3.6%). Gender distribution of foreign partners: male (46%), female (54%). In the HIV-1 RT and PR genes mutations of 72 codons (64 specific resistance codons) - representing 6 NRTIs, 2 NNRTIs and 6 PI drugs - had been analyzed. Resistant mutations to all of the 14 HIV RT and PR inhibitor drugs were detected in various extents. Multiple drug resistant viruses were present in high percentage. Resistance developed mainly towards NRTIs, than NNRTs and in a lesser extent to PRI. The least resistance was detected towards PRIs such as SQV,LPV and ATV. HIV-1 variants harboring resistance mutations belonged to HIV-1 subtypes A,B (25%),C (10.7%),E,F1 (7.2%)G (3.6%) ,J and K. The dominant recombinant forms were: CRF02_AG (28.5%),CRF06_cpx (17.8%) and CRF11_cpx(3.6%). We are monitoring presence ,evolution and distribution of mutant HIV viruses with drug resistance since 2003 in Hungarian individuals. This study identified and confirmed the transmission of drug-resistant HIV during primary infection in therapy-naïve patients. These findings indicate the evolution of drug resistance showing a correlation with the time of introduction

of combination therapy in our country. We could confirm the transmission of non-B HIV clades, CRFs and multi-drug resistant variants in Hungary which raises serious clinical and public health consequences. Development of resistance leads to viruses escaping the control of drug combination therapy and cause disease progression. Drug resistance testing (HIV genotyping) at the time of diagnosis should be the standard of care in countries belonging to low HIV endemic area of Europe.

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EVOLUTIONARY PROCESSES OF FUNGAL FRUITING BODIES

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One of the central questions in fungal evolutionary biology resides around the processes and putative selective forces that generated the overwhelming diversity of fungal fruiting bodies. Identification of the trends and major processes in fruiting body evolution has several potential implications ranging from biodiversity conservation to systematics. Limitations imposed by the lack of adequate statistical methods that hampered the evaluation of several evolutionary hypotheses has recently been largely eliminated by Maximum Likelihood and Bayesian approaches. The development of sound phylogenetic hypotheses about fungi has accelerated the study of morphological evolution on the basis of phylogenies inferred from molecular data. Here we present new findings about the evolution of deliquescent fruiting bodies in the family Psathyrellaceae (past Coprinaceae) and review some of the major problems (such as gastromycetation) addressed recently in this field. Based on a hierarchical Bayesian reconstruction of the ancestral type of fruiting bodies, we conclude that autodigesting types found in several clades of the Psathyrellaceae are derived from non-autodigesting ancestors basal to clades of deliquescent taxa. We show that the transformation of fruiting body types is evolutionarily correlated with changes in several morphological features, such as anatomy of the basidia, cap surface, or the morphology of hymenial cystidia (spacer cells). Using well-known and new calibrations in a Bayesian relaxed molecular clock framework, we estimated minimum divergence times of autodigesting fruiting bodies. These results evidenced that the transformations of fruiting bodies cannot be attributed to specific geologic events or ages, individual transformations being dated from the Cretaceous to the Tertiary (ca. 300 to 23 Mya). We developed an Markov model of the evolution of fruiting body types, suggesting that autodigesting is the preferred state in the Psathyrellaceae, putatively evolved by repeated gain of chitinase enzymes. We suggest that recent debate about gastromycetation, the distribution of mycorrhizal taxa and other questions can be addressed by the framework outlined above. Unfortunately, to date no statistical inferences were made on gasteroid (puffball-like) fungi, which could shed more light on the evolution of these fungi and could clarify several paradoxical observations about their phylogeny and distribution.

WHAT ARE PHYLOGENETIC COMPARATIVE METHODS AND WHAT CAN MICROBIOLOGISTS USE THEM FOR?

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Development in theoretical phylogenetics now allow molecular phylogenies to be used to address

several evolutionary hypotheses in addition to what is the tree that relates the organisms of interest. Such hypotheses might require the researcher to make comparisons between terminals (species, strains, sequences, etc.) of trees, such as the examination of host-parasite coevolution, inference of morphological or physiological features of extinct species, detect evidence of natural selection, testing for correlated change in two traits in an evolutionary context, or detecting shifts in diversification rates as in adaptive radiations or large-scale extinctions. The above approaches, termed phylogenetic comparative methods (PCMs), have considerable history and are commonplace in many fields of evolutionary biology. In this paper we briefly review the analytical framework PCMs use and their potential applications in evolutionary microbiology. PCMs are common in that they take robust, well-supported phylogenies (tree topologies with branch lengths) as a basis to statistically examine the question of interest. The phylogeny may be inferred from molecular or other types of data, but is assumed to adequately represent the underlying pattern of descent of the group to be examined. Modern methods of tree estimation, such as Bayesian methods employing highly elaborate evolutionary models, provide a reliable framework to produce plausible estimates of the phylogenetic relationships of the taxa. This, in combination with recent advances in the PCM algorithms and the availability of used friendly software has accelerated the understanding of a number of biological processes. We review some of the potential applications of PCMs including ancestral character state or sequence reconstruction, the estimation the rate of gain and loss of a physiological trait, as well as several hypothesis tests associated with such questions. Based on examples from external field, we would like to give an overview of the evolutionary questions that can be addressed via PCMs.

SIMULATION STUDIES TO INVESTIGATE LACTIC ACID FERMENTATION

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Lactic acid is one of the oldest fermentation products known by the menkind. It already had a renaissance in the '50s of the last century when first industrial scale lactic acid fermentations started, but nowadays, it is also newly developed to use in production of PLA, green solvents and other derivatives. There is also a need in Hungary, to develop an efficient lactic-acid-production technology on the basis of carbohydrate fermetnation, where carbon source is of agricultural residues or surpluses origin. The Hungarian lactic acid based biorefinery will be planned to use the surpluses of the Hungarian Agriculture in wheat, but should be easily transformed to other grain resources. From fermentation point of view, in most cases the carbon source will be glucose, which can be converted into lactic acid with very high yield (90-98%). In the case of most grain resources glucose must be obtained from simple sugars like sucrose or polysaccharides like starch. Using our own producing strain in both cases hydrolisis of raw material is needed before bacterial fermentation. The efficiency of fermentation processes is indicated usually with volumetric productivity which is the ratio of the final titer to the process time. In our case the process time is considered as the hydrolysis time plus fermentation time. To improve the efficiency two ways arise: incresing the final titer (for example by strain improvements), or shortening the process time. To shorten the process tiem the hydrolysis and fermentation can be driven either separated (SHF) or simultaneously (SSF) or partially simultaneously (pSSF). In the latter case, a hydrolysis started, and the fermentation (inoculation) will start only after a time delay. Thus the hydrolysing enzyme have time to accumlate glucose, which is important because the fermentation rate depends strongly on the initial substrate concentration.

However, the remaining part of the hydrolysis is running parallel with the fermentation. After carrying out inoculations at different timepoints of hydrolysis the optimal time delay was determined by kinetic simulation of starch hydrolysis and fermentation of glucose.

EXAMINATIONS TO DEVELOP AN ALTERNATIVE PASTEURISATION METHOD

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Pasteurisation, means for egg products a several minutes long heat treatment at about 60 °C in the heat exchanger. Two important issues shall be kept in mind: to destroy as much contaminating micro-organisms as possible and at the same time not to damage the valuable substances - mainly proteins - of the egg. Experience shows that the number of micro-organisms in pasteurised liquid eggs is between 10^2-10^3 cfu/ml and from time to time Salmonella bacterium can be found among the survivors. Consequently, such kind of pasteurisation technique is needed, which makes maintenance of the viable cell counts at a steadily low level (possible preferably making the product sterile) and guarantees that the products in every case and under all circumstances are free from *Salmonella* sp. In the research we conducted microbiological examinations to develop an alternative pasteurisation method, which can be used in the manufacture of egg products. We studied the effect of 24-hour incubation at 55 °C in the given cases. The samples were artificially infected with *Enterobacteriaceae*. The samples were raw liquid whole egg, liquid egg white and liquid egg yolk coming from an egg products manufacturing plant. In the detailed investigations on heat destruction we studied 3 bacteria species from the most frequently occurring contaminants in egg products. They were *Serratia marcescens*, a common contaminant, *E. coli*, which is significant from hygienic point of view and Salmonella bacteria, which are the most risky for egg products. Having determined the reduction in viable cell counts against time, the data obtained were in good agreement with literary ones, as the destruction of bacteria is faster in liquid egg white than in products containing egg yolk. In the case of all three *Enterobacteria experience* shows that during the incubation for 12 hours the initial cell count decreased by 4-5 log cycles. My results showed that in 24 hours of incubation at 55 °C we can obtain germ-free products with high probability. We compared the incubation treatment with the widely used pasteurisation procedure for these products. We found marked differences, i.e., while pasteurisation only slightly decreased cell counts, we measured 0 cfu/ml in the investigated egg products after 24 hours of incubation at 55 °C.

ISOLATION AND PRELIMINARY CHARACTERIZATION OF CARBENDAZIM-DEGRADING BACTERIA

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Carbendazim-degrading bacterium consortia were enriched from Hungarian agricultural soil samples by the use of carbendazim-containing minimal medium. The molecular analysis revealed that the members of these consortia belonged to the *Agrococcus, Acinetobacter, Acidovorax, Arthrobacter, Bacillus, Brevundimonas, Caulobacter, Microbacterium, Micrococcus, Pseudomonas* and *Variovorax*

genera. Some strains from the investigated twenty isolates were able to use carbendazim as sole carbon and nitrogen source. The best carbendazim degrader microbe proved to be *Variovorax paradoxus*. The pH optimum and temperature optimum for growth of this strain were at pH 6.3 and 30°C, respectively. This isolate seems to be a potential tool for bioremediation of carbendazim polluted agricultural soils. In the case of many strains the presence of highly active esterases could be detected in the periplasmic space or in the cytoplasm but never in ferment broths. It was proved that these enzymes are able to hydrolyze the carbamyl-methyl ester group in carbendazim.

POWER OF RESPONSE SURFACE METHOD: OPTIMISATION OF MEDIUM COMPOSITION FOR FERMENTATIVE PRODUCTION OF INULINASE

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Recently the interest in enzymes from microorganisms such as inulinase has increased because of wide application of it in the production of bioethanol from inulin and of high fructose content syrups or of prebiotic fructo-oligosaccharides. The development of fermentation medium with adequate composition is a necessary and important step for production of biocatalysts. The conventional "one-factor-at-a-time" approach is laborious and time consuming. Moreover, it seldom guarantees the determination of optimal conditions. These limitations of a single factor optimization process can be overcome by using statistical-based approach. Experimental design techniques are very useful tools for the selection of nutrients, as they can provide statistical models which help in understanding the interactions among the process parameters at varying levels and in calculating the optimal level of each parameter for a given target (i.e. maximal enzyme production). Response surface methodology (RSM) is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for the optimum conditions. It is a statistically designed experimental protocol in which several factors are simultaneously varied. The number of successful applications of RSM suggests that second-order relation can reasonably approximate many of the fermentation systems. In this study, powerful of RSM was investigated for optimisation of medium composition for enhancing the production of inulinase. In the case of *Kluyveromyces thermotolerans* Y00715, firstly two factors (inulin and urea) with five levels technique was applied. After 5 steps of experimental design, 1 (w/v) % of inulin and 0,21 (w/v) % of urea was found to be optimised concentration. Taken consideration that pure inulin from Dahlia is quite expensive, so concentrated liquor of Jerusalem artichoke (CLJA), yeast extract and ammonium phosphate were used in medium for production of inulinase. Three factors (CLJA, yeast extract and ammonium phosphate) at five levels per each were investigated. Results proved that the second order polynomial model was adequate to predict the inulinase activity. Optimal concentration of CLJA, yeast extract and ammonium sulphate were 0,4 (w/v) %, 0,22 (w/v) % and 0,16 (w/v) %, respectively. Applying this medium, about 1.7 U/ml enzyme activity was obtained that means about 3 times higher than before optimisation. In the case of *Thermomyces lanuginosus* thermophilic fungus, after subsequent optimization and checking steps, 1 % of JA and 0.6 % of peptone were found to be optimum concentration of main carbon and nitrogen sources. In this case, about 6.5 U/ml enzyme activity was measured at 48th hour of fermentation meaning 4 times higher than before optimization. Summarising, RSM was successfully adapted and applied to optimise the quantities of medium components for enhancing production of inulinase in the case of selected thermophilic and mesophilic fungus strains.

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ANTIMICROBIAL SUSCEPTIBILITY, INTEGRON AND VIRULENCE-RELATED GENE CARRIAGE AND GENETIC RELATIONSHIP OF SHIGELLAE ISOLATED IN HUNGARY FROM 1998 TO 2008

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The antimicrobial susceptibility and its connection to integron carriage, the presence of some important virulence-related genes and the genetic relationship of selected *Shigella* strains isolated in Hungary between 1998 and 2008 was investigated. *Shigella sonnei* (n=230) and *Shigella flexneri* (n=22) strains from stool samples of patients of outbreak and sporadic cases were tested against 10 antimicrobial agents by disc diffusion method. The presence of class1 and class2 integrons and the virulence-related ipaH, sen, stx and cdt genes was investigated by PCR. The integron-related resistance genes were sequenced. The genetic relationship of the integron carrier strains was investigated by PFGE and by plasmid profiling. Most of the *Shigella* strains were resistant to sulfamethoxazole/trimethoprim (66%) followed by streptomycin (47%), ampicillin (32%) and tetracycline (28%). A few strain was resistant to chloramphenicol (8%), kanamycin (2,8%), nalidixic acid (1,6%) or gentamicin (0,8%) either alone, or in combination. Sixteen percent of the strains exhibited multidrug resistance, mostly showing AmpSSxtTet resistance profile. None of the strains had ciprofloxacin resistance. A *S. sonnei* strain of imported origin was resistant to cefotaxime and harboured a blaCTX-M-55 type ESBL gene. Thirty-three percent (77/230) of the *S. sonnei* and 14% (3/22) of the S. flexneri strains had either class1 or class2 integrons, or both. Two types of class2 integrons were detected: a 2158 bp (dfr1-sat1-aadA1) was the most widespread found in 56 *S. sonnei* and 3 S. flexneri strains. Five *S. sonnei* strains had a 1456 bp (dhfr1-sat1) class2 integron. An 855 bp class1 integron (aadA1) was detected in 3 *S. sonnei* strains. Eleven *S. sonnei* strains had a 1586 bp class1 integron (dfrA1-aadA1) characteristic to the *S. sonnei* serotype only. Several *S. sonnei* strains had ipaH (40/77), a few had sen (10/77) and most interestingly one strain had stx1 gene, but none of them had cdt gene. Seven out of the nine investigated *S. flexneri* strains had ipaH, 3 had sen and 3 had cdtB, but none of them carried stx gene. PFGE analysis revealed that strains having different integrons represented different genetic clusters as well, but further correlation with either the geographical origin, or the isolation time, or dominance of any pulsotype was not detected. Plasmid analysis resulted in diverse profiles without a dominance of any profile. All strains had at least two, but usually more plasmids. In this study we identified the most frequent antimicrobial resistance patterns, determined the incidence of class1 and 2 integrons and provided data about the virulence characteristics and the genetic relationship of *Shigella* spp. isolated in the past decade in Hungary. Emergence of the blaCTX-M-55 ESBL gene in Hungary is reported for the first time. Knowledge of the antimicrobial resistance pattern of *Shigella* strains may be helpful in the antimicrobial therapy.

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INVESTIGATION THE *IN VITRO* ANTIFUNGAL ACTIVITY OF VARIOUS STATINS IN COMBINATION WITH PRIMYCIN AGAINST SOME CLINICALLY IMPORTANT FUNGI

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Recently, there is a growing interest in the investigation of antifungal activities of non-antifungal drugs. Statins are a group of drugs that are used to reduce the level of cholesterol in the human blood, but some studies revealed that they also have substantial growth inhibitory effects on different pathogenic bacteria and fungi. The aim of the present work was to investigate the *in vitro* antifungal activities of these lipid-lowering drugs. The investigated drugs were fluvastatin (Lescol), lovastatin (Mevacor), simvastatin (Vasilip), rosuvastatin (Crestor), atorvastatin (Atorvox) and pravastatin (Sigma). Primycin is a non-polyen type macrolide lactone antibiotic-complex, which has a specific effect on membrane permeability. It has broad antimicrobial spectrum, it is effective against Gram-positive bacteria in low concentration, while it has inhibitory effect on yeast and filamentous fungi in higher concentrations as well. In this study the *in vitro* interactions between primycin (PannonPharma Ltd.) and such statins in their effects also were evaluated.Twenty-six fungal strains, representing six species (*Candida albicans*, *Candida glabrata*, *Rhizopus oryzae*, *Paecilomyces variotii*, *Aspergillus fumigatus and Aspergillus flavus*) were investigated. The broth microdilution antifungal susceptibility testing and the checkerboard titration method were carried out according to CLSI guidelines. The interaction ratio was calculated using the Abbott formula. Our results showed that fluvastatin and simvastatin had the strongest antifungal activity among statins; atorvastatin, rosuvastatin and lovastatin were slightly effective, while pravastatin was completely ineffective. Natural statins (simvastatin and lovastatin) were active only in the hydrolyzed form. Primycin completely inhibited the growth of *Candida albicans* and *C. glabrata* in the range from 32 to 64 μg/ml, and was very effective against *P. variotii* (MIC: 2 μg/ml). *Aspergillus fumigatus* was moderately sensitive to this compound, but it had a little effect on *Aspergillus flavus* or *Rhizopus oryzae*. When primycin was combined with fluvastatin, lovastatin or simvastatin, both synergistic and additive effects were observed. Several primycin-statin combinations could be determined, which inhibited the fungal growth in lower concentrations when they were applied together. These observations suggest that fungal colonization can be affected on statin therapy and these compounds may be used also as antifungal agents. The activities observed for certain primycin-statin combinations highlight the promise of a combination topical therapy for patients with mucocutaneous infections.

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IN VITRO SUSCEPTIBILITY OF DIFFERENT ZYGOMYCETES TO STATINS AND COMBINATIONS OF STATINS AND AMPHOTERICIN B

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Various members of the Zygomycetes are among the most frequently isolated agents of mycotic diseases caused by non-*Aspergillus* moulds. The number of such diseases has increased dramatically over the last decades, especially in immunocompromised hosts and in patients with diabetes mellitus. Treatment with amphotericin B (AMB) and its lipid complexes is the standard and only effective

therapy, in spite of the fact that these are quite toxic and may have serious side-effects. Combined application of AMB with other effective antifungal agents allows decrease its therapeutic concentration and less toxic therapy. Statins (which were originally applied as cholesterol lowering drugs in human therapy) are interesting in this respect, because earlier reports presented that they are exhibiting inhibitory potential against some filamentous fungi inhere Zygomycetes.
The aim of the present study was to investigate the *in vitro* antifungal effect of different statins (atorvastatin, rosuvastatin, simvastatin, fluvastatin, lovastatin, pravastatin) and the combinations of the two most effective ones (fluvastatin and rosuvastatin) with amphotericin B against clinically important Zygomycetes. Six fungal isolates representing 4 clinically important genera (*Absidia*, *Rhizomucor*, *Rhizopus*, and *Syncephalastrum*) were involved in this study. The antifungal effect of statins and statin-AMB combinations was evaluated by broth microdilution assays at neutral pH. Statins showed substantial differences in their antifungal activity. The synthetic statins (atorvastatin, fluvastatin and rosuvastatin) proved to be more effective than the fungal metabolites (lovastatin, simvastatin and pravastatin). All investigated isolate proved to be sensitive to fluvastatin. Fluvastatin and rosuvastatin acted synergistically and additively with AMB on the growth in clinically available concentration ranges. The observed antifungal activity of the investigated combinations has a therapeutic potential against fungal infections caused by Zygomycetes species. Furthermore it would create new potentials in the treatment of such diseases without serious side-effects.

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IN VITRO SYNERGISTIC INTERACTIONS BETWEEN STATINS AND VARIOUS AZOLE ANTIFUNGALS AGAINST SOME CLINICALLY IMPORTANT FUNGI

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In the last years several studies have focused on the development of combination therapy to improve efficacy of antifungal treatments. Azoles are frequently used to control fungal infections: these compounds target the ergosterol biosynthesis by inhibiting cytochrome P450-dependent 14α-lanosterol demethylation. Statins are currently used for hyperlipidaemia control and protection from cardiovascular events, but they known to have pleiotropic (e.g., anti-inflammatory, immunomodulatory and antioxidant) effects. In addition, there are more and more evidence for the potential of statins to prevent and treat microbial infections. In the present study, the *in vitro* interactions between various statins (lovastatin, fluvastatin, simvastatin, rosuvastatin, atorvastatin, pravastatin) and various azole antifungal agents (miconazole, ketoconazole, fluconazole, itraconazole) have been studied by the checkerboard broth microdilution method. Isolates of *Candida albicans*, *Candida glabrata*, *Rhizopus oryzae*, *Paecilomyces variotii*, *Aspergillus fumigatus* and *Aspergillus flavu*s were investigated. The interaction ratio between statins and the antifungal agents was calculated using the Abbott formula.When statins were combined with azoles, synergistic interactions were observed in several combinations, when the investigated strains were sensitive to only the azole or to the statin compound. For example, atorvastatin and itraconazole acted synergistically on the inhibition of growth of nearly all fungal isolates. When the investigated strains were sensitive to both compounds, additive interactions were generally noticed. Similar interactions were observed when the variability of the within-species sensitivities against some selected drug combinations was investigated.
The combinations of statins and different azole antifungals resulted in additive or synergistic

interactions in many cases at levels which can be achieved clinically in human serum. However, azole-statin combinations might be applicable only as topical therapy, because severe drug interactions can arise, when these compounds are administered simultaneously.

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PHYLOGENETIC AND PHYSIOLOGICAL CHARACTERIZATION OF FILAMENTOUS FUNGI BELONGING TO THE ORDER MORTIERELLALES

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Members of the order Mortierellales constitute a remarkable group of Zygomycetes fungi. The lipid accumulating species of this group have biotechnological significance as industrial producers of polyunsaturated fatty acids, such as arachidonic acid; other species are known as biotransforming agents of different organic compounds. In spite of their practical importance and high ecological and physiological diversity, evolutionary relationships among the species of Mortierellales remained unresolved. Actual classification of the order is highly unnatural and based mainly on morphological observations. The aim of the present study was the characterization and comparison of representative species of the main Mortierellales genera using different microscopic, physiological and molecular approaches. Therefore, sequence of the ITS region (including the 5.8S rDNA flanked by the internal transcribed spacer 1 and 2) was determined from more than 60 independent isolates representing about 25 different species; sequences found in international data banks were also involved into the analysis. Phylogenetic analysis suggested a variety of natural groups with monophyletic origin and revealed that the traditional, morphology-based family system of the order substantially deviates from the molecular phylogeny. Some of the established monophyletic groups contain species currently distinguished at a genus level (e.g. the genera *Mortierella*, *Gamsiella* and *Dissophora*).
Carbon assimilation of about 50 isolates was also examined testing 67 different compounds as sole carbon sources. Compounds able to discern groups among the involved isolates could be identified.

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IMMUNOSENESCENCE

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The growing ratio of ageing world population and the steady increase of life expectancy place new challenges for the public health system, because the elderly suffer from more frequent and severe infections than young people. Vaccination could protect the elderly against several infectious diseases, but it can be effective if cells that are capble of responding are still present in the repertoire. Due to normal age-related immune impairment, vaccination may fail in many cases.

Immunosenescence affects both innate and adaptive immunity. Frequent comorbidities in the elderly augment immune defects. Abnormal macrophage-derived cytokine production, NK and T cell cytotoxicities lead to respiratory and gastrointestinal infections. Thymic atrophy results in reduced output of new T lymphocytes and consequently accumulation of anergic memory cells. Ageing dampens production of naive B cells. Instead of ineffective vaccination campaigns, prevention and/or reversion of age-related immune impairment have come into focus. Nutritional interventions, boosting telomerase activity and expression of toll-like receptors can be achieved by chemoterapy. Thymic atrophy could be reversed by thymus transplantation, depletion of dysfunctional naive T cells and herpesvirus-specific exhausted memory cells. Immunostimulatory and anti-inflammatory cytokines can also be adminstered, e.g. IL-7, IL-2, IL-10 as well as leptin and growth hormone. More efficacious vaccines such as DNA vaccines, virosome vaccines and new adjuvants might come into the medical practice in a short time.

GROWTH INHIBITION EFFECT OF CENTRAL EUROPEAN CULTIVATED AND WILD FRUITS AGAINST ACNE-INDUCING BACTERIA

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Acne vulgaris is the most common skin disease in the world. It is not a serious disease but it often involves both physical scarring and social embarrassment. *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* have been recognized as the most important acne-inducing bacteria. Numerous topical and oral drugs are available to treat acne vulgaris, but the numbers of antibiotic-resistant strains have been increasing in the past years. Natural substances from plants might be promising candidates to treat acne vulgaris.

In the present study, *in vitro* biological activity of the juice, as well as water and methanol extract of the pomace of 20 Central European cultivated and wild fruits were investigated on 4 acne-inducing bacteria (*P. acnes*, *S. aureus*, *S. epidermidis* and *S. pyogenes*). The antibacterial effect of juices and pomace extracts was evaluated by broth microdilution assays at pH 7 and at skin natural pH 5.5. Red, purple berries (*Ribes rubrum*, *R.* x *nidigrolaria*, *R. uva-crispa*, *Rubus fruticosus*, *R. idaeus*) and *Prunus armeniaca* revealed a substantial antibacterial effect. *Staphylococcus* strains were the most sensitive to the investigated juices, and *S. pyogenes* to the methanol extracts. *P. acnes* was the most insensitive bacterium in this study. The exerted growth inhibition effect by *Ribes uva-crispa* juice was stronger at acidic pH (MIC=0.40 mg/ml) than at neutral pH (MIC=5.30 mg/ml). Significant difference was not observed between the antibacterial effects of the other fruits at the investigated pH values. The antibacterial activity of the investigated fruits suggested that they have promising natural antibacterial compounds to treat acne vulgaris. Our results urge the need of further studies to prove the practical efficiency, and to reveal the antibacterial compounds from these fruits.

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EVOLUTION IN THE LIGHT OF GENOME PROJECTS

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In 2009 the world is celebrating Charles Darwin for two reasons: Darwin was born 200 years ago on February 12, 1809 and his most important work, "The Origin of Species", was published 150 years ago, on November 24, 1859. The significance of the theory outlined in this book can be compared only to that of the Copernican Revolution. In fact, the Copernican and Darwinian Revolutions are usually considered to be two distinct stages of the same Scientific Revolution. Following the Copernican Revolution mankind's conception of the universe was schizophrenic till the middle 19th century. In this conception of the universe scientific explanations dominated the nonliving world, however, supernatural explanations were accepted as explanations for the origin of living creatures. Darwin's theory resolved this conceptual schizophrenia: he has shown that the origin and evolution of species may be also explained by natural laws. Darwin's theory of 'natural selection' gave a surprisingly simple explanation for the diversity of species: variants arise as a result of random changes and of these variants those will survive and proliferate that prove to be more viable in the struggle for survival. Soon after the publication of this book the theory became generally accepted by the scientific world and has triggered the rise of modern biology. The famous dictum of Theodosius Dobzhansky („Nothing in biology makes sense except in the light of evolution") expresses most clearly the enormous influence of Darwin's theory on Biology.

Today Darwin's theory has greater influence on biological sciences than ever as evidenced by scientometry. In the middle of the 1990s the number of citations of Darwin's Book and theory started to experience a sharp increase coinciding with the start of the genomics revolution. There are three main reasons for this correlation: 1) For the first time, genomics provides large amounts of high quality biological data that permit a systematic analysis of questions raised by Darwin's theory; 2) Darwin's theory provides tools for the interpretation of genomic information; 3) New information from comparative genomics and system biology necessitates the reevaluation of some major dogmas of neo-Darwinism. It must be emphasized, however, that the key elements of Darwin's theory are unaffected by these changes and his 150 year old.

DEVELOPMENT OF A NOVEL MOLECULAR QUANTIFICATION METHOD FOR PROBIOTIC BACTERIA, CULTIVATED IN PREBIOTIC-CONTAINING LIQUID MEDIUM

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Though the beneficial effects of probiotic bacteria were discovered by Metchnikoff in the first third of the last century, a couple of decades elapsed until the industrial production of probiotic products started. Later on it turned out that there are a couple of substances, the so-called prebiotics that help the preservation of human health via their beneficial impact on bacteria.

At the beginning of the work our aim was to elaborate such a molecular method that makes the verification of the presence and the quantification of probiotic bacteria faster and simpler. Traditionally quantification of and presence verification of bacteria are made on selective media but this method is time consuming, especially in case of slowly growing bacteria. Contrary to the traditional method there are molecular methods, like PCR (polymerase chain reaction) and real-time PCR, used by us that allow us to realize the investigations within a few hours.

In our cultivation experiments we use three probiotic bacteria (*Bifidobacterium bifidum*, *Enterococcus faecium*, *Lactobacillus acidophilus*) and the *Escherichia coli* as indicators, to

investigate the impact of prebiotic materials on these bacteria. We isolated DNA from the bacteria by the use of three different kits: with one of them the DNA is precipitated by isopropanol, whereas in the other two kits the DNA is bound to the filters. The precipitation based kit can be used efficiently in those cases where solid materials (e.g. flour) are present in the liquid cultivation medium, whereas the filter based kits are efficient if bacteria are cultivated in a medium that does not contain solid materials. For the PCR based identification of bacteria we used primers that were collected from the literature. We had full success in the real-time PCR based identification and quantification of *E. coli*, but we need to do more experiments and optimization to get the same result with the other three bacteria, however we have some reassuring results. The expected result of our work is the elaboration of such a quick and reliable practical method that can replace the work-, tool- and time-consuming microbiological methods in this kind of examinations.

ETIOLOGICAL INVESTIGATIONS OF NATURALLY OCCURRING RUNTING-STUNTING SYNDROME IN HUNGARIAN FLOCKS

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Runting-stunting syndrome (RSS) is a major enteric disease complex of broiler chicken, causing severe economic losses worldwide. The syndrome is characterized by various clinical signs, such as diarrhoea, depression, ingestion of litter, increased vocalization and huddling. The level of morbidity and mortality are variable, while the economic impact from RSS is primarily due to poor production, failure of affected birds to grow, as well as increase in costs of therapy, and poor feed conversion efficiency. The etiology is still disputed; viruses from numerous families (*Astroviridae, Coronaviridae, Reoviridae, Rotaviridae*, and more recently *Parvoviridae*) have been identified in the intestinal tracts of poultry with enteric disease. These viruses were also isolated from clinically healthy chickens, suggesting that a certain mixture of pathogens combined with environmental factors, may be needed for the disease to appear. No vaccine preventing RSS has been developed so far. Carcasses from five Hungarian broiler flocks experiencing increased mortality were sent to the Department of Pathology and Forensic Veterinary Medicine (Szent Isván University, Budapest, Hungary) for diagnostic purposes. RSS was confirmed in all five cases based on the macroscopic examination and histopathology, furthermore presence of viruses in different organs was demonstrated by electron microscopy (EM) and polymerase chain reaction (PCR) techniques, employing a wide range of diagnostic primers, targeting the involved viral pathogens. Our investigations also revealed the presence of the scarcely known chicken parvovirus (ChPV) in all five flocks. The ChPV nucleic acid sequence analysis revealed that the Hungarian strains share a very high level of identity among themselves.

The phylogenetic analysis has shown that the Hungarian strains are more closely related to a previously described ChPV strain of turkey origin, than to the also described chicken isolate. This finding seems to indicate the potential role of turkey flocks in the epidemiology of ChPV. The scope of this study was to determine the pathogens involved in the flocks naturally affected by RSS, and also to perform the nucleic acid sequence analysis of the scarcely known ChPV.

EFFECT OF PRIMER SELECTION, PREFERENTIAL LIGATION AND CLONE LIBRARY SIZE ON THE BACTERIAL COMMUNITY COMPOSITION PICTURE OF ENVIRONMENTAL SAMPLES

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Clone library analysis of the full or partial 16S rDNA region is still one of the most commonly used methods describing microbial communities. Concerning the community structure, many investigations draw consequences from the composition of the clone library. Experiments on model communities proved that size a sequence dependent preferential ligation may occur, possibly biasing the community picture obtained with this technique. Two widely used cloning systems were tested in parallel with a semi quantitative method on a sediment sample of Lake Hévíz to reveal these biases. DNA extracted from the sediment was amplified by PCR using universal eubacterial primers targeting the first third of the 16S rDNA gene (positions: 63-519). Community fingerprints were made by length heterogeneity PCR, using TET labeled forward primes and capillary electrophoresis. The PCR product was cloned into pGEM (Promega) and TOPO (Invitrogen) vectors in parallel. Two ~300 CFU large clone libraries were constructed, clones were grouped by ARDRA and insert length of all OUTs were measured by capillary electrophoresis. This allowed us to compare the community structure shown by the LH-PCR fingerprints and the two libraries, proving the preference of clone libraries for average-sized inserts. Sequence analysis of all OTUs provided information on phylogenetic selectivity of different cloning systems.
Comparison to parallel clone libraries using different primers on the same sampling site showed strong selectivity of the primer pairs used as well. These findings indicate that members of a diverse microbial sample may be excluded during clone library construction because of preferential amplification and ligation, this way biasing the true community picture.

IMPROVEMENT OF CAROTENOID PRODUCING ZYGOMYCETES FUNGI

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Currently, there is an increasing interest in the development of new carotenoid sources from microbial origin and serious efforts are made to improve the fermentation pigment production. The aim of our study was to investigate the possibility and the biological requirements of the future application of *Mucor circinelloides* (a model organism of the fungal carotenoid biosynthesis) as a carotenoid producer. Therefore, transformation experiments were carried out using endogenous and exogenous carotenogenic genes to obtain *M. circinelloides* strains with higher carotenoid content and/or able to produce new valuable compounds. The resulting strains were then used to characterize the introduced and modified genes and to examine the effect of the culturing conditions on the carotenoid production. Different genes encoding the steps of the general mevalonate-isoprenoid pathway was over-expressed in the fungus using the gene-dose effect or by the modification of their promoter and strains with elevated beta-carotene content could be created. At the same time, a beta-carotene ketolase gene from a bacterial origin was expressed in *M. circinelloides* and production of

new carotenoid compounds, such as canthaxanthin, echinenone and astaxanthin, was detected in the transformants. Co-transformation experiments with the modified isoprenoid genes in combination with the exogenous beta-carotene ketolase gene resulted in strains producing high levels of canthaxanthin and echinenone. Several transformation systems were tested to obtain stable transformants; transformation experiments were performed in different autoreplicative and integrative systems. Chromosomal integration events were verified and mitotic stability and the copy number of the introduced DNA was also analysed as the expression levels of the introduced/modified genes.

MOLECULAR IDENTIFICATION AND PHYLOGENY OF *MORTIERELLA WOLFII*

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Mortierella wolfii is a thermophilic soil fungus, the only species in the order Mortierellales (Zygomycetes) that is recognized as pathogen. This toxin producing fungus is an important casual agent of bovine mycotic abortion, pneumonia and systemic mycosis (mortierellosis) in New Zealand, Australia, Europe and the USA. Currently, species determination of *M. wolfii*, as well as of other *Mortierella* spp., requires a detailed morphological study carried out by a practised mycologist, which has usually not been performed in the reports found in the literature. The aim of our study was to establish molecular markers useful for stable species identification and to examine the phylogenetic relationships among this thermophilic fungus and other species belonging to the genera *Mortierella*, *Gamsiella*, *Dissophora* and *Echinosporangium*. Sequences of five regions including the SSU and LSU rRNA genes, the ITS region and two protein coding genes (tef and ftr) for several isolates of abovementioned genera were determined and involved in the phylogenetic analysis. Sequences useful for the identification of *M. wolfii* isolates and those of related species could be determined. Results of the phylogenetic analysis suggest that the currently accepted taxonomy of the order Mortierellales (based primarily on morphological studies) is highly unnatural.
In our analysis, *M. wolfii* highly differed from the other *Mortierella* species and seemed to be an outermost group within the order Mortierellales.

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BIOSYNTHESIS OF CYCLOPHILIN FKBP12-BINDING NATURAL PRODUCTS, NEW WEAPONS IN MEDICINE

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The biosynthesis of the medically important and structurally related compounds rapamycin, FK520 and FK506, all FKBP12 binding natural products, has generated a pronounced interest, due to their immense pharmaceutical potential. They represent a new versatile weapon to fight cancer as well as to treat cardiovascular, autoimmune and neurodegenerative diseases. A significant demand has therefore arisen during the past decade for the development and production of these compounds and

their analogs. A number of semi-synthetic analogs have been developed. However, semi-synthetic approaches are relatively limited due to the structural complexity of these compounds. The biosynthetic engineering approach, on the other hand, represents a unique opportunity for the generation of novel analogs, thus potentially extending and/or complementing semi-synthetic efforts. The biosynthetic pathways involved in the formation of these natural products have only been revealed to a great detail during the last decade. The complexity of these mixed polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) systems is seen as a great opportunity to develop the technology and to generate a large number of potentially useful analogs. The understanding of the biosynthetic pathways and the approaches which have been developed to generate medically useful analogs through synthetic chemistry and/or biosynthetic engineering will be presented. Finally, the regulation of the biosynthesis by *Streptomyces* strains will be discussed using the rapamycin biosynthetic pathway as a model system.

ANTIFUNGAL ACTIVITY ASSESSMENT OF FINISHED COTTON FABRICS

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An investigation into the antifungal activities of antimicrobial, hydrophobic and oleophobic finished cotton fabrics designed for tents and marquees was performed according to the AATCC 30-1999 (IV) test method [1]. The growth of test moulds *Aspergillus niger*, *Penicilium pinophilum* and *Trichoderma harzianum* on differently treated cotton fabric samples was evaluated according to the standard procedure and additionally using image processing and analysis, which has, to a great extent increased the accuracy of standard organoleptic estimation. Image processing and analysis enabled an exact evaluation of test microorganisms' growth over a percentage area of a fabric sample covered by fungi, after a defined exposure period. The introduction of an evaluation technique using image processing and analysis was shown to be suitable for the purpose of assessing fungal attack. The results of the preformed tests on selected antifungal compounds showed significant distinctions even between those cotton fabric samples treated with different amounts of antifungal agents.

[1] AATCC Test Method 30-1999. Antifungal Activity Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials. 2002. In: AATCC Technical Manual 2002. Research Triangle Park, American Association of Textile Chemists and Colorists, pp. 81-84.

TWO NEW METHANOL ASSIMILATING YEAST SPECIES FROM HUNGARY

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The number of methylotrophic yeast species has steadily increased since the first report [1] on methanol utilization by yeasts, and the current number of known methanol-assimilating yeast species is approaching 60. The majority of them are distributed in three clades in the phylogenetic trees constructed from nucleic acid sequences. These are the *Ogataea*, the *Kuraishia* and the *Komagataella*

clades. The methanol assimilating yeasts are often associated with the decaying wood of trees and the necrotic soft tissues of succulent plants [2] or with tree exudates.
During a survey of methylotrophic yeasts, several strains of two undescribed yeast species (three strains of each) were recovered from rotten wood, tree leaf and freshwater samples collected in Hungary. One of the new species is an anamorphic member of the *Kuraishia* clade and is very closely related to *Candida hungarica*. However, the comparison of the sequences of the D1/D2 domain of large subunit rRNA gene, the ITS and the mitochondrial small subunit rRNA gene revealed that the three strains represent a distinct species. We proposed *Candida ogatae* sp. nov. to accommodate these strains [3]. The other new species is an ascosporulating member of the *Ogataea* clade, and genetically is most closely related to, but is distinct from *Pichia pilisensis*. The phenotypic characteristics of the three strains representing this species are also very similar to those of *P. pilisensis*. We proposed *Ogataea nitratoaversa* sp. nov. as a new species based on these strains [4]. As *O. nitratoaversa*, like a few other *Ogataea* species, do not assimilate nitrate as a sole nitrogen source, the emendation of the diagnosis of the genus Ogataea Yamada, Maeda & Mikata [5] from "Potassium nitrate is assimilated." to "Nitrate assimilation is variable." was also proposed, in order to allow inclusion of the phylogenetically related species which do not assimilate nitrate [4].

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ELECTRICITY GENERATION IN A BIOHYDROGEN FUEL CELL USING A FERMENTATION GAS PRODUCED BY RUMEN CILIATES

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Microbial fuel cells (MFCs) convert the chemical energy of natural, organic compounds directly into electric energy with the aid of live microorganisms. Generally, metabolic cell processes associated with anaerobic oxidation of nutrients mainly glucose can supply fuel for direct oxidation in the anode compartment, or they can be a source of electrons directly transferred to the anode. To date, several different ways have been proposed to use microorganisms for producing electrical energy. One of them involves the biological and electrochemical parts being separate and working independently. *E.g.*, a hydrogen-rich, fermentative gas produced in a bioreactor by microorganisms can be transported to a proper fuel cell and be used there as the fuel. This concept, as well as analogous systems with the hydrogen bioreactor and the CFC combined, is often considered not to be a typical MFC because the fuel cell is in fact a chemical one. However, the hydrogen is still generated microbially). Despite that, in this work the term "biohydrogen fuel cell" (BHFC) is used.
Here we present a high-performance BHFC that has been constructed and operated on glucose. The fuel cell consisted of a bioreactor and a chemical fuel cell (CFC) of the polymer-electrolyte type. The bioreactor supplied gaseous fuel for the chemical fuel cell. The fuel gas was a hydrogen-rich gas produced from glucose fermented by rumen ciliates mainly of the *Isotrichidae* family. The protozoa were isolated from the rumen fluid of cows and separated from intra- and extracellular bacteria and methanogens by incubation with antibiotics. A good match between the CFC and the fuel gas was

proven by the MFC performance being equal to the performance of the CFC running on pure hydrogen as well as by the lack of any fuel cell poisoning effects in the course of a one-day continuous experiment. A maximum power density of 166 mW cm^{-2} (geometric area) obtained at room temperature is amongst the highest values obtained in MFC studies. The configuration of the bioreactor limited the continuous operation time of this MFC to *ca.* 14 hours. Nevertheless, it was shown for the first time that the rumen protozoa could be successfully used to produce biohydrogen and to build a high-power-density MFC.

REDUCTION OF *CAMPYLOBACTER JEJUNI* BY NATURAL ANTIMICROBIALS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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To assure food quality and safety, many different preservative and/or decontamination agents are used for control of pathogenic and spoilage organisms in food production and supply chain. Recent consumer demands favour natural antioxidant and antimicrobial agents, such as phenolic plant extracts. In our experiments, we tested the antimicrobial activity of commercial rosemary extract formulations against different gram-positive and gram-negative bacteria [1]. Thermophilic *Campylobacter* species have become the most prevalent food-borne pathogens, mainly associated with fresh poultry meat, but transmitted also with red meat and other cross-contaminated food products [2]. Thus, we tested the effect of selected rosemary extracts against *Campylobacter jejuni* under different environmental conditions, either alone or in combination with nisin. Tests were performed in laboratory media and in a chicken juice, which resembled a natural food environment. The effect of low storage temperature (8°C) and freezing on the activity of rosemary extract against *C. jejuni* was also assessed. Rosemary extract proved to be more effective in laboratory media than in a chicken juice. Low temperature storage conditions prolonged the survival of *C. jejuni* in a chicken juice and decreased the extract activity. Nisin has not been effective alone and neither did a combination of extract and nisin display the expected antimicrobial activity against *C. jejuni*. However, freezing in combination with plant extract addition proved to be the most effective combination in reduction of *C. jejuni* cells. Thus the experiments in a chicken meat model system [3] were also carried out. The results again demonstrated the synergistic activity of freezing and plant extract antimicrobial activity in reducing the number of *C. jejuni* cells, which proved to be promising for reduction of *Campylobacter* risk in poultry meat supply. This work was carried out in the frame of Biotracer EU project (Improved bio-traceability of unintended microorganisms and their substances in food and feed chains FP7-036272).

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NEW FEATURE OF *MICROCOCCUS ROSEUS* SOIL INOCULANT BACTERIUM: HIGHLY EFFECTIVE INDOLE-3 ACETIC ACID PRODUCTION

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An increasing need for environment-friendly crop production worldwide promotes the development of alternatives to synthetic agrochemicals (chemical fertilizers and pesticides). The use of plant growth promoting rhizobacteria (PGPR) as soil inoculants denotes an alternative to chemicals. Besides the environment friendly consequences, soil inoculation with effective PGPR may result in increased plant biomass production and crop yield. These microorganisms are promising tools for sustainable agriculture. Plant growth promotion is a complex phenomenon, including biofertilization by biological nitrogen fixation, biocontrol effect by preventing deleterious effects of phytopathogens in the rhizosphere, nutrient solubilization in the root zone, and the phytostimulation by production of plant growth regulating phytohormones. The commercial soil inoculants, - for the efficient phytostimulation -, commonly contain the well known *Azospirillum brasilense* bacterium. *Azospirillum brasilense* produces and releases a considerable amount of indole-3-acetic acid (cytokinins and gibberellins in less amount) during the root and rhizosphere colonization.

In our work we conducted research on the soil inoculant bacterium *Micrococcus roseus* ssp. NCAIM/P/B 001294. This strain is a unique component of some commercial microbial soil inoculants (BactoFil® products). It is known that it exerts remarkably advantageous effect on the growth and development of inoculated plants, both in field and greenhouse. However, the background of this effect was not elucidated. It was supposed that the ability of the strain to improve the soil physical structure - by production of viscous polysaccharide material - certainly contributes to the beneficial effect (patent nos. US20050060930, WO03016241).

We present a new feature of this bacterium strain, its indole-3 acetic acid production of high-capacity, which is comparable to that of *Azospirillum brasilense*, the highly efficient IAA producer and phytostimulator. We examined the IAA production capacity of the bacterium in different culture media and drew conclusions on the effects of the sole carbon sources (organic acid, sugars and different proteins sources). Kinetics of the production of IAA was also followed for 10 days in liquid cultures, and incubation times required for the maximal production were determined. All experiments were performed both in the presence and absence of added L-tryptophane (100 microgram/ml medium). Results suggest possible tryptophane-dependent pathway/s of the IAA biosynthesis.

Results presented here are new scientific additions, supporting the particular beneficial effect of BactoFil® on plant growth, consistently manifesting in the field applications.

This work was supported by Kozma Lászlo Program TUDAS_06-SANI0609 grant of National Office for Research and Technology, Hungary.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *SIDERITIS REASERI* SPP. *REASERI* ETHANOL EXTRACTS

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Ethanol extracts obtained from aerial parts of cultivated *Sideritis reaseri* spp. *reaseri* have been investigated for their antimicrobial activity. Plant material in five different stages of flower development were collected from experimental field of Institute for medical plants research in Pancevo (Serbia). Antimicrobial activity was determined with *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Sarcina lutea* ATCC 9341, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 by broth microdilution method using 5 or 2,5% water solution of vacuum dried extract residues. Minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) was determined according to the National Committee for Clinical Laboratory Standards. The extracts of *Sideritis reaseri* L. exhibited antimicrobial activity of various degrees against the tested strains. Maximum activity all five different extracts was observed against *Staphylococcus aureus* and *Salmonella enteritidis* but had no activity against two tested fungal strains (MFC>2,5%).

RAPID DETECTION OF ENTEROVIRUSES IN CLINICAL SPECIMENS BY SHELL VIAL ASSAY: COMPARISON WITH CONVENTIONAL VIRUS ISOLATION METHOD

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The human enteroviruses (HEV) can cause a wide spectrum of human illness, from mild nonspecific fewer, upper-respiratory infections to paralytic poliomyelitis, severe myocarditis, hepatitis, diabetes mellitus and aseptic meningitis. Rapid enterovirus detection is important for decisions about antibiotic administration and length of hospital stay. At present, the diagnosis of enterovirus infections is usually carried out by virus isolation in tube cultures inoculated with appropriate clinical samples. In the present study we assessed the efficacy of rapid shell vial culture technique for detection of HEV in clinical samples, in comparison to that of the conventional virus isolation procedure. A total of 70 clinical specimens (29 cerebrospinal fluid specimens, 27 stool specimens, 9 rectal swabs and 5 throat swabs) were obtained from 52 patients with clinical suspicion of aseptic meningitis. For both, conventional culture and shell vial culture, Vero, Hep-2 and RD continuous cell lines were used. In conventional virus isolation procedure, inoculated cell lines were examined daily for 21 days or until the appearance of cytopathic effect. Rapid shell vial detection of enteroviruses in clinical specimens was carried out using centrifugation (600×g for 60 minute at 35°C) after inoculation, followed by 72h incubation. In both methods, at the end of incubation period all cell cultures were subjected to immunofluorescence staining with genus-specific 2E11 monoclonal antibodies (Chemicon International, Temecula, CA). Thirty-six samples were positive by traditional tube-culture isolation (51.4%), whereas 34 samples were positive by shell vial assay (48.6%). Detection time of HEVs by conventional method ranged from 2-13 days (mean time, 6.1) compared to 3 days for shell vial method. After 72h from inoculation, the sensitivity of shell vial assay was 94.4% and specificity 100%, with respect to conventional virus isolation method. Within the same period, a cytopathic effect compatible with the presence of an enterovirus occurred in only 15 of the

36 (41.7%) specimens that eventually became positive. Although shell vial assay displayed slightly reduced sensitivity compared with conventional cell culture after 21 days, the markedly superior 72h enterovirus detection rate supports incorporation of shell vial assay into the routine clinical setting.

THE PREDICTIVE VALUE OF P16/INK4 PROTEIN STAINING FOR CERVICAL DYSPLASIAS ASSOCIATED WITH HUMAN PAPILLOMAVIRUS (HPV) INFECTION.

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Human papillomaviruses (HPV), members of the Papillomaviridae family, have been characterized mainly as DNA species traditionally grouped into 118 genotypes based the nucleotide dissimilarity of their capsid polypeptide (L1) sequence. Genotypes frequently associated with cervical dysplasias and subsequently developing squamous cell carcinomas (SSC) of the female genital tract (5, 8. 16, 18, 30, 31, 33, 35, 39, 45, 51, 52, 56, 58, 66 and 70) were categorized as high risk (HR-HPV) and ordered into 12 out of 15 species within the Alphapapillomavirus genus. During latency, especially the DNA of HR-HPV genotypes gets integrated into the host cell chromosomes. During this, disruption of the circularized episomal genome at the E2/E1 polypeptide sequence occurs. Following integration therefore, the expression of HPV oncoprotein E7 increases. If the E7 protein is present in high levels, the expression of a cellular division regulating protein, p16/INK4A (inhibitory kinase 4) also increases. Detection of p16 protein by immunohistochemical staining is much easier than that of the E7 oncoprotein. In our hands, parallel sections from 432 randomly selected blocks representing biopsies of 178 women with the diagnosis of cervical dysplasia were stained for the p16 polypeptide. In mild dysplasia (CIN I/LSIL), the p16 antigen was found in 65 out of 80 cases (81%). The p16 protein was typically seen in dysplastic basal and suprabasal cells forming a confluent layer in the lowest segment of the stratified epithelium. The typical distribution of p16 antigen in HSIL encompassed over two thirds and/or the whole dysplastic stratified epithelium. The positive rates for p16 antigen presence in medium (CIN II) and severe (CIN III) dysplasias (both HSIL), were 95% (45 out of 47) and/or 100% (each of 27), respectively. Extensive staining for p16 antigen was seen within nuclei as well as cytoplasm of neoplastic cells in all 6 cervical squamous cell carcinomas, which were examined in many sections when being used as positive controls. The detection of p16 in cervical smears from women with mild dysplasia (LSIL) and/or uncertain epithelium cell atypia (ASCUS = atypical squamous cell of undetermined significance) was made using both, the same kit as for bioptic sections and a new (CINTec) cytology kit. The letter was found more specific at handling smears. The p16 staining using both kits was compared with HPV DNA detection (by the HC-2 method) aiming to evaluate the significance and the predictive value in cervical smears.

MURID HERPESVIRUS 4 (MUHV 4): STORY OF THE MOUSE GAMMAHERPESVIRUS MODELLING EBV INFECTION

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Murid herpes virus 4 (MuHV 4), a natural pathogen originally isolated from free-living rodents, encompasses 7 isolates identified and described at the Institute of Virology, SAS, in Bratislava. Based on DNA sequence homology, it was classified to the subfamily Gammaherpesvirinae. In

addition to the genomes of two human viruses, the Epstein-Barr virus (EBV, HHV-4), the Kaposi sarcoma herpesvirus (KSHV, HHV-8), the primate herpesvirus saimiri (HVS) and of the murid herpesvirus 4 (MuHV 4) genomes were fully sequenced and shown to possess closely related genes. The author shall review the functional significance of the genetic assemblage of above mentioned gammaherpesviruses (KHSV, EBV, MuHV 4 and HVS) regarding to their role in pathogenesis and oncogenesis. At least two MuHV 4 isolates, namely MHV-68, MHV-72, were widely studied and became routinely propagated laboratory strains. Following intranasal inoculation, both induced an acute infectious mononucleosis-like syndrome with elevated levels of peripheral blood leukocytes, shifts in the relative proportion of lymphocytes along with the appearance of atypical mononuclear cells. During latency (until 24 months post-infection), which was observed with the majority of isolates, the carrier mice infected with either MuHV 4 strain and/or isolate (except of MHV-76) developed lymphoproliferative disorders. Genes the expression of which is inevitable for productive virus replication were usually analyzed separately from those, which are expressed during latency. The lack of tumor formation in MHV-76 infected mice was associated with persistent virus production at late post-infection intervals. At least two MuHV 4 isolates revealed spontaneous deletions at the 5´-end of their genome, resulting in the absence of at least M1 and M2 M3 as well as of the M4 gene (strain MHV-76). Based on DNA sequence amplifications only, another two isolates (MHV-Zum and MHV-60) were shown to possess similar deletions of varying length. In addition to careful analysis of spontaneously occurring 5´-end genome defects, our knowledge of the function of 5´-end genes relies on the behaviour of mutants with corresponding deletions and/or insertions. While M2 and M3 genes encode immune evasion proteins, the M4 codes for a soluble glycopeptide acting as immunomudolator and/or immunostimulator

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CURRENT CHALLENGES FOR FOOD SAFETY FROM MICROBIOLOGIST POINT OF VIEW: HOW MUCH WE CAN GET FROM GOOD PRACTICE APPROACH IN FOOD MICROBIOLOGY EDUCATION?

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Food microbiology is applied science dealing with practical attitude of microbiology in food area from food producer to food consumer. It is constantly on crossroad between food science and microbiology and is still fighting for full independence, although at least theoretically is established as independent discipline. Food technology, and in particular food microbiology, is facing fast development in the last century. Consequently we have food mycology, food bacteriology, food zymology, and lately also food virology, what reflects new professions in the area of food microbiology who should speak the same language with all different food professionals along food supply chain. Currently food safety occupies the top of this fast growing pyramid. However, education creates basic communication network necessary for its functioning and has to assure the same knowledge and skills principles in this field. The question is how much novelty it tolerates and how to introduce it via education to assure relevant and efficient start up learning/teaching but also later lifelong learning/teaching. Education of food safety should be based on good practice approach.

Education in food microbiology is based on natural science principles. However, food technology education is based on historical experiences enriched with process and engineering principles. We have lately published conceptual approach towards total food safety management, which seems relevant for this issue. Complexity of food area is asking for a standardized approach. For this reason, good practices entered the field very efficiently [1], however this is not sufficient to manage its complexity. It is proposed to cluster practices under comprehensive shield of good nutritional practice. Likewise in food cluster, also in education we face new streams and new practices on learning and teaching side. They are, in principle, coming to the food education from pedagogical area. Consequently, we see the possibility to fuse experiences from various sides in a novel model of education in food microbiology area which would harvest good practice principles from food management with principles arising from pedagogical background. To our opinion, both concepts can be integrated into new platform good practice in education. On vertical level we erect seven main principles based on contacts, cooperation, active learning, regular respond, management skills, personal goals, diverse talents and personal attitude to learning. To be efficient, this is not enough; we need to network these principles with personal practice of students and educators to realize relevant skills. For that reason seven drivers are proposed: professional activity, expectations, cooperation, interaction, diversity, responsibility and self-management. However, the ways how different institutions implement good practice in reality, depend very much on their students and their circumstances. Being (food) microbiologist and educator is a challenge to analyze this approach and to try to understand how good practice is implemented in education, but also in food supply chain, where food microbiology is actively involved in food safety management from producer to consumer.

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PURIFICATION AND PARTIAL CHARACTERIZATION OF AN EXTRACELLULAR BETA-GLUCOSIDASE FROM *MUCOR CORTICOLUS*

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Beta-glucosidases (β-D-glucoside glucohydrolases) play important roles in biology, including the degradation of cellulose biomass by fungi and bacteria, degradation of glycolipids in mammalian lysosomes, and the cleavage of glycosylated flavonoids in plants. Several members of the genus Mucor are well known from different biotechnological applications in consequence of their effective extracellular enzyme production. Filamentous fungi are known to be good producers of beta-glucosidases and a number of fungal glucosidases have been isolated and analyzed. Zygomycetes, however, are poorly characterized from this aspect. In the frame of a recent study, beta-glucosidase activities of several Zygomycetes fungi were analyzed by solid state fermentation methods. Among the tested Mucor isolates *M. corticolus* (syn.: *M. circinelloides* f. *corticolus*) showed intensive extracellular enzyme activity. The aim of our present study was the isolation and biochemical characterization of a beta-glucosidase from the fungus *M. corticolus*. For production of the extracellular beta-glucosidase enzyme in high amount, *M. corticolus* was grown on wheat bran medium for seven days at 25ºC. The enzyme was purified from the crude extract to homogeneity by

ammonium sulphate fractionation and size exclusion and anion exchange chromatographic separations were performed. The optimum temperature and pH for the action of the enzyme were at 50°C and 5.0 to 5.5, respectively. The beta-glucosidase was highly stable at temperatures up to 40°C, but was almost inactivated at temperatures above 55°C. The enzyme was fairly stable at pH 4.5 to 6.0. The enzyme activity was enhanced about 10% by the addition of 5 mM $AgNO_3$, $MnCl_2$ and $MgSO_4$ to the reaction mixture. Similar concentration of Ca^{2+}, Co^{2+}, K+ and Na+ ions had no significant effect on the enzyme activity, but strong inhibition was observed after the addition of Cu^{2+}, Zn^{2+} and Hg^{2+} ions. Enzyme activity was increased about 37% by using final concentration of 50 mM EDTA in the reaction mixture. This research was supported by the ETT grant 214/2006.

ESSENTIAL OILS AGAINST FOOD SPOILAGE BACTERIA AND YEASTS

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Essential oils are widely used for a variety of purposes in the food, aroma and fragrance industry; and some of them are known to possess antimicrobial properties. In this study, the antimicrobial effects of 9 essential oils (*Citrus lemon, Foeniculum vulgare, Hippophae rhamnoides, Juniperus communis, Origanum majorana, Pimpinella anisum, Salvia sclarea, Vitis vinifera* and *Zingiber officinale*) and their combinations with juniper oil against 4 bacterial (*Bacillus subtilis, B. cereus, Escherichia coli* and *Serratia marcescens*) and 4 yeast (*Geotrichum candidum, Hansenula anomala, Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) isolates were evaluated. The antimicrobial effects were tested by agar diffusion method. Essential oils had different antimicrobial effects on the microbes in the concentration range tested. Juniper oil and citrus oil acted synergistically in most of their combinations against the isolates tested.

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HEAVY METALS REMOVAL IN A HORIZONTAL ROTATING TUBULAR BIOREACTOR

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Mixed microbial culture was isolated from heavy metals contaminated ground soils located inside iron, vinyl, and cement factory area. Isolated mixed microbial culture was used for the heavy metal ions (Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}) removal process in horizontal rotating tubular bioreactor (HRTB). In this research, the effect of bioreactor process parameters on the bioprocess dynamics in thr HRTB was studied. Results of this research have shown that profiles of heavy metals concentration were gradually reduced along HRTB for all combinations of bioreactor process parameters [inflow rates (0.5-2.0 L h-1) and rotation speed (5-30 min-1)]. Hydrodynamic conditions and biomass removal

capacity have main impact on the metal ions removal efficiency that was varied in the range of 38.1% to 95.5 %. In this investigation, volumetric removal rate of metal ions (QM) was in the range of 2.408-14.989 g L-1 h-1 and the highest values were observed at medium inflow rate of 2.0 L h-1. On the bases of obtain results it is clear that medium inflow rate (F) has higher impact on the heavy metals removal process than bioreactor rotation speed (n). Notable pH gradient (cca 1 pH unit) along the HRTB was only observed at the inflow rate of 2.0 L h-1. Results of this research have also shown that heavy metals ion removal can be successfuly conducted in a HRTB as one-step process.

IDENTIFICATION OF *CAMPYLOBACTER JEJUNI* ANTIGENS ELICITING SPECIFIC IMMUNE RESPONSE

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Campylobacter species have been shown to serve as a major cause of diarrhoeal diseases in both industrialised and developing countries. Widespread studies have been performed to elaborate specific preventive measures to the disease. It has already been shown that application of whole cell vaccines can evoke protective immune response in mice and chicken, but till now no good candidates have been found that could serve as a subunit vaccine. In this study two anti-sera were raised in rabbits against three human isolate *C. jejuni* strains by immunising with boiled and formalin treated bacteria. Specificity of both sera was tested against 92 clonally different human isolate *C. jejuni* strains and 25 different Gram + and Gram -, pathogenic and non-pathogenic bacteria by ELISA. No significant cross reaction was detected by this method with bacteria other than *C. jejuni*. Campylobacter specificity of the serum was also confirmed by Western blot. In the second part of the work those antibodies that only weakly recognised the *C. jejuni* antigens were affinity absorbed from the antiserum in order not to interfere with the further analysis. Up to now eight strongly immunogenic compounds could be detected by immunoblot.
Analysis of the molecules and their functions are under way.

PREDICTION OF FUNGAL FRUIT SPOILAGE VIA DETECTION OF VOLATILE ORGANIC COMPOUNDS

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Moulds are the most important causing agents of post-harvest spoilage of fruits and are responsible for the damaging processes and occasionally mycotoxin production during their growth even under controlled conditions (e.g. in modified atmosphere storage). Growth of fungi is accompanied with the production of a series of different metabolites, among them the volatile organic compounds (VOCs) are released into the atmosphere. By detection of VOCs the spoilage process could be indicated even in the early phase of fungal growth. *Penicillium expansum* was found as dominant spoiling mould during deterioration of the plum *Prunus salicina*, which is especially harmful because of its patulin production. A standardized measuring system has been developed for detection and measurement of fungal VOCs and it was used for studying the characteristic volatile compounds during spoilage of plums and apples. As a model system apple (*Malus domestica L.*) and plum (*Prunus salicina L.*)

inoculated with *Penicillium expansum* were chosen. We have generated samples from the inoculated fruits and from the control fruits over the course of 5 to 9 day periods. Analyses were performed by solid-phase microextraction (SPME) coupled to GC-MS.
Fruits supply a wide range and large amount of nutrients for the growth of moulds. One of our goals was to investigate whether there is a common pattern in the produced compounds on different fruits. The size of data set would make very complicated and extremely long to check manually the characteristics of peaks in different chromatograms to form a peak table. We applied an automated method for peak detection and matching as it has been described in details in [1]. We have found more than 250 different peaks in the 135 measured samples. The lack of the knowing of the exact measured compounds makes the analysis of the components difficult.
Principal component analysis was applied to the overall data set. Healthy and spoiled apples can be distingushed with randomization of running times for the samples. There were only 15 compounds which had important and representable role in this differentiation, mostly alcohols, aldehydes and esters. Some of the indicator compounds were found also in the VOCs of plum samples.

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ISOLATION AND CHARACTERIZATION OF *PSEUDOMONAS TOLAASII* LYTIC PHAGES

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Pseudomonas tolaasii is a well-known mushroom pathogen, Gram-negative aerobic bacterium. The members of the genus *Pseudomonas occurs* almost in all habitats, they are potential human, animal, fungi or plant pathogen, and moreover could be saprotrophs or endophytes. The pathogenic form of *P. tolaasii* secreting tolaasin toxin, it is a lipodepsipeptide, which could disrupt the cellular membrane of the oyster mushroom (*Pleurotus ostreatus*) by forming pores. Our former investigation showed that other *Pseudomonas* strains -mostly belonging to the *Ps. fluorescens* biovar V- could be necrotic to *P. ostreatus* and at the same time others could be essential for the healthy fruit body formation. In the cultivation of the mushrooms, the uses of chemicals against to these pathogens are not allowed, so we should find and appropriate, highly selective biological control agent. The bacteriophages suit this requirement. Phages are estimated to be one of the most widely distributed entities in the biosphere. They are ubiquitous and can be found in all reservoirs populated by their bacterial hosts, for example in the soil, or either in mushroom sporocarps. The isolation of the phages was carried out with differential centrifugation technique from smashed, deformed fruit bodies of oyster mushroom. The resulting 16 distinct phage's physical and molecular properties were investigated in this study.We analyzed the host spectrum of the phages against to different *Pseudomonas* strains from strain collections DSMZ, BCCM/LMG and to our former harmful isolates; and the kinetics of the bacteriolysis in liquid medium was investigated. We classified the isolates based on the nucleic acid type. Our phages belonged to the dsDNA-phages. After restriction enzyme analyzes we could establish that our phage isolates are very similar, there are only host specificity differences between them. On the basis of the sequence analyzes the phage isolates showed a low similarity (short item, in low percentage) with other *Ralstonia* and *Xanthomonas* phages.

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EFFECTS OF LONG-TERM FERTILIZATIONS ON ABUNDANCE AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI UNDER MAIZE CROPPING SYSTEM

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The association between terrestrial plants and arbuscular mycorrhizas (AM) is one of the most widespread mutualistic plant-fungus interactions in natural and cropping systems. AM fungi have been shown to influence plant productivity however the effects of different fertilizations on the structure of AM fungi communities are still poorly understood [1]. We assessed the diversity of AM fungi in a long-term crop production experiment established at Martonvásár to improve knowledge on the effects of inorganic and organic fertilization practices on species diversity and community structure of AM fungi complex with their seasonality.

Polymorphisms in small subunit ribosomal RNA genes amplified by nested PCR were used to identify groups of AM fungi actively colonizing maize roots [2]; amplified products were cloned into *Escherichia coli* DH5α and subjected to RFLP analysis. The phylogenetic diversity of AM fungi was estimated by using the Shannon-Weiner diversity index. The extent of root colonization was also measured. There were no significant changes in the root colonization between different treatments but shifts in the diversity of AM fungi could be detected. Most of the sequences obtained at both sampling dates belonged to the *Glomeromycota* (76 %) but several other fungi (24%) were also observed. Besides the dominant *Glomus*-A fungi only a few members of the *Glomus*-B group, *Paraglomus* and *Archaeospora* were found. The diversity of AM fungi measured by the Shannon-Weiner diversity index at 97% similarity level increased from 1.45±0.23 to 1.9±0.35 due to organic fertilization; higher values were calculated at the second sampling date. Interestingly, inorganic fertilization had a stronger influence on AM fungus community composition as expected. These treatments increased the proportion of *Glomus*-Ad plus *Glomus*-Ac subgroups from 15% to 60%. Corn stalk application caused significant changes in composition of the *Glomus*-Ac subgroup resulting in more diverse AM population than as compared to populations found in the roots of non-fertilized plants. These results demonstrate the seasonality of changes in AM fungi community structures. Both traditional and organic fertilizations were found to affect arbuscular mycorrhizal populations but more research is required to understand the reasons of these changes.

This work was supported by the National Office for Research and Technology, SANI2007.

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MOLECULAR EPIDEMIOLOGY OF VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUSES IN CROATIA

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Infectious bursal disease (IBD) is a highly contagious immunosuppressive disease of young chickens with significant economic impact on the poultry industry. The emergence of very virulent strains in late 1980's resulted in mortality up to 60% in infected flocks. Very virulent IBD (vvIBD) was recognized in Croatia in 1995 and still occurs in farm and backyard chickens. In order to reveal phylogenetic traits of the epidemic in Croatia, we analysed the VP2 variable region of vvIBD virus isolates collected in north-western and southern Croatia during the period 1996-2009. All analysed isolates had amino acids in the VP2 variable region typical for very virulent viruses at the positions 256 (isoleucine), 294 (isoleucine) and 299 (serine). Nevertheless, significant genetic diversity of isolates from 1996 indicates not only possible multiple introduction of the virus into Croatia, but also its circulation in Croatian poultry before 1995. In contrast, some molecular markers and high similarity of certain isolates from the same region, obtained over a span of almost a decade, indicate enzootic character of the virus in certain areas of the country. Clustering of several Croatian isolates with isolates from neighbouring countries implicates spreading of the viruses between the countries.

PERSISTENCY OF ALCAN-DEGRADING INOCULANTS IN COMPOSTS PROCESSED FROM WASTES OF BIOENERGY PRODUCTION

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Energy demand of the world can be supplied from several sources simultaneously. The most beneficial ways of the production of biomass energy are focused in worldwide researches. Composting seems to be the most appropriate method in respect of the agriculture, ecology and hygiene. Persistency of alcan-degrading inoculants in composts must have a great importance in environmental technologies. We tested the survival of the cells of different microorganisms in laboratory examination. The survival of the propagules of 2 Gram-negative (*Pseudomonas putida*, *Chryseobacterium hungaricum*) and 4 Gram-positive bacteria (members of the genus Rhodococcus), as well as of 2 fungal species (*Cladosporium cladosporoides*, *Yarrowia lipolytica*) (all of them are representatives of alcan-degrading inoculants) was followed with the method of quantitative cultivation. The starting quantity of the relating microbes was standardized at the level of 107 CFU/g in the compost-based incubation mixtures. Different values of the survival of the examined microorganisms, at 20-26°C and 60% of water content concerning the environmental practice, were experienced. The viability of the Gram-negative bacteria proved to be more permanent than that of the Gram-positive bacteria and fungi: after 20 week incubation the amount of the *Chryseobacterium hungaricum* cells remained 4.5-8.5 x 10^3 and that of the *Pseudomonas putida* cells did 2.8-6.5 x 10^3 CFU/g. Among the 4 Rhodococcus species involved in the experiments, the quantity of *R. pyridinivorans* decreased to the level of 101-102 while that of the *R. rhodochrous* decreased under the limit of culturing. The composition of starting mixtures evidently influenced the survival of all tested microorganisms. In a definite compost processed from straw waste, biogas waste slurry and wood-ashes not only the cells of Gram-negative bacteria and those of rhodococci but even those of the yeast *Yarrowia lipolytica* and those of the filamentous fungus *Cladosporium cladosporoides* could be demonstrated at least of the quantity of 101-103 g/CFU. The effect of the water content (20-80%) of the incubation mixture was surprisingly low on the survival of each applied microorganism

species. The persistency of alcan-degrading inoculants related to the survival of different microbe cells in certain composts can be concluded as a base of the inoculants of second generation.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF ECTOMYCORRHIZAE OF *PINUS* SPECIES FROM A SEMIARID SANDY GRASSLAND

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The mycorrhizal interactions of different fungi with vascular plants play a fundamental role in terrestrial ecosystems. One of the main mycorrhiza types is the ectomycorrhiza (ECM) generally formed by woody plants and different basidiomycetes and ascomycetes. The main aim of our investigation was to characterize the ECM of invasive Pinus species and to compare the ECM of the *P. sylvestris*/*P. nigra* forest bordering the semiarid sandy grassland close to Fülöpháza with the ECM of the pines invading the grassland. Morphological characterization of the ECM was carried out using a stereomicroscope. The anatomy of the ECM, the mantle-structure, the hyphal and rhizomorphal characteristics, was studied by Nomarski (DIC) microscopy. In course of the molecular identification of the ECM the internal transcribed spacer (ITS) region of the nrDNA was amplified and sequenced using the ITS1F-ITS4 fungus-specific primer pair. The BLASTn was used to compare our ECM sequences with sequences deposited in public databases. More than 90 ECM were collected and studied from the forest and invading trees of the grassland area. More than 80 microscopy slides were prepared to study their anatomical characteristics. Several different ECM forming basidiomycetes and ascomycetes have been identified by the ITS sequences. Here we present these results and the comparison of ECM colonizing the pine trees from the forest and the grassland. This comparison could reveal habitat specific ECM of the pine from this semiarid sandy area.

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HYDROGEN PRODUCTION BY CONTINUOUS CULTURE OF FERMENTATIVE BACTERIA ON WASTE SUBSTRATES

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Hydrogen is thought as an attractive energy carrier that, in future, can replace conventional fossil fuels. Current methods of hydrogen production are based on conventional fossil fuels like natural gas, petroleum and coal, so, they are energy-expensive and cause pollution of the environment. That is why there is a great interest in hydrogen production from biomass (a versatile and renewable energy source) by microorganisms under anaerobic conditions. That approach can be a method of utilization of various organic wastes. Among known biological processes leading to hydrogen production two types of bacterial dark fermentation (enteric bacteria type fermentation and clostridia type fermentation) seem to be the most promising for the future applications. Actually, many

investigations are focused on improvement of hydrogen yield during fermentation.
We have selected a consortium of anaerobic fermentative bacteria producing hydrogen-rich gas in a continuous culture inoculated with a sample of the lake bottom sediment on the medium containing waste substrate – molasses. The bacterial culture is conducted in a bioreactor of a special design filled with stones (Φ 2-3 cm) as a solid phase to develop a biofilm on their surface. We have established optimal conditions for hydrogen production by the culture. The fermentative gas contains 46% of hydrogen. The molar yield of hydrogen is 2.1 mol of hydrogen per 1 mol of sucrose from molasses. Moreover, the fermentative gas was recognized as a fuel by a chemical fuel cell.
Using classical microbiology methods we isolated culturable bacteria from the bioreactor. Analysis of 16S rRNA gene sequence revealed that most of them belong to *Enterobacteriaceae* family and are related to *Citrobacter freundii, Klebsiella oxytoca* and *Candidatus cuticobacterium kirbyi.* We also detected two Gram (+) bacteria: *Leuconostoc mesenteroides* and *Enterococcus* spp. However, only a small fraction of microorganisms can be routinely cultivated from natural environments. Analysis of the non-gaseous fermentation products pointed to the presence of other than *Enterobacteriaceae* bacteria in the culture. The results warrant further investigations on molecular analysis of bacterial biofilm responsible for hydrogen production.

CONTINUOUS CULTURES OF FERRIC (FE^{+3}) REDUCING ANAEROBIC MICROORGANISMS MIXED POPULATION

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Ferric ion-respiring microorganisms (FRM), are a group of prokaryotes that use iron (III) as well as other metals as terminal electron acceptors in the process of anaerobic respiration. The best known ferric ion-respiring bacteria are the members of *Geobacteraceae* and *Shewanellaceae*. Numerous bacteria are capable to reduce Fe(III) but the process does not lead to energy conservation. Such a dissimilatory iron-reduction often accompanies fermentation and is thought as a secondary respiratory pathway where ferric ions serve as a sink for excess reducing power. Special attention is paid to a biotechnological significance of ferric-reducing microorganisms because of their potential role in electricity production in microbial fuel cells where the terminal acceptor of the electrons during anaerobic respiration is not a ferric ion but the anode.
We have selected two consortia of iron-reducing bacteria in continuous cultures inoculated with samples of the lake bottom sediment on the media containing waste substrate – molasses and different sources of Fe(III), a soluble ferric citrate and an insoluble ferric oxide. The bacterial cultures were conducted in a bioreactors of a special design filled with stones (Φ 2-3 cm) as a solid phase to develop a biofilm on their surface for 30 months. For the first time molasses was used to select iron-reducing bacteria. Large amount of ferrous ion Fe(II) in the probes taken from the bioreactors was observed pointing at intensive Fe(III)-reduction processes. Presence of iron-reducing bacteria in both cultures were confirmed by a modified MPN method. Using classical microbiology methods we isolated culturable bacteria from the bioreactors. Analysis of 16S rRNA gene sequence revealed the following genera of bacteria: *Shewanella, Klebsiella, Citrobacter, Pseudomonas, Ochrobactrum, Azospirillum, Acinetobacter, Aquaspirillum, Stenotrophomonas.* However, only a small fraction of microorganisms can be routinely cultivated from natural environment. Moreover, electrochemical

activity of the obtained ferric-reducing bacterial consortia was confirmed in microbial fuel cells.

THE APPLICATION OF THE MDA METHOD IN MICROBIAL COMMUNITY ANALYSIS OF TCE CONTAMINATED GROUNDWATER

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Dehalogenating microbial communities of PCE and/or TCE contaminated groundwater were examined on the basis of T-RFLP and clone library analyses. During an *in situ* bioremediation process, the group-specific detection of microbes capable of dehalogenation is the deterministic factor of chlorinated ethene degradation potential. Therefore, we also performed 16S rRNA gene based *Dehalococcoides* tests, demonstrating the presence (rDNA) of this important genus reported to carry out reductive dechlorination. We found that a positive *Dehalococcoides* test result often did not manifest itself in T-RFLP fingerprints or clones in the 16S rRNA gene libraries. For more detailed investigation concerning e.g. the *dehalogenase* gene pool of such communities we need to capture possible dehalogenating microbes present in low copy numbers in highly contaminated samples. The MDA (*multiple displacement amplification*) method demonstrated that it could be a useful pre-PCR step for processing samples of minute quantity, or when the target sequences are in low copy numbers, and also when substances are present that inhibit standard PCR amplification. The technique proved to successfully amplify rRNA genes from 10 times lower genomic DNA concentrations than standard PCR and in the presence of 10-fold higher humic acid concentrations. The MDA method is a type of whole genome amplification technique applying phi29 DNA polymerase enzyme. The isothermal strand displacement reaction is carried out with random hexamer oligonucleotides that non-specifically bind to the genomic DNA. The protocol is very simple and produces microgram quantities of genomic DNA from nanogram amounts of starting material, however intact templates are necessary for successful amplification, therefore a DNA isolation method that enables minimum shearing is required.We have optimized our DNA isolation method as well as the MDA reaction conditions for acquiring genomic MDA DNA from TCE contaminated groundwater samples, from which microcosm experiments using different electron donors were also set up. We have compared MDA amplification efficiencies from different sources and DNA isolation techniques, and determined the optimum conditions for MDA reactions. We have compared the direct 16S rRNA PCR amplification with the MDA pre-amplified 16S rRNA PCR for the microbial community analysis of a TCE contaminated groundwater sample by T-RFLP fingerprinting and 16S rDNA clone library construction. A significant difference in the relative abundance of T-RFs in the chromatograms was observed between the direct and two step (pre-MDA) PCR approach, which was further confirmed by sequence analysis of the clones.

GENETIC DIVERSITY OF THE *LACTOBACILLUS* COMMUNITY IN AUTOCHTHONOUS ISTRIAN EWES CHEESE

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Istrian cheese is semi-cooked, three month ripened cheese made from thermized or raw whole ewe's milk without addition of selected starter culture. The cheese making technique at the farmhouse level

has remained unchanged over time resulting in typical features which depend on local and regional traditions. Among different microbial communities involved in the fermentation process of traditional cheeses lactobacilli represent an essential part of it promoting their health and technological benefits. The objectives of this work were to describe the diversity of lactobacilli in Istrian cheese and to get a collection of well characterized strains. Samples were subjected to microbiological analysis. A total of 150 isolates of mesophilic and thermophilic lactobacilli were collected from 2 dairy farms during the ripening process on monthly base. All Rogosa agar isolates were found to be Gram-positive rods. Biochemical fingerprinting with PhenePlate LB system preliminary grouped 150 tested lactobacilli in 7 distinct PhP types. Only one representative from each PhP type was further analyzed on genotypic level for a reliable identification of lactobacilli to the genus and species level. PCR analyses with genus-specific primers LbLMA1-rev and r16-1 confirmed that all representative isolates were Lactobacillus members. The PCR products were sequenced for identification on species level. The representative strains were identified as *Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus casei and Lactobacillus rhamnosus.*

ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER* : A LINK BETWEEN ANTIBIOTIC AND BIOCIDE RESISTANCE?

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The increasing antimicrobial resistance is a world-wide problem, including transmission via food chain. Campylobacteriosis is the leading food-borne illness and most frequently reported zoonosis in humans. *Campylobacter* as a zoonotic pathogen, mainly transmitted by food, is exposed to antibiotics and other antimicrobials in food animal production, food processing and supply chain as well as in human medicine. As such it is an interesting model for studying transmission and persistence of resistant strains in the environment (along the food chain). The analysis of reported antibiotic resistance of strains from different sources is difficult due to different methodologies and breakpoints used, however, the increasing prevalence of fluoroquinolone (ciprofloxacin), macrolide (erythromycin), tetracycline and also multiple resistant strains from different environments was found in our studies [1, 2, 3], other research works and official national reports [4]. Beside target mutations, also non-specific mechanisms (e.g. active efflux pumps) contribute to bacterial acquired reduced susceptibility and/or resistance and cross-resistance against unrelated antimicrobials including antibiotics, disinfectants, detergents, decontamination agents, bile salts, etc. For studying possible interactions we have introduced several experimental approaches. The efficiency of well-known (e.g. phenylalanine-arginine beta-naphthylamide (PAßN) and 1-(1-naphthylmethiyl)-piperazine (NMP) and presumptive efflux pump inhibitors (EPIs) was studied with phenotypically and genotypically characterized *Campylobacter* isolates from animal, food and human clinical samples as well as with the reference and mutant strains in specific efflux pump genes. The mechanisms involved in microbial resistance and cross-resistance development and the results of adaptation and cross-adaptation tests with *Campylobacter* strains after exposure to sub-inhibitory concentrations of antimicrobials (antibiotics, disinfectants, meat decontamination agents etc.) will be discussed.

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ANALYSIS OF METALLO-BETA-LACTAMASE AND ENTEROTOXIN-PRODUCING *BACTEROIDES FRAGILIS* STRAINS

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Strains of *B. fragilis* can be divided into two divisions (Division I and II) based on DNA homology measurements. Genetic and biochemical fingerprinting data such as multilocus electrophoresis typing, multilocus sequence typing and RAPD-PCR can also differenciate between these two homology groups of *B. fragilis*. Additionally, the gene content regarding β-lactamases (cepA and cfiA) and enterotoxin (bft) genes was found to distribute differentially between Division I (cepA and bft) and Division II (only the metallo-β-lactamase-encoding cfiA).Among 275 Hungarian clinical *B. fragilis* strains 24 and 14 were bft or cfiA-positive, respectively. No coincidence between harbouring the two genes was revealed. According to our experiments among these cfiA-positive *B. fragilis* strains, the incidences of the cephamycinase (cfxA) and tetracycline resistance (tetQ) genes were decreased compared to the incidence among Division I strains. The distribution of these latter two genes was statistically significant for the differential distribution in the two genetic divisions of *B. fragilis*. Surprisingly, a bft and cfiA-positive *B. fragilis* strain has been isolated from a patient in the UK in 2004. The bft genes have been found to be harboured on a genetic element called „patogenecity islet" or BfPAI which can reside on conjugative hybrid transposons (CTn86 and CTn9343). However, these CTns do not carry a BfPAI islet in every case. According to our novel investigations on the detection of resistance genes of Bacteroides species from a pan-European susceptibility survey out of 280 *B. fragilis* strains two from Turkey and Italy were detected that harboured the bft and cfiA genes coincidentally. To account for this behaviour and explain the differential distribution of genes in *B. fragilis* strains, the interaction and differential distribution of the harbouring genetic elements are expected and investigated.

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BIOTECHNOLOGY OF *STREPTOCOCCUS THERMOPHILUS* - POTENTIAL FOR FOOD AND NON-FOOD APPLICATIONS

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Streptococcus thermophilus is an important thermophilic lactic acid bacterium widely used in the industrial production of fermented dairy foods. As a starter culture, it is traditionally used in combination with lactobacilli in the production of yogurt and many Italian- and Swiss-style cheeses. An essential biocatalyst in dairy fermentations, *S. thermophilus* rapidly produces lactic acid from lactose and its key enzyme systems generate peptides from milk proteins to stimulate the growth of lactobacilli. Many strains possess genetic elements responsible for the production of exopolysaccharides that impart desirable rheological properties to yogurt and other fermented milk products. Selected strains also have the capacity to synthesize bacteriocins, the ribosomally produced antimicrobial peptides with potential for applications as food-grade biopreservatives. In addition, ethanol or detergent-treated biomass of *S. thermophilus may* serve as the source of β-galactosidase in the production of low-lactose milk for lactose intolerant consumers. Research on native plasmids in *S.*

thermophilus established their value in the construction of cloning vectors and the subsequent development of strains with improved and/or novel metabolic functions. The presence of restriction-modification elements in some plasmids provides the host with resistance to bacteriophage infection. The food-grade status of *S. thermophilus* eliminates the need for containment and strains producing isoschizomers of several restriction endonucleases used in genetic research are desirable sources of these enzymes. Some strains produce Sth132I, a restriction endonuclease with a unique recognition sequence that is useful in detecting mutations that cause dwarfism in humans. Bacteriocins of *S. thermophilus* strains include the broad-spectrum thermophilins with activity against pediococci (spoilage bacteria in beer fermentation) and propionibacteria (acne) which creates new opportunities for non-food applications in the brewing and cosmetics industries.

DETECTION OF IMPORTANT FUMONISIN B_2 PRODUCER BLACK ASPERGILLI BY HIGH RESOLUTION MELTING POINT ANALYSIS

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Fumonisins are a large group of mycotoxins discovered in 1988. This group of toxins can be divided to several structural analogues including fumonisins A, B, C, and P. The major producers are *Fusarium* species but recent studies clarified that a black *Aspergillus* species, *Aspergillus niger* is also able to produce fumonisin B_2 [1]. In contrast with the well-known fumonisin producer *Fusarium verticillioides*, which produces fumonisins on media containing plant extracts, *A. niger* produces this mycotoxin in media with low water activity. Black Aspergilli are difficult to identify to species level using morphological or physiological criteria, consequently, sequence-based approaches are needed. Up-to-date, partial sequences of the calmodulin gene were found to be the most useful for discriminating black Aspergilli at the species level [2]. We developed a real-time PCR based approach using High Resolution Melting Point analysis to distinguish the fumonisin producing *A. niger* from other fumonisin non-producing species of the *A. niger* species complex including *A. tubingensis, A. acidus, A. brasiliensis* based on the analysis of part of the calmodulin gene. This method does not need sequence analysis and can easily be quantified. The method could also be applied to distinguish ochratoxin producing members of the *A. niger* species complex from non-producers in various foodstuffs.

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APPLICATION OF REAL-TIME FLUOROMETRY TO STUDY EFFLUX PUMP ACTIVITY IN BACTERIA AND CANCER CELLS

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Multidrug resistance plays a crucial role in the failure of cancer chemotherapy and the treatment of infectious diseases. Because multidrug resistant (MDR) bacterial infections involve the over-

expression of efflux pumps that utilize cellular energy to extrude antibiotics or biocides actively out of the cell, it is necessary to characterize the efflux activity of MDR bacteria under physiologically relevant conditions. The real-time fluorometry uses the fluorochrome ethidium bromide (EB), which is considered as a common substrate of bacterial efflux pumps. Ethidium bromide has been shown to be particularly suitable to be used as a probe because it emits weak fluorescence in aqueous solution (outside cells) and becomes strongly fluorescent in non-polar and hydrophobic environments. The methodology and real-time evaluation of accumulation and efflux provides a somewhat closer representation of physiological conditions at sites of infection that afford a more defined assessment of overall activity of the efflux pump system. The method has been successfully applied by us to characterize intrinsic and over-expressed efflux pump systems of *Escherichia coli*, *Salmonella enteritidis*, *Enterobacter aerogenes*, *Enterococcus faecalis* and *Enterococcus faecium*. In tumour cell lines, multidrug resistance is often associated with an ATP-dependent decrease in cellular drug accumulation related to the over-expression of the members of the ATP-binding cassette (ABC) transporter superfamily. Based on former results with bacterial transporters, we employed this methodology to detect and demonstrate the activity of the eukaryotic ABC-transporter P-glycoprotein (Pgp or ABCB1). The clinical importance of P-glycoprotein for MDR and cancer treatment has led to the investigation of the inhibiting properties of several compounds. The aim of the real-time fluorometric method is to easily and accurately detect and quantify the transport of the Pgp substrate EB through the cell membrane, at working concentrations that will not affect cell viability nor perturb cellular function, in order to readily assess efflux activity in neoplastic cells. In our studies we used L5178 mouse T-cell lymphoma cells transfected with human ABCB1 gene and classical MDR modulators such as verapamil and reserpine to standardize the method. The fluorometric assay is a new application of the RotorGeneTM 3000 (Corbett Research, Sydney, Australia) real-time thermocycler and provides information about transport kinetics thereby offers a rapid, high-throughput, reproducible, accurate and inexpensive screening of efflux pump inhibitors.

LOCALISATION OF YEAST CELL WALL MANNOPROTEINS - IMPACT ON WALL BIOGENESIS

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Yeast cell wall contains proteins that are noncovalently (Scw-proteins), or covalently (Ccw-proteins) bound to beta-1,3-glucan, the latter either through GPI-anchors and beta-1,6-glucan, or by alkali labile ester linkages between gamma-carboxyl groups of glutamic acid and hydroxyl groups of glucoses (Pir-proteins, extracted from the cell wall by mild alkali). Disruption of all four genes coding for Pir-proteins revealed that a 67 kDa protein still remained in the NaOH extract. Disruption of the SCW4, a gene coding for one of the most abundant SCW proteins resulted in the disappearance of the band, indicating that Scw4p was partly also covalently linked to the cell wall by an apparently new, so far unobserved alkali sensitive linkage. Structural analysis revealed that the N-terminal part of the protein is required for anchoring in the wall. Both the absence and the increased concentration of Scw proteins resulted in undesirable changes in the wall and in the increase in cell mortality, particularly upon entering the stationary growth conditions. Possibilities for application of the knowledge gained on different mechanisms for incorporation of wall proteins in heterologous expression of proteins in yeast and their "genetic immobilisation" will be discussed.

GENOTYPING OF LONG POLAR FIMBRIAE IN BOVINE *ESCHERICHIA COLI* O157 STRAINS

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Long polar fimbriae are being recognized as an important virulence factor of enterohaemorrhagic Escherichia coli (EHEC), and were recently proved to be contributing to its colonisation of the intestinal tract. So far there are two main types of long polar fimbria genes, lpfA1 and lpfA2. According to a recently proposed typing scheme, there are 5 subtypes of lpfA1 and 3 subtypes of lpfA2. In the present study a previously characterised collection of *E. coli* strains of serogroup O157 (n=42), representing three pathotypes: EHEC, enteropathogenic (EPEC) and atypical (AT), was screened for lpf genes according to the typing scheme mentioned above. We found that all 11 of the EHEC O157:H7/NM (non-motile) strains carried both lpf genes, type 3 of lpfA1 and type 2 of lpfA2. The same gene combination characterised the 22 EPEC O157:H7 strains, confirming that this genotype is well conserved among the O157 serogroup. Seven out of the 9 atypical O157 strains harboured only type 1 of lpfA2, this genotype is characteristic mainly of non-O157 *E. coli* strains. These findings, in harmony with the previous report suggest the existence of a novel genotype and pathotype of *E. coli* O157 strains.

THE SIGNIFICANCE OF SIZE CONTROL IN SINGLE CELLS: HOW SIZE IS MEASURED?

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Size control maintains size homeostasis in cell populations; it enables that (in balanced growth) the size distribution does not change in consecutive generations. The most important requirement is that there must at least one cell cycle event, which cannot be performed unless the cell has reached a critical size. Generally, two events are known to be controlled by cell size, namely initiating DNA replication (S phase) and the onset of mitosis (M phase). As a consequence, two size checkpoints exist in the eukaryotic cell cycle, one in G1 phase (before S), and another one in G2 (before M). The molecular mechanisms of these size checkpoints are still obscured, however, they seem to be evolutionary conserved from unicellular microorganisms up to even human cells. Since the late 1970s the fission yeast *Schizosaccharomyces pombe* is an attractive model organism in size control studies. The cylindrically shaped fission yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. As a consequence, cell length is an easily measurable parameter, which characterizes cell size. Early time-lapse microphotographic studies with fission yeast proved that there is a strong negative correlation between cycle time and birth length, i.e., the larger the cell at birth, the shorter its cell cycle. This ensures a size compensation mechanism, which is an evidence for the existence of size control. In wild-type cells, G2 phase is long and size-dependent, meanwhile G1 is short and constant, therefore, size control seems to operate exclusively in G2 in fission yeast. Later, it was discovered that length growth in fission yeast follows a bilinear pattern during the cycle, meaning that growth is linear, however, there is a point (called rate change point or RCP) in mid G2 where growth rate increases. Separating the cell cycle into a preRCP and a postRCP period, and analysing

their durations as a function of birth length indicated that size control acted in the first part of G2 rather than near the G2/M transition (as previously thought). The wee1 mitotic inhibitor (a cell cycle regulated kinase) was found to be mainly responsible for this size checkpoint. The general view is that in small cells wee1 keeps the cdc2/cdc13 complex (also known as M-phase promoting factor or MPF) in an inactive form. After reaching a critical size, wee1 itself becomes inactivated by some mitotic activator(s) and the cell starts to prepare for mitosis. Former models suggested that MPF accumulated (proportional to the increasing cell size) in the constant sized nucleus, which hypothesis was able to describe quantitatively the phenomenon of size control for many years. However, in 2007 Paul Nurse showed that the nucleus extended during the fission yeast cell cycle, parallel to cell volume, ruling out the above hypothesis. Very recent experimental data seems to give us a new clue how size control might even operate. Developing a new relevant mathematical model of the fission yeast cell cycle, involving these results, is in process in our laboratory.

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MEASURING HEAVY METAL SENSITIVITY OF NEW HYDROCARBON DEGRADING MICROBE STRAINS ISOLATED FROM CONTAMINATED SITES

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The aim of our study was to find new hydrocarbon degrading strains for bioremediation on complex contaminated sites. „Complex contaminated" means the sites have not only hydrocarbon, but other pollutants (eg. heavy metals) also in soil or groundwater. High heavy metal concentrations are toxic (Takács et al., 2003) and able to decrease the growth of aerobic bacteria (Algaida et al, 2007). Our work focused on isolating new hydrocarbon degrading strains that have less sensitivity against toxic elements. In our study approximately 50 aerobic microbe strains were isolated from eight Hungarian hydrocarbon contaminated sites (eg. petrol stations, pipeline breakages and former oil distribution facilities, sites). From this collection 6 strains were determined as hydrocarbon degraders. The strains were identified by 16S rDNA sequencing as *Acinetobacter rhizosphaerae* (TBF2/20.1), *Olivibacter* sp. (TBF2/20.2), *Rhodococcus erythropolis* (ZFM 23.1), *Pseudomonas* sp. (SZM5/4.2), *Rhodococcus erythropolis* (OM 7-2) and *Rhodococcus ruber* (S/8). *Acinetobacter rhizosphaerae* is cannot be used as an inocula member because it's a facultative pathogen in spite of that heavy metal sensitivity of it was determined. By the results of identification TBF2/20.2 strain is probably a new species of *Olivibacter* genus. The effect of the chosen heavy metals on these strains was studied by in vitro, agar diffusion hole tests. This method was used to determine the effect of 10, 100, 1000 and 5000 mg/l concentration of 8 heavy metals (zinc, lead, copper, nickel, chromium[VI], mercury, cadmium and cobalt) on the strains. Based on the counted diameter values of the inhibition zones an own inhibition rating system was generated. By the results of the agar diffusion hole tests the strains are generally less sensitive to lead, copper and zinc because the 10 and 100 mg/l concentration of these elements caused no measurable inhibition on the injected agar plates. Nickel caused „low inhibition" only for one strain (*Pseudomonas* sp.) in 100 mg/l concentration. In the same concentration mercury caused „low inhibition" for two strains (*Rhodococcus ruber*, *Rh. erythropolis* [OM7.2]). Cobalt caused „low inhibition" for *Pseudomonas* sp. in 10 mg/l and for *Rh. ruber* strain in 100 mg/l. Inhibiting effect was caused by cobalt for *Acinetobacter rhizosphaerae*, Olivibacter sp. and

Pseudomonas sp. in 100 mg/l concentration. In the lowest concentration (10 mg/l) cadmium caused „inhibiting effect" for *Acinetobacter rhizosphaerae*, *Rhodococcus erythropolis* (ZFM 23.1) and *Pseudomonas* sp., but there was no measurable inhibition till 5000 mg/l concentration for the other three strains. *Acinetobacter rhizosphaerae* and *Olivibacter* sp. were highly sensitive to chromium(VI) and it caused „low inhibition" for *Rhodococcus ruber* and *Pseudomonas* sp. There was no measurable inhibition for the two *Rhodococcus erythropolis* (ZFM 23.1 and OM-7.2) strains until 1000 mg/l concentration. After assessing the results of our study, strains could be selected that are able to degrade hydrocarbon compounds in bioremediation of complex contaminated sites and are able to be labelled by the results for practical users.

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IMPORTED MELIOIDOSIS FROM INDIA: FIRST CASE IN HUNGARY

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Melioidosis - an infection caused by *Burkholderia pseudomallei* - is endemic in Southeast Asia and Northern Australia. However, it has been observed in the South Pacific, Africa, India, the Middle East, Central and South America. The disease is increasingly recognized around the world and it has acquired the status of a newly emerging infectious disease in India. The disease is very rare in Europe but cases in returning travelers to Europa from endemic areas have been reported. No case of melioidosis has been described in Hungary until now. Melioidosis presents in a variety of forms ranging from asymptomatic infection to localised abscess and fulminant septicemia. Altough several organs may be involved in melioidosis, pulmonary infection is the most common form. Neurological melioidosis is unusual - especially outside Australia - but meningoencephalitis, encephalomyelitis and brain abscess can occur. In cases of sepsis and neuromelioidosis the mortality rate is high. The vast majority of cases have an identified risk factor, the most important of which are diabetes, alcoholism and chronic renal disease. We report the first case of melioidosis in Hungary, which was an imported disease from India. The previously healthy 30-year-old male patient, who spent 3 months in India, was admitted to Petz Aladár Hospital (Győr), because of severe symptoms of encephalitis. His condition was critical, tetraplegia and vigil coma were developed. Gram-negative bacterium was cultured from his blood and urine. Hospital microbiological laboratory has been suggested preliminary result of Burkholderia pseudomallei, on the basis API20NE (Biomérieux) profile. Verification was made by API32GN (Biomérieux) and GN2 MicroPlate using Biolog system with Dangerous Pathogens (DP) Database Supplement, in National Center for Epidemiology. The results were confirmed Burkholderia pseudomallei as the causative agent of melioidosis. The patient's condition improved slowly and after the nine-month-therapy began his rehabilitation.

CHANGE OF THE WEST NILE VIRUS ACTIVITY IN HUNGARY IN HUMAN ASPECT

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West Nile Virus (WNV) spread by mosquito have already occured for 1972 in Hungary but in the last years it has been proved that the virus can cause acute neurologic infections (mainly meningitis and encephalitis) in birds and in mammals. The laboratory diagnostic of human diseases based on serologic methods and it is performed solely in the National Center for Epidemiology (NCE) in our country. The human diagnostic laboratory has to collaborate with veterinarian colleagues in order to be able to find potential infection sources and to prepare for human patients. In 2008, both the human and the animal infections showed, that the virus activity was increased. Furthermore, until 2008, the majority of WNV infections occurred in the Eastern half of Hungary, mostly in the area of the Great Hungarian Plain (non-endemic for tick-borne encephalitis) and there were some patients who became infected in Western Hungary, which means that the virus have spread towards the West. This expansion to the Transdanubian region can cause diagnostic difficulties because of the cross-reactions against the endemic tick-borne encephalitis virus. In the case of sequential infections it can cause more severe disease than that of the usual.

THE ROLE OF MINOR ANTIGENS IN THE FORMATION OF PROTECTIVE IMMUNE RESPONSE AGAINST SHIGELLOSIS

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Enteric pathogens elicit over 4 million fatal diarrhoeal cases worldwide, 1 million of which are caused by *Shigella* pathogens. This high incidence, the increasing resistance to antibiotics and the potential danger of misuse of *Shigella* as a biological weapon justify the emerging necessity of a potent vaccine against shigellosis. In spite of extensive studies, however, there is no licensed vaccine available. The major difficulties in the vaccine development originate from the lack of cross-protection against the numerous serotypes of *Shigella* spp. and from the fact that the nature of protective antigens is not fully clarified. We aimed to elucidate the role of two main factors - the serotype determining O antigens and the invasion plasmid antigens (Ipa) - in the triggering of immune protection. We constructed an isogenic auxotrophic (*Δaro*C) and an LPS rough (*Δrfb*F) mutant of the prototype *S. flexneri* 2a strain 2457T, and selected variants of both mutants, which had spontaneously lost their invasion plasmid (resulting in non-invasive phenotype). The virulence attenuation and the immunogenic potential of the strains were examined upon mucosal immunisation using the mouse lung model. Groups of mice were immunised with the various mutants and a subsequent challenge infection was performed with either a homologous or heterologous *Shigella* strain.

Both the invasive and non-invasive mutants provided significant protection against a homologous infection. However, the histological examination of the lungs of mice infected with invasive and non-invasive mutants proved qualitative difference in the cellular immune response provoked. The determination of the serum and mucosal antibody levels attested that the rough non-invasive "double" mutant devoid of both classes of major immunogenic antigens (i.e., Ipa and O-antigens) not only provoked significant anti-*Shigella* titres, but also raised antibodies against heterologous *Shigella* serotypes as well as other representatives of the family Enterobacteriaceae. This implies that the conserved antigens shared by heterologous bacteria are more immunogenic in the double mutant background. Indeed, the double mutant provided high protection against heterologous strains. Existence of protective antigens, different from the Ipa and O-antigens is likely.

The identification of shared protective antigens could open new perspectives in the development of broadly protective vaccine strategies.

IN VIVO EFFICACY OF FLUCONAZOLE, VORICONAZOLE AND CASPOFUNGIN AGAINST *CANDIDA ORTHOPSILOSIS* IN A NEUTROPENIC MOUSE MODEL

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Efficacies of fluconazole (FLC), voriconazole (VOR) and caspofungin (CAS) against three *Candida orthopsilosis* isolates (CP25, CP85 and CP125) were determined in a neutropenic mouse model. Male BALB/c mice weighing 25-27 g were immunosuppressed with a single intraperitoneal 200 mg/kg cyclophosphamide dose four days prior to infection. Mice were infected intravenously through the lateral tail vein with 6-7 $x10^6$ CFU/mouse in a 0.2-ml volume with each isolate. Intraperitoneal FLC, VOR, CAS treatment was started 24-26 hours postinfection. FLU (1, 5 and 10 mg/kg), VOR (6 and 12 mg/kg), and CAS (1 and 2 mg/kg) were administered for five consecutive days. Efficacy of a single 5 mg/kg CAS dose was also determined. All doses were given in a 0.5 ml bolus. On day seven the mice were sacrificed, the kidneys were removed and kidney pairs were homogenized aseptically. Aliquots were plated on Sabouraud dextrose agar plates and after 48 hours the colony count was determined. The numbers of fungal CFUs from the kidney pairs were compared by Kruskal-Wallis test (with Dunn's post-testing). Values of $P<0.05$ were considered to be significant. One mg/kg daily dose of FLC did not reduce the fungal tissue burden compared to the controls in case of any of the three isolates ($P>0.05$). Ten mg/kg of FLC significantly reduced the tissue fungal burden against all three isolates ($P<0.05$ for all isolates). Both doses of VOR decreased the fungal burden significantly only in case of a single isolate CP85 ($P<0.05$); against isolates of CP25 and CP125 neither 6 mg/kg nor 12 mg/kg of VOR was effective ($P>0.05$). Two mg/kg daily dose of CAS as well as a single 5 mg/kg CAS significantly decreased the fungal tissue burden for all isolates ($P<0.01$ to <0.001). The FLC derivate VOR was ineffective against two of three isolates even at relatively high daily doses (6-12 mg/kg), suggesting that high dose of FLC may be superior for the treatment of infections caused by *C. orthopsilosis*. CAS was effective at therapeutic doses; a single 5 mg/kg CAS dose was not inferior as compared to one or two mg/kg daily doses of CAS.

TRANSGLYCOSYLATION ACTIVITY OF A BETA-GLUCOSIDASE FROM *RHIZOMUCOR MIEHEI*

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Beta-glucosidases catalyse the hydrolysis of alkyl and aryl beta-glycosides as well as disaccharide glucosides and oligosaccharides of glucose. Under defined conditions, they also have a synthetic activity, which may be used for the synthesis of a variety of compounds such as oligosaccharides and different glycoconjugates. Oligosaccharides can be used as therapeutic agents, diagnostic tools, and

growth promoting agents for probiotic bacteria. Their enzymatic synthesis by the transglycosylation or glycosyl-transferase activity of glycoside hydrolases can be performed in one step instead of the several protection-deprotection steps required in chemical synthesis. The genus *Rhizomucor comprises* two well-established thermophilic fungal species, *R. pusillus* and *R. miehei*. In particular, *R. miehei* is important from a biotechnological aspect in consequence of its extracellular enzyme production. Because of the increasing interest of synthetic activity of beta-glucosidases in several biotechnological processes, the aim of the present study was to identify and characterize of the transglycosylation activity of the extracellular beta-glucosidase purified recently from *R. miehei*. Synthesis was carried out with cellobiose as substrate and transglycosylation activity was detected at every 3 hours during 24 h. The incubation temperature was 50°C and the applied enzyme concentration was 0.9 U ml-1. The HPLC analysis was performed using an YMC-Pack Polyamine II column at 35°C and at a flow rate of 1 ml min-1. The mobile phase was acetonitrile: water (60:40), and the detection of the oligosaccharides was carried out with a refractive index detector using glucose, cellobiose, cellotriose and cellotetrose as standards. Increasing amount of cellotriose was observed during the incubation period as a result of the transglycosylation activity; the maximal level was detected at the 24th hours. The quantity of glucose also increased due to the hydrolytic activity. The decrease in the cellobiose concentration observed was likely caused by the action of both activities of the enzyme. Further investigations of parameters influencing the transglycosylation of cellobiose are in progress in order to optimize the reaction.

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EXTRACELLULAR LIPASE ACTIVITIES IN ZYGOMYCETES

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Filamentous fungi are well known by their ability to excrete enzymes into the environment. Several Zygomycetes have important biotechnological potential as producers of industrial enzymes. Lipases are hydrolytic enzymes that catalyze the hydrolysis of triacylglycerols, which are the major constituents of fats and oils, to produce free fatty acids, glycerol and partial acylglycerols. There is a growing interest in microbial lipase production due to its great potential for industrial applications such as food additives, industrial reagents and stain removers, as well as for medical applications. Zygomycetes are known to be good producers of lipases and several *Mucor*, *Rhizomucor* and *Rhizopus* lipases have been isolated and utilized in the industry. However, other Zygomycetes are poorly characterized from this aspect, and our knowledge in reference to the activity and production of the enzymes is also limited. The objective of this work is to find new producer strains potentially applicable in further basic studies and biotechnological applications. This work evaluated the extracellular lipase activity of 143 strains representing the Zygomycetes genera *Rhizomucor*, *Rhizopus*, *Gilbertella*, *Mortierella*, *Mucor* and *Umbellopsis*. The culturing media contained tributyrin to monitor the lipase activity and the level of the enzyme production was evaluated by measuring the diameter of the halo around the colonies that formed in consequence to the lipase activity. The halo was measured daily during the incubation period (6 days). The enzyme activity of the tested strains showed high variability and several isolates showing high activity were detected in each genus. Isolates with good lipase production were selected for further submerged culture studies to investigate the effects of different inductors on the enzyme activity.

THE ROLE OF B-PROTEOBACTERIA IN BTEX DEGRADATION: DIVERSITY OF CATECHOL 2,3-DIOXYGENASE AND 16S RRNA GENES IN HYPOXIC, PETROLEUM HYDROCARBON CONTAMINATED GROUNDWATER

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Monoaromatic hydrocarbons, like benzene, toluene, ethyl-benzene and xylenes (BTEX compounds) are major components of petroleum products, therefore these hydrocarbons are always detectable in gasoline contaminated environments. Bioremediation of the contaminated groundwater is a promising low-cost approach; thus investigation of microbial communities involved in this process is in focus today. Detection of catechol 2,3-dioxygenase genes (C23O) in aromatic hydrocarbon contaminated environments gives the opportunity to measure the diversity of bacteria involved in the degradation of these contaminants. In this study a primer set was designed to detect mainly Comamonadaceae family (β-Proteobacteria) related catechol 2,3-dioxygenase genes in hypoxic, petroleum hydrocarbon contaminated groundwater and diversity of these genes was analyzed with Terminal Restriction Fragment Length Polymorphism (TRFLP). These catabolic genes coded enzymes supposed to function under hypoxic conditions as well, and may play a key role in BTEX degradation in the groundwater investigated by us. C23O genes were detected in every groundwater sample and it was observed, that different genotypes are dominant in the contaminated and in the background samples. To link taxonomic identity to the TRFs, the C23O PCR products of the samples were cloned and sequenced. Phylogenetic analysis based upon the nucleotide sequences showed, that all of the detected genes belong to the I.2.C subfamily of extradiol dioxygenases.To link the metabolic activity to the microbial structure 16S rRNA gene based clone libraries were generated from the samples and it was found that β-Proteobacteria dominated the groundwater samples. These results support the opinion that β-Proteobacteria play a grate role in BTEX degradation under hypoxic conditions.

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USING *SACCHAROMYCES CEREVISIAE* CELL WALL PROTEINS AS A ANCHOR FOR EXPRESSION OF HETEROLOGOUS PROTEINS AT THE CELL SURFACE

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In the past several years much efforts have been devoted to the study of expression systems for the display of heterologous proteins at the surface of microorganisms, opening new perspectives in biotechnology. Yeast cell surface systems have the advantages of simplicity of genetic manipulation and ability for proper post-translational modifications and folding of mammalian proteins. Yeast whole-cell biocatalysts displaying enzymes on their cell surface can be produced at a low cost and

show a high enzymatic activity. Recently a number of surface-engineered yeasts, displaying different heterologous proteins interesting for biotechnological or medical applications, have been constructed. *S. cerevisiae* cell wall proteins that are covalently bound to the carbohydrate components of the wall can be divided in two main groups. Majority of proteins of this class are bound at their C-termini through a remnant of the GPI-anchor. A smaller group of proteins are directly covalently bound at their N-termini to β-1,3-glucan by the alkali labile ester linkage between the glutamic acid γ-carboxyl group and hydroxyl groups of glucoses (Pir-proteins). Almost all heterologous proteins constructed so far for yeast surface display are GPI-anchored to the cell wall using C-terminal part of α-agglutinin as anchor. Some enzymes, whose active sites are located near their C-termini are not suitable for display through GPI anchor that must be fused at their C-terminal region.
Possible approach for such enzymes is to use Pir-proteins as a cell wall anchor. In this work Pir4p was used as anchor for N-terminal immobilization and Ccw12p for C-terminal immobilization of heterologous proteins to the yeast cell surface.

INVESTIGATION OF THE DISTRIBUTION OF VIRULENCE-ASSOCIATED GENES IN *KLEBSIELLA* SPECIES FROM URINARY TRACT AND BLOOD STREAM INFECTIONS

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Klebsiella species are important opportunistic pathogens causing infections mainly in immunocompromised patients suffering from underlying diseases. However, recently new information appeared about isolating more virulent clinical strains than before causing serious life threatening infections i.e. primary liver abscess, meningitis in patients without suffering from known underlying diseases. These data indicate that *Klebsiella* spp. especially *K. pneumoniae* has become an emerging pathogen. Previously, we have already reported results of a study on phenotypic characterisation of *Klebsiella* isolates at this forum. The aims of our present study were to extend our investigations to the prevalence of those genes potentially associated with virulence capability of *K. pneumoniae* and *K. oxytoca*. The collection and selection of both clinical isolate types fulfilled the principle of being relevant clinical isolates. All specimens were collected at different clinical wards of Pécs University. The group of urinary tract isolates consisted of 114 (83,3%) *K. pneumoniae* and 23 (16,7%) *K. oxytoca*, totally 137 isolates, and the group of blood stream isolates 115 (82,7%) *K. pneumoniae* and 24 (17,2%) *K. oxytoca*, totally 139 isolates. Seven genetic loci potentially associated with virulence characters were examined by polymerase chain reaction (PCR). The *Irp1* and *Irp2* genes encode proteins which are involved in yersiniabactin siderophore biosynthesis, and their orthologues are localised in the High-pathogenicity island of *Yersinia pestis* (YHPI). The integrase gene (*Int*) is integrated in the *asnT* tRNA gene in the flanking region of YHPI. The *Irp* genes and *asnT-int* loci showed almost the same prevalence in the blood borne and urinary tract isolates (29.5%, 29.9% and 20.1%, 18.2% respectively). The *kfuB* gen is a part of the 20-kb kfu/PTS locus, and lays a role in the iron uptake of *Klebsiella*. The *kfuB* gene could be shown on 43.1% and 40.8% of the blood and urinary culture strains, respectively. The *allS* gene is responsible for the anaerobic assimilation of allantoin as a nitrogen source and the presence of the gene increases the virulence in intragastric inoculation in animal models. This gene could be showed on 14,3% and 11,6% of isolates originated from urinary tract infection (UTI) and blood stream infections (BSI), respectively. The *rmpA* gene, known as a positive regulator of extracapsular polysaccharide synthesis confers a mucoid phenotype. Only one BSI strain showed positive reaction for that. Equal prevalence (2,8%) was found for the

k2A gene, the genetic determinant of K2 capsule serotype in both isolates types. The *magA* gene is the genetic determinant of K1 capsule serotype. We could show the presence of the *magA* gene in neither group of the isolates. The plasmid profile analysis and above data will be discussed in detail and substantiated by statistical dissection in the presentation. Further phenotypic and genotypic analysis of potential virulence factors of the isolates is in progress.

WHOLE-GENOME ANALYSIS OF PSEUDORABIES VIRUS BY REAL-TIME RT-PCR

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Pseudorabies virus (PRV) belongs to the *Alphaherpesvirinae* subfamily. PRV is an important causative agent of Aujeszky"s disease in swine, this is an excellent model organism in herpesvirus research and this virus is used as a "live" tracer of neuronal pathways.
The gene expression of Herpesviruses can be divided into three classes during a productive infection, namely immediate early (IE), early (E) and late (L) genes. Real-time RT-PCR is a sensitive and reproducible method that can be used to detect very small amount of RNA molecules. We have developed a *reverse transcriptase-based* real-time PCR technique to analyze the the expression of different viral genes of PRV. We characterized the expression kinetics of the genes of this virus, using a new calculation technique (relative expression ratio (R)).
We divided the genes into kinetic classes on the basis of temporal expression, as well as, dependency on viral DNA replication and *de novo* protein synthesis.

A MULTIPLEX PCR PHAGE TYPING SYSTEM BASED ON DETECTING ENTEROHAEMORRHAGIC *ESCHERICHIA COLI* O157 (EHEC) SPECIFIC PHAGES

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Since the frequently applied molecular typing methods like multi locus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) are expensive and time consuming. The aim of the study was to establish a novel molecular typing method for pathogenic, first of all for *E. coli* O157 strains. We presumed that EHEC O157 Sakai specific prophages (Sp) could characterize further *E. coli* strains. We monitored twelve Sakai phage (Sp) marker genes among different intestinal and extra-intestinal *E. coli* (ExPEC) strains. The Sp gene based typing system was used as triplex PCR. All the investigated *E. coli* strains carried at least one phage gene. Most Sp genes were detected in *E. coli* O157 strains, and different gene patterns were observed among the different pathotypes of O157 strains. Less phage genes were detected in non-O157 intestinal and in ExPEC strains. PCR results were converted to four digit numbers according to the Farmer's scheme. In summary, the PCR based phage typing is a novel molecular epidemiological method. This method could be not only a supplementary typing method, but an alternative one as well.

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OCCURRENCE OF *ALTERNARIA* SPECIES ON WHEAT LEAF LESIONS IN HUNGARY

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We evaluated the occurrence and composition of the necrotrophic pathogens of wheat across locations in Hungary since 2000. Altogether 340 monosporic isolates were selected from the several hundred samples examined between 2004 and 2007 for molecular studies. Species assignment of the monosporic isolates collected from wheat leaf samples has been carried out using morphological methods and ITS-based sequence analysis. Besides *Pyrenophora tritici-repentis* and *Pyrenophora teres*, several other pathogens have also been identified, including *Pithomyces chartarum*, *Alternaria* sp., *Epicoccum* sp., *Ascochyta* sp., *Curvularia* sp., *Ulocladium* sp. Altogether 51 isolates were found to belong to the *Alternaria* genus. Among these, 19 (37.25%) belonged to the *A. alternata*/*A. tenuissima*/*A. longipes*/*A. abrorescens* species group, and 28 (54.90%) to the *A. triticina*/*A. infectoria* species group. Species belonging to these species groups cannot be distinguished by ITS sequence data. Since previous studies clarified that *A. triticina* is an important pathogen of wheat causing *Alternaria* leaf blight in several parts of the world including India, Iran and Argentina, further studies are in progress to assign these isolates to species using other molecular and pathogenicity data in order to clarify the potential occurrence and pathogenicity of *A. triticina* in Hungary. 4 additional isolates (7,85%) were found to belong to a previously undescribed species, Alternaria hungarica based on morphological examinations and ITS sequence data. These isolates were collected from wheat leaf lesions in 2007 near Debrecen. Although the solitary conidia of *A. hungarica* resemble those of *A. mouchaccae* and *A. molesta*, the growth and sporulation patterns are more close to those of *A. geniostomatis* and *A. soliaridae*. Phylogenetic analysis of ITS sequence data indicated that this new species can be distinguished from all other examined *Alternaria* and *Embellisia* species. Pathogenicity tests revealed that *A. hungarica* can be considered as a minor pathogen of wheat.

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EPIDEMIOLOGICAL ANALYSIS OF PNEUMOCOCCAL CARRIAGE IN HEALTHY CHILDREN ATTENDING DAY-CARE CENTRES

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The carriage of *Streptococcus pneumoniae* (pneumococcus) plays a major role in the transmission of bacteria to sensitive individuals. The carriage rate can reach 50-100% in small children, especially attending day-care centres. Prevenar was proven also to have an effect on the carriage. As this vaccine became widely available in Hungary for children < 2 y in Oct 2008, it was important to examine the carriage of pneumococcal strains before the vaccination programme was implemented. Sixty-four pneumococcal isolates were collected from the nasal passages of children attending 4 day-care centres in Hungary. The species identity of all strains was confirmed by the presence of the lytA

gene. Their antibiotic sensitivity was determined by E-test, applying the EUCAST breakpoints. Serotyping was done by the combination of the conventional method (with antisera) and a PCR-based method. The genetic relatedness of the strains was examined by PFGE. The overall pneumococcus carriage rate was 40.9% among the children. We could serotype 59 out of the 64 strains (=92.2%). The detected serotypes were (n): 14 (27), 6A (13), 6B (5), 23F (3), 19F (3), 18C (3), 3 (2), 19A (1), 13 (1), 15B (1). The strains were generally very sensitive, except for macrolides. None of them were resistant (R) to penicillin, the highest MICs (0.5-0.75 mg/L) were detected in sero 14, 23F, 19F and 18C isolates. 17 strains showed high-level R to erythromycin (ery), these were of different serotypes, and 13 carried the erm(B) gene. Four strains showed the M type (= ery MIC 6-24 mg/L, clinda S), all these were of sero 14, identical by PFGE and had the mef gene. We could identify several definite PFGE clones. Based on our data, and taking certain cross-protections into account, Prevenar (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) would cover about 85 % of all isolates. The presence of a mef+ serotype 14 clone (= England14-9 PMEN clone) and another mef+ clone with unusually high ery R (= Taiwan19F-14) were observed, both of which were detected earlier in Hungary. We could clearly show the presence of firm clones within the nurseries, indicating the intensive exchange of bacteria between children. This is the first study on pneumococcal carriage in Hungary.

HCV GENOTYPES AMONG INJECTION DRUG USERS IN HUNGARY

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More than 170 million people are infected by hepatitis C virus (HCV) worldwide, in Hungary approximately 70 000. Unfortunately, the infection leads mostly to chronic hepatitis, cirrhosis and it is one of the main causes of hepatocellular carcinoma. The high degree of genetic variability is an important feature of HCV, which led to its classification into six major genotypes and a large number of subtypes. The prevalence and genotypic pattern of HCV vary by geographic locations. Response to interferon (IFN)-based therapies in patients infected with different HCV genotypes is different. Genotype 3 responds much better to IFN treatment than genotype 1. Therefore genotyping of HCV is important for predicting treatment responses and for determining the duration of antiviral therapy. HCV genotyping is also of public health importance as it can be useful for investigating outbreaks and for understanding the epidemiology of the infection. HCV is transmitted through exposure to contaminated body fluids and blood especially by needle-sharing. Injection drug usage accounts for a significant proportion of HCV infections in Hungary. The aim of this study was to determine the prevalence and genotypic data of HCV infections among Hungarian injection drug users (IDUs), which is an important step in the prevention and treatment processes. We measured anti-HCV antibodies from dried blood spots of IDUs to determine the seroprevalence. Anti-HCV positive specimens were subjected to RNA isolation, reverse transcription and nested PCR using universal HCV primers specific to the 5' non-coding region of the virus genome. PCR products were sequenced and sequences were compared with reference sequences to determine the genotype. We found that the genotype distribution is different amongst IDUs compared to the general population: genotype 3 occurs more often in people of drug abuse. Moreover there are certain regions of the country where the frequency of genotype 3 is very high (10/13) amongst HCV PCR positive IDUs. Most of the HCV positive IDUs rejected the offered treatment. One cause of the rejection can be the serious side effects. Knowing the high frequency of the presence of genotype responding better to the treatment could help IDUs accept the treatment.

FORMATION OF FUNGICIDE RESISTANCE LEVELS IN *BOTRYTIS CINEREA* IN DIFFERENT WINE REGIONS OF HUNGARY

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Botrytis cinerea (de By.) Pers. (teleomorph: *Botryotinia fuckeliana,* Whetz) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants by infecting various tissues. In grapevine, the frequent occurrence of *B. cinerea* prior to harvesting results serious losses of fruits and deterioration of wine quality. *B. cinerea* has been shown to have several variable genetical and physiological traits. It is able to act as a saprophyte as well as a pathogen, and it has developed resistance to most of the fungicides used to control it. Numerous isolates of grapevine berry-infected *B. cinerea* were collected from various locations of four Hungarian wine regions: Eger, Tokaj, Badacsony and Villány. Individual strains were obtained by single-spore isolations. Potato dextrose agar (PDA, Sharlau) was used as a base medium, fungicide added minimal medium and fruit rotted test for resistance levels. Isolates were tested for iprodione (Rovral 50WP, BASF), fenhexamide (Teldor 500SC, Bayer) and pirimethanil (Mythos 30 SC, BASF). Fungicide resistance was described toward was described the fungicide resistance or possibility of fungicide resistance in *B. cinerea* in different international publications. In the course of our examinations we found different fungicide resistance levels as well. The presence of this resistance is a remarkable hazard, because these chemicals are used in every day plant protection routine against *B. cinerea*. In our examinations, using of one sided and not purposive plant protection can eventuate the attendance and permeation of high number of fungicide resistant *B. cinerea* strains in fields against new fungicides as well.

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MICROBIAL COMMMUNITY STRUCTURE CHANGES DURING OYSTER MUSHROOM SUBSTRATE PRODUCTION

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Although oyster mushroom (*Pleurotus* spp.) is a valuable food, and is cultivated worldwide in industrial scale, the microbial dynamics during oyster mushroom substrate production is still unknown. Thus in the presented study the aim was to characterize the microbial dynamics with chemical and biological tools. During substrate production enzymatic digestibility of the substrate improved with 77%, whereas the cellulose and hemicellulose to lignin ratios decreased with 9 and 19% respectively. Fungal species played a role in the initial mesophile phase of substrate production process, but they disappeared during pasteurisation in the tunnels at constant elevated temperatures. Changes in the bacterial community showed a noticeable succession during substrate production investigated with terminal restriction fragment length polymorphism (T-RFLP) based on 16S ribosomal deoxyribonucleic acid (rDNA). Mature samples represented the least variance, which indicated the effect of the standardized production protocol. The relation between mushroom yield

and the bacterial community T-RFLP fingerprints was investigated, but the uniformity of mushroom yields did not support any significant correlation. For identifying the dominant microbes in the community-fingerprints one clone library was constructed from each phase of a high mushroom-yielding production series. The first phase was dominated by Proteobacteria: Sphingomonas species, which occur typically in soils, Pantoea and Pseudomonas species, but also the members of Actinobacteria (*Saccharopolyspora* spp.) and Bacteriodetes (*Pedobacter* spp.) could be found. In the following two phases the dominance of thermophilic microbes were more characteristic. The most abundant bacteria in the second phase were the widespread *Flavobacterium* spp., *Geobacillus debilis*, *Bacillus thermozeamaize*, Sphingobacterium composta and *Ureibacillus* spp. The third phase was dominated by *Microbispora bispora*, uncultured *Chloroflexi clones*, *Geobacillus thermodenitrificans*, *Thermobacillus xylanilyticus* and some uncultered clones from matured compost.

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COMPARISON OF AVIAN *PASTEURELLA MULTOCIDA* ISOLATES BY CONVENTIONAL CHARACTERISATION METHODS AND BY THEIR OUTER MEMBRANE PROTEIN PATTERN

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Pasteurella multocida, a coccoid Gram negative facultative anaerobic bacterium can infect a wide range of wild and domesticated mammal and avian species causing a variety of diseases all over the world. It was recognized from natural infection of more than 180 bird species. As a primary infectious agent it is responsible for fowl cholera in poultry and as a secondary invader it is associated with respiratory diseases and local inflammations of birds. *P. multocida* strains show high heterogeneity when biochemical characteristics, capsule and somatic serotype, pathogenicity, and antibiotic sensitivity are examined and display great genotypic variations. The three distinguished subspecies (*P. multocida* subsp. *multocida*, *P. multocida* subsp. *septica*, *P. multocida* subsp. *gallicida*) were all isolated from birds. All of five capsular serogroups (A, B, D, E and F) have been recognized among avian *P. multocida* isolates but serogroup A strains were most common. Eleven reference strains of the 16 somatic serotypes are originated from birds. According to various biochemical characteristics, biotypes have been recognised within the species. Nevertheless, these parameters do not provide enough details to recognize and distinguish the virulent strains from the avirulent. Outer membrane proteins (OMPs) of bacterium are very variable. More than 35 different OMP types are known in *P. multocida*. The phospholipids and lipopolysaccharides make their structure even more variable. It is confirmed, they play important role in bacterium-host cell adhesion, in the bacterial conjugation and they are responsible for bacterial cell integrity. Pattern of the outer membrane proteins in SDS-PAGE showed high heterogeneity within avian *P. multocida* isolates. Results of various traditional and molecular biological characterisations may provide a better understanding of the heterogeneity and pathogenicity of avian *P. multocida* strains.

We compared with the above mentioned methods over hundred avian *P. multocida* isolates from various bird species suffering from different illnesses. Majority of avian *P. multocida* isolated from acute fowl cholera showed well distinguishable OMP pattern. All of them displayed A:1 serotype and they were similar to each other in their carbohydrate fermentation pattern. The isolates from acute fowl cholera are possessed of clone population characters. Contrary to it, the OMP patterns of isolates from chronic cases presented heterogeneity both in fermentation features and protein pattern.

RECENT DEVELOPMENTS IN THE TAXONOMY OF CLINICALLY RELEVANT ASPERGILLI

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The genus *Aspergillus* includes more than 250 species, among which about 20 have been reported to cause opportunistic infections in humans. The most important human pathogens in this genus are assigned to sections *Fumigati*, *Flavi*, *Nigri*, *Terrei*, *Nidulantes* and *Usti*. An overview will be given on recent developments in these medically important taxa. In section *Fumigati*, *A. fumigatus* is the most common cause of invasive aspergillosis. Recently, a significant increase has been observed in the incidence of multiple azole-resistant *A. fumigatus* isolates in patients with invasive aspergillosis. A dominant resistance mechanism was found, and preliminary data indicate that azole resistance might have developed through azole exposure of the fungus in the environment. Recent molecular studies have also revealed that several clinical isolates previously identified as *A. fumigatus* exhibit altered antifungal susceptibility profiles against several drugs, and actually belong to other species including e.g. *A. lentulus*, *Neosartorya udagawae* or *N. pseudofischeri*. Among black Aspergilli, recent sequence-based identification of about 200 clinical isolates indicate that besides *A. niger*, other black Aspergilli including *A. tubingensis*, *A. awamori*, and *A. acidus* are also important human pathogens. Preliminary data indicate species-specific differences in the antifungal susceptibilities of the clinical isolates. Besides the species listed above, the recently described *A. brasiliensis* has also been identified as causative agent of fungal keratitis. In section *Usti*, *A. ustus* has been considered previously as a relatively rare human pathogen. However, recent reexamination of several clinical isolates revealed that they represent a new species, *A. calidoustus*. Antifungal susceptibility testing showed that this species has decreased susceptibilities to several antifungal drugs. The triazoles are inactive in vitro, including the new azole posaconazole. In section *Flavi*, several species including *A. tamarii*, *A. nomius* and *A. pseudonomius* have been identified as causative agents of fungal keratitis in India. Sequence-based identification and antifungal susceptibility testing of about 130 clinical isolates belonging to section *Flavi* is in progress. *Aspergillus terreus* is another important human pathogen with decreased susceptibilities of amphotericin B. This species is the predominant one causing invasive aspergillosis in some medical centres. Using multilocus sequencing for analyzing species diversity within a large number of clinical and environmental *Aspergillus terreus* isolates representing diverse geographic locations, a new species, *Aspergillus alabamensis* has been described within section *Terrei*. Most members of this new species were recovered as colonizing isolates from immunocompetent populations, and were morphologically similar to *A. terreus* with decreased in vitro susceptibilities to the antifungal drug amphotericin B. Invasive infections caused by *E. nidulans* are uncommon in animals and humans; in humans they appear to occur predominantly in patients who have chronic granulomatous disease. Recent sequence-based examination of a set of clinical *Emericella* isolates have identified a role of *E. quadrilineata* as an opportunistic fungal pathogen, especially in patients with CGD and in those with hematologic malignancy. *E. nidulans* was less susceptible than *E. quadrilineata* to amphotericin B, but more susceptible to caspofungin, indicating that correct species demarcation and in vitro susceptibility testing may affect patient management.

MYCOTOXIGENIC BLACK ASPERGILLI IN RAISINS OF DIFFERENT ORIGIN

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Ochratoxin A is a mycotoxin produced by several *Penicillium* and *Aspergillus* species, including black Aspergilli. This mycotoxin is a common contaminant of various foods including cereal products, spices, dried fruits, coffee, beer and wine. Recent data indicate that black Aspergilli including *Aspergillus niger* and *A. carbonarius* are responsible for ochratoxin contamination of grape and grape-derived products. The ability to produce another important mycotoxin, the carcinogenic fumonisin B2 has recently also been described in *A. niger*. In contrast with the well-known fumonisin producer *Fusarium verticillioides*, which produces fumonisins on media containing plant extracts, *A. niger* produces this mycotoxin in media with low water activity. Data on the occurrence and role of this species in fumonisin contamination of agricultural products with high sugar or salt content are needed to clarify the importance of *A. niger* in human health. We examined the mycobiota of 30 raisin samples of different origin to clarify which species could be responsible for mycotoxin contamination. All except six raisin samples were contaminated with black Aspergilli. Besides *A. carbonarius*, ochratoxin producing *A. niger* and *A. tubingensis* isolates were identified in the samples. *Aspergillus niger* dominated in most of the samples, except for 2-2 Chinese and Turkish samples, and in 1-1 Iranian and Indian samples. Since most isolates of *A. niger* are able to produce both ochratoxin A and fumonisin B2 in media with high sugar content, our data indicate that mycotoxin contamination of raisins is a real threat to the consumers.

Ochratoxin A content of some of the raisin samples was also examined by an immunochemical technique, and the results were confirmed by HPLC analysis using fluorescent detection. Altogether 20 raisin samples were analyzed. Ochratoxin A was detected in all but two samples with ochratoxin concentrations ranging from 0 to 6.2 mg kg-1. The most heavily contaminated raisin sample came from Iran. However, none of the raisins contained ochratoxin A above 10 mg kg-1, the European Community maximum allowable limit in raisins.

THE ROLE OF IGG AVIDITY IN DIAGNOSIS OF CYTOMEGALOVIRUS INFECTION IN NEWBORNS AND INFANTS

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Cytomegalovirus (CMV) is the most common cause of congenital viral infection. CMV IgM antibody detection is a good indicator of acute or recent primary infection. However, some congenitally infected babies do not produce IgM antibodies or IgM antibody production can be delayed. Determination of IgG avidity could help in determination of primary CMV infection. The aim of this study was to evaluate the value of IgG avidity in diagnosis of CMV infection in newborns and infants. Serum samples from 40 infants under 12 months of age with congenital/perinatal CMV infection were tested for IgM, IgG and IgG avidity using an enzyme-linked immunosorbent assay (ELISA). The determination of IgG avidity was carried out with urea as denaturing agent. For 25 of them, virus isolation and/or polymerase chain reaction (PCR) on urine specimens were performed. Thirteen (32.5%) patients showed the presence of CMV IgM antibodies, 3 (7.5%) had equivocal IgM result, and 24 (60.0%) patients had IgG antibodies only. Using IgG avidity, primary CMV infection (low avidity antibodies) was documented in 61.5% IgM positive and 54.2% IgM negative patients. Eight of nine (88.8%) IgM positive patients were positive either on virus isolation or PCR. In IgM

negative patients, 46.6% urine cultures were positive for CMV and 66.6% were PCR positive. IgG avidity demonstrated acute/recent primary CMV infection in 58.8% patients younger than three months compared with 91.7% and 81.8% in 3-6 and 6-12 months old babies, respectively. In conclusion, IgG avidity can identify primary CMV infection either in IgM positive or IgM negative children older than 3 months of age. In infants less than 3 months, transplacentally derived maternal IgG antibodies of high avidity influence on the IgG avidity result. CMV infection should be confirmed by direct virologic methods such as virus isolation or PCR in these children.

MIG IS AN ANTICHLAMYDIAL MEMBER OF THE MOUSE EPITHELIAL SECRETOME

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Chlamydia trachomatis is an obligate intracellular bacterium that primarily targets columnar epithelial cells of the conjunctiva and the genitourinary tract. The major cytokine involved in the elimination of the bacterium is the IFN-gamma produced by CD4+ and CD8+ T-lymphocytes. In order to discover the potential elimination mechanisms, the combined impact of *Chlamydia trachomatis* infection and IFN-gamma on the transcriptome of murine epithelial cells was measured using the Affymetrix 430 A2 whole-genome mouse chip. Functional analysis of the microarray data revealed that the genes of various cytokines, chemokines and other secreted molecules were highly upregulated in the infected, IFN-gamma treated and infected+IFN-gamma treated cells. MIG (monokine induced by gamma interferon) was among the significantly induced host genes with a 3,14 and 64 fold upregulation in the IFN-gamma treated and infected+IFN-gamma treated cells respectively. A modest, but detectable antibacterial activity of MIG against both Gram+ and Gram- bacteria has been described before. We tested the direct antichlamydial activity of MIG in different concentrations and incubation times. Distinctly from the previous literature the measured direct antichlamydial activity of MIG was substantial, reaching a 95-97% reduction in infectivity. According to our results, the secreted MIG could have a role in the antichlamydial defense.

GENOME MINING TO IMPROVE BIO-ETHANOL PRE-TREATMENTS

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The first step in bio-ethanol production involves hydrolysis of plant polysaccharides to fermentable sugars using either chemical or enzymatic treatment. The currently available enzyme cocktails for bio-ethanol pre-treatment leave a significant part of the biomass unused. The availability of a growing number of fungal genomes provides a large variety of potential novel enzymes that may improve these enzyme cocktails. Detailed analysis of fungal genomes enables reliable function prediction for many putative plant polysaccharide degrading enzymes as was evidenced recently for *Aspergillus nidulans*, where the function prediction was confirmed by large scale enzyme analysis. Fungal comparisons highlight enriched enzyme families that can be related to the natural biotope and the ability of the species to grow on different polysaccharides. Identification of genes encoding

enzymes for which no previous genes have been identified is a more complex issue. In addition, characterization of the in vivo function of novel enzymes relies on specific substrates that can distinguish between small differences in activity between iso-enzymes. Finally, production of the enzymes depends on improved (fungal) production systems that can not only produce enzymes of ascomycete origin, but also those derived from basidiomycetes and zygomycetes. Recent strategies and examples will be discussed to highlight the possibilities and limitations of genome mining for the identification of novel enzymes that could be used to improve bio-ethanol pre-treatments.

LEISHMANIOSIS IN AUSTRIA? CLIMATE CHANGE AND AUTOCHTHONOUS *LEISHMANIA* INFECTIONS IN CENTRAL EUROPE

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Two different disease entities are caused by *Leishmania* spp., the visceral leishmaniosis (VL) and various forms of cutaneous leishmaniosis (CL). Worldwide around 12 million people are infected and 60.000 people die from leishmaniosis - predominantly from VL - annually. *Leishmania* spp. are transmitted by sandflies (Phlebotominae), *Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World. In Europe, sandflies are widely distributed in more than 20 species in many parts of Southern Europe; but their occurrence in Central Europe north of the Alps was excluded until recently. However, in the past years, there has been increasing evidence of autochthonous leishmaniosis cases in regions where there previously had been imported cases only, and in 1999 sandflies were recorded in Germany for the first time. Since then sandflies have been found in several parts of Germany and Belgium and it has been discussed, whether this is due to climate change. Meanwhile, the more likely assumption is that sandflies have been in Central Europe perpetually, but sporadically since the Holocene climate optima (ca. 5000 and 3000 B.C.), that global warming, however, will certainly lead (and maybe has already led) to an extension of the distributional areas of sandflies, temperature being the key factor to their distribution. A comparison of climatologic parameters of Central European sandfly habitats with the climatic conditions in Austria (where sandflies have not yet been found) has shown that an increase of temperature by 1°C in January (*Phlebotomus mascittii*) or 1°C in July (*Ph. neglectus*), respectively, would lead to suitable conditions for the occurrence of sandflies and thus the possible occurrence of autochthonous leishmaniosis in certain parts of Austria.

CHARACTERIZATION OF YEAST *SACCHAROMYCES CEREVISIAE* IN THE STATIONARY GROWTH PHASE AT CELLULAR AND MOLECULAR LEVEL

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Yeast *Saccharomyces cerevisiae* is being widely used as a model organism for investigating fundamental cellular processes, stress responses and metabolic pathways of the human [1, 2]. The high homology between yeast and mammals for basic cell processes gives great opportunity for understanding human cell biology. Specifically in the stationary growth phase the cells are

homologous to the most cells of multicellular organisms [3]. To better understand the action in the stationary growth phase, the yeast *Saccharomyces cerevisiae* was characterized at the cellular and molecular level. Cell energy metabolic activity, cell viability, intracellular oxidation and protein expression at different time points during stationary phase were investigated. Results obtained at both levels were statistically evaluated and compared to each other. This gave better insight into yeast metabolism in long-term stationary phase culture. Additionaly, results enabled us to choose the optimal time point in the stationary phase reflecting appropriate metabolic and oxidative state of the yeast being a good model.

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THE INVASIVE GROWTH OF *SACCHAROMYCES CEREVISIAE*: NEW APPROACHES AND FINDINGS

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The increased incidents of *Saccharomyces* invasive infections in immunocompromised adults and newborns, the symptoms clinically indistinguishable from that of invasive candidiasis, and the insusceptibility to some antifungal drugs, like fluconazole (Enache-Angoulvant, A., 2005, Clin Infect Dis 41, 1559), raised the question about the GRAS (generally recognized as safe) status of the species. From the beginning of the previous decade, the number of scientific articles related to virulent traits of *Saccharomyces cerevisiae*, like invasive growth, has rapidly increased. However, the pathogenesis of this yeast is still not well understood. One of the reasons for the lack in the knowledge on the field is surely the lack of methodology. Therefore, new approaches have been developed recently in our laboratory. Qualitative agar invasion assays have evolved to the Quantitative agar invasion assay (Zupan, J., et al., 2008, J Micr Meth, 73, 100), which was used to clarify the significance of a wide specter of extrinsic factors controlling the adhesion and the invasion of virulent and non-virulent *S. cerevisiae* strains. The results showed a stimulative effect on the invasiveness of virulent strains at nutrient starvation (resulting in relative invasion above 80% on nitrogen- and above 30% on glucose-depleted medium), and at temperatures typical for human fever (39°C), which indicates the virulence potential of those strains in the particular condition. On the other hand, a strong repressive effect on the invasion was found in the presence of salts, anoxia and some preservatives (manuscript in submission). This quantitative approach opens new possibilities for gene/protein-expression studies related to yeast invasion, which was exploited in our next, proteomic research on yeast cell wall (CW) proteins, mostly important as adhesins and flocculins. A new, simple and non-destructive method for miniprep extraction and rapid quantitative SDS PAGE analysis of *S. cerevisiae* CW proteins was developed and used to study the yeast invasive growth phenomenon. The new approach revealed a presence of up to 20 CW protein bands and showed significant changes in the protein profile expression relevant to different cultivation temperature, cell morphology (invasive vs. non-invasive growth) and yeast strain (manuscript in submission). Additionally, the method on animal epithelial cell cultures, adapted from the bacterial cytotoxicity assays, was introduced to determine the virulence potential of *S. cerevisiae* strains.

KLEBSIELLA INFECTIONS AND SUSCEPTIBILITY TESTING

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Klebsiella are ubiquitous in nature, a genus of rod-shaped bacteria of the family Enterobacteriaceae. In humans may be regarded as normal flora in many parts of the intestinal tract and the biliary tract. They may colonize the skin, pharynx, gastrointestinal tract, also wounds and urine. But, *Klebsiella* species cause a variety of clinical syndromes, including upper respiratory tract infection, pneumonia, ozena, wound infection, urinary tract infection, diarrhoea, meningitis, bacteremia etc. *Klebsiella* infections tend to occur in people with weakened immune system. Bacteremia and significantly increased mortality have resulted from infection with these species. Uncontrolled and extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and subsequently the development of multidrug-resistant strains. The objective of this study was to evaluate the antimicrobial susceptibility tested in vitro of *Klebsiella* isolates, based on the agar disc diffusion method on Mueller Hinton agar, of the NCCLS standard method. A panel of 86 clinical *Klebsiella* isolates was tested on antimicrobial susceptibility test discs: AN -AmC - SAM - FEP - CTX - CAZ - CRO - CXM - CIP - GM – IPM - MEM - NOR - TZP -PIP. This is important because different *Klebsiella* bacteria are resistant to different antibiotics. Pathogenic species include: *K. pneumoniae*, *K. rinoscleromatis*, *K. oxytoca* and *K. ozaenae*. All kinds of bacterial isolates were clinically isolated from different patient samples in the routine microbiological work. All isolates were grown on Columbia blood agar and MacConkey agar. Revaluated and identified as *Klebsiella* species with no automated biochemical tests for Enterobacteriaceae: triple sugar iron agar - lysine (MIL) - ornithine - citrate - urease production - arabinose - lactose - sacharose - manitol and adonitol-fermentation, l-sorbose test - malonate - oxidase test. In the present study the results have shown that sensitivity of *Klebsiella* specieson to the AN and IPM are 100%, followed by the MEM (97,7%) and FEP (92,6%). These antibiotics can be used in the treatment of Klebsiella infections. With 62% sensitivities on: TZP - GM - CXM - CRO - CAZ - CTX -SAM and 60,5 % with PIP and 60% on AmC, finally are NOR (53,5%) and the CIP (48,8%). In flow of this study 36,6% of strains was multiply resistant on the antibiotics (SAM - CTX - CAZ - CRO - CIP - GM - NOR - PIP - AmC). This multidrug resistant *Klebsiella* species were isolated from clinical samples. *Klebsiella pneumoniae* is the most medically important species of the group (82,5%), followed by *Klebsiella oxytoca* (14%) and *Klebsiella rinoscleromatis* (3,5%).These findings may have important therapeutic and epidemiological implications. Ongoing studies will reveal the true entity of these microorganisms, which could be of concern for infections in immunocompromised patients. This information is useful to perform targeted interventions (considering, antimicrobial agents and medical specialty), to control the further development of resistance. Clinicians have to carefully evaluate the use of combination when coexistence of resistance is observed.