# ABSTRACTS

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## EFFECT OF HERBAL EXTRACTS ON THE GROWTH OF PATHOGENIC *KLEBSIELLA PNEUMONIAE* STRAINS

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*Klebsiella pneumoniae* infections with multiresistant character of this nosocomial pathogen constitute a word-wide challenge in the clinical practice. The frequently multiresistant character of the nosocomial pathogen justifies the efforts to search for alternative prospects in order to control such manifestations. In this study we give an accout on the antibacterial potential of several plant educts on a set of *Klebsiella pneumoniae* strains producing extended spectrum betal-lactamases (ESBL). Effect of 14 commercially available herbal extracts (peppermint-, lemon-, melissa-, eucalyptus-, fennel-, cinnamon-, spearmint-, grapefriut-, indian lemongrass-, lavender-, clary-, sage-, rosemary-, and clove-oil) were tested on the growth of different human pathogenic multiresistant *Klebsiella pneumoniae* strains (KPC-1, KP0787, MGH78578). All tests were carried out in liquid cultures by using different herbal extract oil concentrations. Spearmint, indian lemongrass and clove proved to be the most effective in retarding the proliferation of the investigated strains. To reveal if the observed antimicrobial effect of these three herbal extracts is general for *K. pneumoniae* we tested 15 additional and formerly characterised human isolate *K. pneumoniae* strains. We have revealed that all the three herbal extracts have exhibited hindering effect on the proliferation of the tested human isolate *K. pneumoniae* strains. To visualize possible groups of active components a bioautography was carried out where the investigated herbal extracts were separated by thin layer chromatography (TLC). The TLC plate was sopped into the bacterium suspension that was followed by the development procedure. By this method active componnets or groups of active components could be revealed that should be further analyzed.

## DETECTION OF THE *aprX* GENE CODING FOR THE ALKALINE METALLOPROTEASE AND SCREENING FOR THE PROTEASE ACTIVITY IN *PSEUDOMONAS* STRAINS OF MEAT ORIGIN

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In spite of that bacterial spoilage of meat causes significant economic loss, the role of hydrolytic enzyme activities of the evolving microbiota in the progress of spoiling is not well studied. *Pseudomonas* spp. are the predominant spoiling bacteria, which were frequently identified as producers of extracellular heat-resistant proteases in dairy products. Alkaline metalloproteases are produced by several bacteria, which are zinc-dependent metalloproteases and requiring $Ca^{2+}$ (or $Sr^{2+}$) for stability. Soil derived *P. fluorescens* strains were reported as producers of alkaline metalloprotease encoded by the *aprX* gene and specific primers were designed by Bach et al. (2011) for detection of the gene in different *P. fluorescens* biotypes. Our purpose was to examine *Pseudomonas* strains isolated from chilled-stored meat for the presence of the *aprX* gene and determine its sequence

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polymorphism in case of positive isolates. Traditional enzyme assays were used to screen for the proteolytic capacity of different *Pseudomonas* isolates. Protein digestion was tested by determination of the clearing zones around the macrocolonies developed on special indicator media (SM - Skim Milk Agar and SMC - Standard Method Caseinate Agar). Fifty-five % of the analyzed strains showed proteolytic activity, *P. fluorescens* and *P. lundesis* were found as the main producers, while *P. fragi* strains were weak or null from this respect. Set of primers designed by Bach et al. (2001) for detection of the alkaline metalloprotease gene *aprX of P. fluorescens* were used for PCR screening. According to our results only 23 out of the 27 *P. fluorescens* strains were positive for the generation of PCR amplicons. For determining the polymorphism of the PCR products the amplicons were examined with DGGE (Denaturating Gradient Gel Electrophoresis) and HA (Heteroduplex Analysis) assays. High conservation in the nucleotide sequences of the *aprX* amplicons was observed among the *Pseudomonas* strains involved in the molecular analysis. *In silico* analysis of the *Pseudomonas* alkaline metalloproteases were examined using the UniProt database. The proteolytic domain includes the active-site (HEIGHTLGLxH) in which „x" represents an arbitrary amino acid residue. The three histidine (245, 250, 256) are zinc ligands and the Gly is responsible for the catalytic activity. Every residue in that motif showed more than 90% homology. X is represented mostly by Ala or Ser. The calcium binding motif GGxGxD is responsible for binding more than one $Ca^{2+}$. The eighth $Ca^{2+}$ binding motif (SGDAHAD or SGQGVAD) has a role in the optimal temperature of alkaline metalloproteases. The primers designed by Bach et al. (2001) are flanking the region around the active centre showing high nucleotide sequence conservation, which could be the explanation of the low degree of polymorphism indicated by the DGGE and HA analysis.

## SCREENING AND APPLICATION OF NEW GENETIC MARKERS FOR *THERMOPLASMA ACIDOPHILUM*

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In our previous research we have developed the basic genetic tools for *Thermoplasma acidophilum*, a thermo-acidophilic archeon: solid medium for clonal selection, screening for suitable selection markers, shuttle and integrative vectors based on the novobiocin resistant (novR) gyrase B (*gyr*B) gene, originating from a naturally occurring cell line, and the homologous *T. volcanium* novR *gyr*B gene and an effective transformation method. The aim of the current research is to bring the existing genetic tools to application level. Firstly, the effectiveness of gelrite solid medium was increased by addition of a native yeast extract. Application of this growth promoting substance decreased the clonal selection time by 25 %. Parallel, the sensitivity of *T.acidophilum* against a dozen of antibiotics was tested for putative use as selection marker; among others thiostrepton and rifampicin were the most appropriate candidates. A successful transformation was carried out with a thiostrepton resistance mediating vector (pSES1) from *Thermobifida fusca*. Moreover, *T. acidophilum* was reported to be resistant to rifampicin, but unexpectedly in our hands it showed great sensitivity ($C_{inhibition}$=2.5 µg/ml). Rifampicin resistance gene from *Pseudomonas aeruginosa* (*arr2*) and a newly constructed synthetic novR *gyr*B were employed as selection markers in our newly rebuilt KO and His-pulldown vectors created by the "overlapping-extension PCR-based method". Rifampicin resistant *T.acidophilum* clones could be generated with the *arr2*-based construct pTA-Arr. The

successful transformation was verified by *arr2*-specific PCR and sequencing in all *T. acidophilum* transformants. Synthetic novR *gyr*B gene was designed to avoid homologous recombination with the wild type *gyr*B gene. It was created by silent mutations based on the codon degeneration in a way that stretches of no more than 5 base pairs were identical with the original gene. Novobiocin resistant cell lines could be generated based on the synthetic novR *gyr*B gene and the maintenance could be also confirmed. By the use of this maker a vector-based knock-out mutagenesis system was developed and tested in order to deplete two proteins involved in protein turnover (ubiquitin-like archaeal protein, tricorn protease). The three new and tested antibiotic (novobiocin, rifampicin and thiostrepton) resistance marker genes have considerably advanced the development of *Thermoplasma acidophilum* genetic tools. The new genetic markers can raise high possibility of the application not only the classical genetic tools but also the recombineering tools.

## ANTI-CHLAMYDIAL EFFECT OF PLANT PEPTIDES

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Members of the family Chlamydiaceae are obligate intracellular bacteria that can cause both human and animal diseases. *Chlamydia trachomatis* causes trachoma and genitourinary infections, *Chlamydophila pneumoniae* causes acute and chronic respiratory tract infections. Although antibiotic therapy eliminates chlamydial infection, it does not ameliorate established pathology. Furthermore, the administration of antimicrobial agent may blunt the development of natural immunity to *C. trachomatis* in human. Anti-Microbial Peptides (AMPs) that represent ancient host defense effector molecules present in all organisms across the evolutionary spectrum. To date, more than 800 AMPs have been discovered in various organisms, including 270 from plants. One part of the antimicrobial peptides exhibit a narrow spectrum, while others show highly broad-spectrum of activity against Gram negative and Gram positive bacteria, fungi as well as viruses and parasites. Nodule specific Cysteine Rich (NCR) peptides produced by symbiotic plants have AMP-like structure and activities. The NCR gene family encodes at least 350 highly divergent peptides in *M. truncatula* (Mergaert et al. 2003, Alunni et al. 2007). We tested synthetised NCR peptides activities on *Chlamydia* sp. and our work demonstrated that some of these peptides have in vitro antimicrobial affect.

Elementary bodies of different *Chlamydia* strains were incubated with different NCR peptides at various concentrations. The time course of the anti-chlamydial effect of plant peptides was tested after incubation periods of 0.25, 0.5, 1, and 2 h. To quantify the anti-chlamydial effects NCR peptides, HeLa cells were seeded in tissue culture plates with cover glasses. After 24 h, the confluent cells were infected with *Chlamydia* elementary bodies or with NCR peptides-treated *Chlamydia* elementary bodies. After 48 h, the cells were fixed and stained with monoclonal anti-*Chlamydia* LPS antibody and FITC-labelled anti-mouse IgG. The number of *Chlamydia* inclusions was counted under a UV microscope, and the titre was expressed in IFU/ml.

Seven of the tested peptides exerted dose-dependent antibacterial activity against *C. trachomatis* D. Significant anti chlamydial activity of NCR peptides was observed after a 15-min incubation period. Since the NCR peptides exerted anti-microbial effect not only against *C. trachomatis* D, but against *C. pneumoniae* and *C. trachomatis* L2, our results suggest that the anti-chlamydial effect was not strain or genus-specific.

Further investigations are needed to reveal whether these peptides can be potential alternative of

supplementary agents in the therapy of chlamydial infections.

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## ANALYSIS OF TTV SEQUENCES IN LUNG CARCINOMAS AND NON-TUMOROUS HUMAN LUNG TISSUES

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Torque Teno Virus (TTV) has been found to be unexpectedly widespread in the human population. Although TTV was implicated in the generation of several neoplasms, there is no conclusive evidence yet supporting such an idea. There is a huge DNA sequence variegation among TTV isolates and according to these, several genogroups were created. In our experiment we screened lung carcinomas and non-tumorous lung tissues derived from lung cancer patients for the presence of TTV. 24 out of 28 lung carcinoma samples and 16 out of the 28 non-tumorous samples were positive for TTV based on a PCR reaction specific for the UTR (untranslated) region of the genome, that amplifies all known TTV genogroups. The 40 UTR PCR-positive samples were further characterized using N22 (ORF1) region-specific primers, that detect group 1 TT viruses only. The N22 region could be amplified in 15 out of 24 lung carcinoma samples and 5 out of 16 non-tumorous samples. Amplified N22 regions were sequenced for genotyping to assess the incidence of different genotypes in tumor and non-tumor tissues. The phylogenetic analysis is in progress. TTV-positive samples were examined to determine strand polarity of TTV genomes using a strand-specific amplification reaction (SSPCR). In the first amplification reaction only one, strand-specific primer is used that carries a tail sequence at the 5' end, creating thereby the single-stranded template for the second run. This tail sequence does not have similarity with the TTV sequence. Using the tail sequence as an oligo in the second amplification reaction and an appropriate TTV-specific second oligo allows us to amplify TTV sequence form either the positive or the negative strand, depending on the primer set used. Our experiments showed, that all the examined TTV genomes were of negative polarity.

## ANTIBIOTIC RESISTANCE AND VIRULENCE OF *PSEUDOMONAS AERUGINOSA* ISOLATES FROM HEATLH-CARE ASSOCIATED WATER SYSTEMS

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*Pseudomonas aeruginosa* is one of the most relevant nosocomial pathogens. It is known as a causative agent of a variety of infenctions including wound, respiratory and urinary tract or blood stream infections. *P. aeruginosa* can colonize water distribution systems, proliferating in biofilms in pipelines and tap faucets. Epidemiological evidence suggests a multidirectional transfer between the patients, the water systems and other environmental reservoirs; water origin was confirmed for a large

proportion of infections, while pathogenic strains contaminate water outlets during hand or instrument washing. The frequency and morbidity of water related *Pseudomonas* infections depends on the virulence of the infectious strains. The aim of the present study was to assess the antibiotic resistance profile and the presence of virulence genes in *Pseudomonas aeruginosa* isolated from health-care facilities. A total of 78 *Pseudomonas aeruginosa* strains - isolated from 16 facilities - were investigated. Majority of the strains (86 %) were cultured from potable water samples, the rest from dialyzers. Antibiotic resistance was tested on Mueller-Hinton agar using disc diffusion test with 10 antibiotics regularily used in clinical treatment of *P. aeruginosa* infections. Results were evaluated based on the EUCAST protocol. The prevalence of virulence genes was examined by PCR. Five of the 78 strains were resistant to 9 or all 10 antibiotics, 37 % of the strains to at least 7. Carbapenems were the most effective, majority of the strains were sensitive or showed intermediate reaction. On the contrary, almost all strains were resistant to aminoglycosides. The rate of resistance to penicillin, quinolons and cefalosporins was between 70 and 90 %. The presence of exoenzyme S gene was confirmed in all tested strains. Over 80 % was positive for exoenzyme Y, and about one third for exotoxin U. Results indicate that multiresistant *Pseudomonas aeruginosa* strains (17 % of all isolates) are prevalent in the water distribution system of health care facilities. The presence of virulence genes was also confimed in many of the isolates. These factors multiply the health hazard associated with the colonization of water outlets by *Pseudomonas aeruginosa*, especially in the high risk areas, such as intensive care, neonatal, or haematology units. Monitoring and control of *P. aeruginosa* in the water system should be the part of the good hygiene practice in health care facilities.

## ECHINOCOCCOSIS - HYDATIDOSIS TREATED IN COUNTY MURES

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Cystic echinococcosis, CE, called also hydatidosis is a zoonotic disease that occurs mainly in rural areas. Cases are reported from all over the world; in Europe the most affected countries are in the Mediterranean and the Balkans, especially those with low socio-economic standards. Infection is highly related to occupation and is present in regions were animal husbandry is the main occupation. Because in many cases CE is asymptomatic it remains underdiagnosed and untreated. Romanian official data are missing, notification of cases is not compulsory. The aim of our work is tracking the frequency of CE in the records of the Pathology Unit of the Mures County's Emergency Hospital and follow up the evolution of patients treated at the 2nd Infectious Diseases Hospital and at the Surgical Units of Târgu- Mures between 01.2009- 04.2011. For that, we studied the cases of 82 hospitalized patients, 43 (52.43%) female and 39 (47.57%) male, following up their residence, location and diameter of the cyst and the incidence of complications, the response to surgical removal and treatment with antiparasitic medication. Diagnoses were based on serological (IgG detection and Western Blot), parasitological methods (wet- mount examination of cyst- content after centrifugation) and were confirmed by pathologists. Our results showed that 47 (57.31%) of the patients arrived from rural while 35 (42.69%) from urban areas. The patients' age varied between: 9-79 years, the mean age was 42.89. In most cases the location of the cyst was hepatic (76.82%), in 9.75% was pulmonary, cerebral in 2.43% and in the spleen, the gastro- colic ligament, pancreas, kidneys, medullar each in

1.21%. Complications occurred in 26 cases (31.70%). of the 63 patients with hepatic localization 22 presented suppurations, biliary duct fistulas, hepatic abscesses, penetration in other organs, right pulmonary fistulas and peritonitis. Two of the pulmonary locations were complicated with pulmonary abscesses. Postoperative recurrences appeared in 5 patients (6.09%), they received antiparasitic prophylaxis with Albendazole only for a month.
In conclusions, we can affirm that the patients came mostly from rural areas, in accordance with data from the literature, but their average age was higher and the female gender dominated. The most frequent localizations were in the liver, followed by the lungs. The prevalence of complications was elevated, which could have been avoided by an early diagnosis with introduction of screening methods in the affected areas and association with proper antiparasitic prophylaxis.

## HUMAN MYASIS – CASE REPORT

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Myasis is caused by the larval stages of flies living in tropical and subtropical regions of Africa and South America, which feed on live or dead mammalian tissues and fluids. The most implied species in myasis is *Dermatobia hominis*, from the order Diptera, family Cuterebridae, but accidentally other flies may intervene, too. Infestations are very rare and imply body sites exposed to fly- attacks. Cases involving the genitourinary system are even scarcer and they present unusual symptoms. In this abstract we report the case of a male, 25 year old shepherd who underwent a surgical intervention on his urinary tract for renal lithiasis. Following the intervention the patient was catheterized for several days and had a drain dwelling in his left kidney. As the wound healed, the drain was removed and the patient was released from hospital. A few days later, the patient started to have dysuria, polyuria and polakisuria. No macroscopic haematuria was present. Because of aggravation of his condition urine samples were collected and summary testing and urine culture were performed. Lower urinary tract infection with *E. coli* was diagnosed and treated according to the antibiotic sensibility test. The symptoms persisted and, a few days later, maggots of fly larvae were released repeatedly in the urine of the patient and sent for analysis. First, the laboratory staff considered the larvae as contaminants and asked for fresh samples. The elimination of the larvae was very painful for the patient so he asked for a rapid diagnosis and treatment and attended to our laboratory. Examinations of larvae recovered from the patient's urine were performed by macroscopic analysis and microscopy. We detected 3-4 mm long, rice-shaped, white, live organisms. The wet mount preparation showed fly larvae feeding on bacteria present in the urine. Identification on species level could not be performed. For that purpose, we should have fed the maggots until they had reached the adult stage of fly. That was not possible in our laboratory. Because in our region the most common flies causing myasis are *Lucillia sericata* and *Wohlfahrtia magnifica*, we suppose that the detected maggots belonged to one of these species. The patient was explained that the parasites would not multiply in his urinary tract and he was recommended to assure an elevated fluid intake to help release the fly- larvae. Antibiotics were given to prevent bacterial growth. A few days later the patient returned confirming that he was cured and related that he eliminated more than 40 maggots. No other body site was affected by larvae; the infestation remained localized in the urinary tract. He could not explain the circumstances of the infestation. In conclusion, even if maggot urinary tract infestations are extremely rare in our region, we should consider them in order to provide a rapid and correct diagnosis. Collaboration with

specialists is necessary for identification on a species level. Personal hygiene and improvement of general sanitation are important to prevent such cases.

## EMERGING PATHOGENS IN WATER SUPPLIES – *LEGIONELLA* COLONIZATION PATTERNS IN THE LIGHT OF EXTERNAL FACTORS AND MANAGEMENT PRACTICES

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In Hungary, operators of the water supplies and water distribution systems are as a rule not aware of the risk associated with *Legionella*, probably due to the lack of legal regulation. The effect of external factors on *Legionella* colonization in hot and cold water systems thus can be investigated without confounders derived from the risk management practices which are implemented in countries with higher *Legionella* awareness. Even when risk management is applied (e.g. after confirmed cases of legionellosis in a hospital or a hotel), intervention often fails due to inexperience. Usually a single remedial measure is implemented (e.g. chemical disinfection), while *Legionella* colonization is the result of multiple external factors and thus its management calls for an integrated approach. In the present study, our aim was to associate *Legionella* prevalence data with external factors, such as water source, system design and maintenance etc. Between 2006 and 2010 a total of 177 water distribution systems were investigated, 98 (55.4 %) were colonized by *Legionella*. Excluding the samples which were collected after the disinfection of the system, 1952 samples were analyzed. *Legionella* was detected in 843 samples (43.6 %). Counts exceeded the 1000 CFU/L guide value (defined by the European Working Group for Legionella) in 25.1 % of the samples. The highest observed count was above $10^7$/L. Most heavily affected facilities were the large, new buildings (e.g. hotels) or multi-wing hospitals with joint hot water distribution system. Sufficiently high water temperature (>55°C) was a strong predictive factor; where water temperature was between 20°C and 55°C (79.6% of the samples) both the rate of positive samples and the average counts were significantly higher than outside this range.

Correlation was found with the size and complexity of the building and the population of the city. Source water also associated with the colonization intensity. Water distribution systems using potable water derived from surface water and bank wall filtered wells were more frequently and more heavily colonized than those using ground water sources. Water treatment was only efficient when a complex, multiphase approach was used (combination of water temperature setting, heat shock disinfection, optimization of recirculation and system design). When only one risk factor was targeted, *Legionella* counts were often higher than before intervention. Results indicate a combined effect of water system design and environmental factors in *Legionella* colonization, thus they should be taken into account during the assessment and management of *Legionella* risk.

## INFECTION CONTROL FOR THE 21ST CENTURY – NEW MEDICINES FOR NEW AND OLD AILMENTS

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The 20th century has seen the emergence of new and the re-emergence of old diseases. The changing environment, largely resulting from increasing population and resource consumption, is resulting in an altered relationship between man, his livestock and the wider environment. There is more rapid transmission of pathogens around the world with the possibility of genetic exchange between related and unrelated microorganisms, in addition to our exposure to new sources of infection including wild animals. The continued high use of antibiotics for therapy and prophylaxis in animals and man, the absence of regulation and their use for growth stimulation in many countries is leading to increased frequency of isolation of multi-resistant microorganisms. All these issues require a change in our approach to diagnosis and surveillance in addition to infection and disease control. Diagnosis is required to be rapid, sensitive and specific. Current DNA–based technologies, including PCR, are moving into more multiplex systems including microarrays which themselves may be replaced eventually by sequenced-based analysis with the continually reducing cost of this technology. This technology is able to identify the presence of several pathogens in any sample simultaneously in addition to detecting completely novel microorganisms facilitating the study of real-time evolution. Although it is difficult to envisage how infection and disease control might be addressed in completely novel ways in the future we are currently looking at new ways to adapt well-understood methods including stimulating adaptive or innate immunity. Thus adaptive immunity can be modulated in ways to guide the host response in particularly desired directions. Administration of cytokines or using live vaccines can stimulate a Th1 response away from a Th2 response. This might be used to enhance immunity to parasites or to bacterial pathogens which enter the carrier state. Although innate responses are generally short lived they can also be stimulated by live vaccines and resistance is profound and generated to a variety of unrelated pathogens. Under some circumstances they might also be used therapeutically! We know so little about what makes vaccines effective. Inactivated and sub-unit vaccines are currently less than effective and although ways exist to improve them so much remains to be understood. Biological control is also being considered. Bacteriophages are being examined for their potential in controlling systemic and enteric infections in addition to having potential in reducing entry of food-borne pathogens into the human food chain. The parasitic bacterium *Bdellovibrio* has also been used in this way experimentally.
We should not limit our imagination to existing combinations of approaches. “He that will not accept new remedies must accept new evils” Francis Bacon 1561-1626

## MICROBIOLOGICAL MONITORING IMPACT OF SOME INSECTICIDES IN CLAY LOAM BROWN FOREST SOIL

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At least about 70% of agriculturally used soils all over the World are treated with insecticides in order to protect the plant crops from insects and ensure that high-quality agricultural food production will be produced to meet the increasing of global population. Soil processes are affected by a number of environmental variables such as moisture content and pH. As these parameters not only control the

dynamics of soil processes but also the availability, degradation rate and persistence of insecticides, the measurement of moisture and pH is essential for the interpretation of insecticide impact on soil processes. Also, The composition, number and activities of soil microbiotas vary widely between biome and with seasons, due to climatic changes and soil treatments. The majority of soil microorganisms live by consuming and decomposing organic matter. In this way, soil microorganisms are responsible for maintaining the natural fertility of soils through processes that cycle plant nutrients and facilitate the flow of energy. The aim of the study is to identify microbiologically significant changes in soil processes that are attributable to the actions of the insecticides belonging to Carbamates (Carbosulfan, Carbaryl and Methomyl), Organophosphates (Dimethoate, Endosulphan and Malathion) and Pyrethroids (Cypermethrin and Deltamethrin) at field recommended rate in 2 kg pot experiment. The insecticides were applied to clay loam brown forest soil with low clay and organic matter content. Evaluation of the impact of insecticides on the clay loam brown forest soil by assaying for non-target effects on single species or microbial communities was carried out during 9 weeks. Soil and insecticides properties greatly influence the behaviour, availability and subsequent ecotoxicity of insecticides to soil microbiotas and their functions. Since the distribution of insecticides in soil is far from uniform and the natural variability of soil populations and processes is high, pot experiment monitoring of insecticides effects on soil microbial functions is often impeded by unmanageable four sample replication. This monitoring impact of insecticides on soil microbial communities is evaluated in view of their role in sustaining the global cycling of organic matter and their varied functions in supporting plant growth. Carbon and nitrogen mineralization serve to complement the interpretation of the results for the microbial biomass-related parameters. Breakdown of organic matter by soil microorganisms is accompanied by the uptake of oxygen and production of carbon dioxide as soil microbes respire. According to the results, soil respiration is a useful indicator of insecticide impact on organic matter breakdown. The efficiency of microbial carbon mineralization over an extended period can be stimulated by the mineralization of soil organic matter or the degradation of the insecticides themselves. Assaying for the enzymatic activity e.g., dehydrogenase, conveniently monitors the metabolic activities of microbial cells in oxidizing organic matters. This parameter seems to indicate an impact on microbial activity most significantly. Also, results showed that inhibition of microbial biomass and related activities and stimulation of carbon and nitrogen mineralization were the most significant effects caused by the application of insecticides.

## PURIFICATION AND CHARACTERISATION OF DIFFERENT OPHIOBOLIN COMPOUNDS

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Ophiobolins, a group of sesterterpenoid secondary metabolites produced by certain filamentous fungal species show various biological activities, such as antibacterial, fungicid nematocide, phytotoxic and cytotoxic effects. Until now, ophiobolin A is the most intensively studied member of this metabolite family. The main objects of our research are the purification and characterization of different ophiobolin analogues produced by *Bipolaris* species and their teleomorphs in the genus *Cochliobolus*. Identification of new bioactive substances is also planned. We have succesfully developed an isocratic HPLC method for the detection of the different ophiobolin compounds and a preparative layer chromatographic method for the purification of these metabolites. Using our new preparative method we have cleaned up four potential ophiobolin analogues (p1 - 4) from a *B. oryzae*

strain and one of them was identified as ophiobolin A. The purity of the compounds was determined by HPLC technique, and proved to be 93% for p1, 90% for ophiobolin A (p2), 97% for p3 and 80% for p4. The in vitro antimicrobial activity of the isolated substances was determined with 96-well microtiter plate bioassay by measuring the absorbance of fungal and bacterial cultures at 620 nm. According to the first antimicrobial tests, filamentous fungi proved to be more sensitive to ophiobolins than yeasts and bacteria.
Our results provide a basis for the search for novel, undescribed bioactive ophiobolin derivatives and an opportunity to start an extensive assay to determine their biological effects.

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## CO-CULTIVATIONS OF FUNGI IMPROVE THE PRODUCTION OF SPECIFIC ENZYMES

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During their natural life cycle most fungi encounter other microorganisms and live in mixed communities with complex interactions, such as symbiosis or competition. Industrial fermentations, on purpose or by accident, can also result in mixed cultures. Fungal co-cultivations have been previously described for the production of specific enzymes, however, little is known about the interactions between two species that are grown together. *A. niger* and *A. oryzae* are two of the most important industrial fungi worldwide and both have a long history of strain improvement to optimize enzyme and metabolite production. Co-cultivation of these two Aspergilli with each other and with the ascomycete phytopathogen *Magnaporthe grisea*, and the basidiomycete white rot fungus *Phanerochaete chrysosporium*, has recently been described by our group (Hu et al, 2010). Total secreted protein, enzymatic activities related to plant biomass degradation and growth phenotype were analyzed from cultures on wheat bran demonstrating positive effects of the co-cultivation compared to the individual cultivations. In a follow-up study the morphology and mechanism of the interaction if currently studied. Data from the paper and follow-up studies will be presented.

## SEQUENCE ANALYSIS OF BOVINE ADENOVIRUSES THAT BELONG TO THE GENUS ATADENOVIRUS

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Bovine adenoviruses (BAdVs) are an economically important group of rather diverse viruses that might be involved in respiratory and enteric diseases of cattle. BAdVs are assigned to two genera of the family Atadenoviruses were named on account of the high A+T content of their DNA. Atadenoviruses possess biological features and genomic arrangement markedly different from those characteristic for mastadenoviruses. They require primary (bovine testicle or other fibroblast type) cell cultures for and the lack of the common complement-fixing antigen have also been described as distinguishing features from mastadenoviruses. The atadenoviral genome is compact. The E1A and E3 regions as well as the genes of protein V and IX are missing. A genus specific gene coding for a

novel protein (p32K) was identified. Bovine atadenoviruses occur worldwide, BAdV-4, -5 and -8 were first isolated in Hungary, whereas BAdV-6 (strain 671130) in the Netherlands, and BAdV-7 (strain Fukuroi) in Japan. One yet un-typed strain (Rus) originates from Russia. Later, bovine atadenoviruses have been isolated in a number of other countries (Germany, UK, USA). The GenBank contains only one complete genome (BAdV-4) and several shorter sequences of bovine atadenoviruses. Initially, strain Rus had been described as a Russian isolate of BAdV-7 but serology did not confirm this. Later it was considered as a candidate new BAdV type, and its whole genome sequence was determined in our laboratory. Partial sequences from the genes of the DNA-dependent DNA polymerase, penton base and hexon of BAdV-8 are almost identical to the corresponding sequences of Rus. We concluded that Rus is indeed a new isolate of BAdV-8. Phylogenetic calculations based on hexon sequences of ruminant AdVs rendered BAdV-6 and BAdV-7 to be discrete species, proposed as BAdV-E and BAdV-F, respectivelly. To confirm the species level classification, we determined the sequence of the full genome of strain 671130 and a large part the strain Fukuroi. The viruses were propagated in calf testis cells and the purified viral DNA was digested with restriction enzymes. The fragments were molecularly cloned and sequenced by primer walking. Genome regions not contained by the clones were sequenced after amplification by specific PCRs. Fragments coding viral proteins were identified with the BLASTX software. The sequences were assembled in the STADEN program. The genome of BAdV-6 was found to consist of 30,024 bp with a G+C content of 35% and ITRs of 42 bp. The variable E4 region of BAdV-6 has 3 RH genes and two hypothetical genes, which have no homology in the GenBank. The partial genome of BAdV-7 has G+C content of 34%. The gene of p32K was identified in both genomes. The phylogenetic calculations, based on the DNA polymerase, penton base and hexon sequences, justified registering BAdV-6 and BAdV-7 as members of two new species.

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## SOIL-PLANT-MICROBE INTERACTIONS AND SESSION OF „AGRICULTURAL AND FOOD MICROBIOLOGY" FOUNDED BY M. KECSKÉS

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Microorganisms are basic elements in every ecosystem in our World. Studies on the interrelation between higher plants and those ubiquitous microbes have started at the 60-year-old "*Sopron School of Soil-biology*" and also at the "*Gödöllő School of Microbiology*". The key person at those two schools is Professor Mihály Kecskés, who is being 80 years old in these days. Prof. Kecskés was the founder of session "*Agricultural and Food Microbiology*" at Hungarian Microbiological Society and he headed it up till his retiring. Three generation of Prof. Kecskés students become microbiologists and studying the aspects of interrelation between higher plants and microbes among various environmental conditions. There were two main directions of the focused research activities so far. *1. Interrelations among higher plants and microorganisms in a changing world.* This subject is studying the role of microorganisms in soil-fertility and in crop-production in correlation with biotic and abiotic environmental factors, more particularly the role of man-made activities. There are different parts of plants, with outstanding activities of microbes. Plant wellness is starting from the seed environment, the *spermosphere* followed by the root environment, the *rhizosphere* and finally an

effluence of microbes should happen in the *phyllosphere*. Those subjects were all considered and currently studied by M. Kecskés and his coworkers. *2. The soil-plant-(microbe)-animal-human food chain*, as important subject not only of soil, but also of human health. Several biotic and abiotic pollutants are having a bioaccumulation potential in food chain. Microorganisms, especially if they are adapted to specific environments, can reduce the uptake of heavy metals and toxic elements and they can be involved in the degradation of other organics. There is an increasing necessity also in controlling the threat of microorganisms of food quality and safety importance. The bioprocesses are also highly depends on the microbes in the food chain. the applicability of beneficial microorganisms in the phyto(rhizo)technologies, as widely accepted methods of the soil-plant-(microbe)-animal-human food chain. Both main topics are intensively studied in Session of *"Agricultural and Food Microbiology"* at the Hungarian Microbiological Society, so far. Welcome of Professor Mihály Kecskés founding the Session and providing research directions and outstanding results. Young and elder colleagues are following the paves, stepped by Him.

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## HORIZONTAL MICROBIOLOGICAL CHARACTERIZATION OF FOREST SOILS TARGETED BY DEICING FLUIDS

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In European airports large amount of deicing fluids (DF) containing propylene-glycol (PG) are being used in the severe winter conditions between October and April. The DF infiltrate the soil surface when the snow begins to melt, so it is important to ensure that these chemicals do not contaminate the soil and the groundwater. Horizontal microbiological characterisation was performed on samples obtained from forest soils of the Oslo airport , the distance of the samples increase from the runways (from 0 to 150 m). Total culturable aerobic/anaerobic heterotrophic bacteria, microfungi (yeasts and moulds) and catabolic microbial activity measured by fluorescein diacetate hydrolysis (FDA) were estimated. The ratio of pollutant-decomposing microbial components was assessed and cultured by most probable number (MPN) method. PCR-based identification of bacterial strains was achieved by using genus-specific primers. The highest microbial activity (culturable aerobic, anaerobic bacteria, microfungi and FDA) was found in the top 20-30 cm segment of the soil surface. Countable aerobic heterotrophic bacteria were ≥2 orders of magnitude, more abundant in the examined soil samples than the anaerobic bacteria and microfungi. Further experiments using the MPN method demonstrated that one of the key limiting factor in the biodegradation of the DF during snowmelt infiltration is low soil temperature (about 0.2-9 °C). Bacterial strains biodegrading DF at 4 °C could be cultured and isolated from the soil samples, and molecular identification revealed that these isolates belong to *Pseudomonas* spp. After further identification the isolates involved in the biodegradation of this type of organic pollutants can be utilized in the development of new bioremediation technologies.

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## DIFFERENCES IN HUMAN MICROBIOTA AND BACTERIOCIN PRODUCTION OF YOUNG SLOVAK PEOPLE WITH DIFFERENT NUTRITION HABITS

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Human microbiota includes a wild range of anaerobic and facultative anaerobic microorganisms, which are classified into hundreds varieties and about 50 genera. Food composition has a strong impact on colon microbiota as well as on the immune system and human health. In this work we have focused on changes in microbiota of people aged 21-40 with different nutrition habits (vegetarians and non-vegetarians) using cultivation on selective diagnostic media. We have studied qualitative and quantitative microbial representation presented in colon that could affect human physiology, as well as development of some diseases (colon carcinoma, some inflammation processes). We have riveted on lecithinase-positive clostridia, which are most presented in faeces of colon carcinoma patients. Also *Bacteroides* strains appear to contribute to the conversion of heterocyclic aromatic amines to DNA-reactive carcinogens. Concerning this fact, our second aim was to compare incidence of LP clostridia and *Bacteroides* with presence of potentially mutagenic compounds in faeces of people with different nutrition habits. We have observed no significant difference between microbiota of meat eaters and vegetarians. Changes were only in case of potentially mutagenic compounds presence. The lowest percentage of people with potentially mutagenic compound was in category of older (31-40) meat eaters. Our last aim was determination of thermo-stable bacteriocins.

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## CIPROFLOXACIN RESISTANCE COULD BE CONNECTED WITH MUTATIONS OCCURRING IN STATIONARY PHASE

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In this phase cells are responding to non-lethal stresses by promoting adaptive mutations. Some of these mutations can result in resistance to different environmental stresses or antimicrobial agents. *Salmonella enterica* subsp. *enterica* serotype *Typhimurium* has been iivestigated. In isolated resistant mutants we were interested in occurrence of mutations in gene encoding gyrase subunit A as well as in changes in susceptibility to other antibiotics. In *gyrA* already after 24 hours of cultivation; all tested strains had mutation in 83. position. In 96 hour we have detected also some strains with mutation in 87. position. We have also registered changes in susceptibility to gentamicin, chloramphenicol and tetracycline. Mutation frequency to ciprofloxacin resistance is increasing in cells of *gyrA* gene in 83. position.
This change is usually connected with resistance to antibiotics with different mode of action.

## PHYLOGENETIC DIVERSITY OF BACTERIAL AND ARCHAEAL COMMUNITIES INHABITING THE SALINE LAKE RED LOCATED IN SOVATA, ROMANIA

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Lake Red is one of the saline to hypersaline lakes which were formed as a consequence of salt massif dissolution at the foot of the Gurghiu Mountains (Central Romania) at the end of the 19th century. In order to reveal and compare the phylogenetic diversity of Bacteria and Archaea, water and sediment samples were collected in spring 2009. According to the results of 16S rRNA gene based DGGE, no differences were found between the diversity of the bacterial and archaeal communities originated from the lake water at two different sampling sites. From DGGE bands, sequences affiliated with Gammaproteobacteria (*Halomonas* and *Alkalilimnicola*) and Bacteroidetes (*Psychroflexus*) as well as Euryarchaeota (*Halopelagius* and *Halobacterium*) were retrieved. Cultivation resulted in altogether 67 water and 34 sediment bacterial strains from five different saline media. Besides the most abundant strains of Gammaproteobacteria (*Halomonas, Marinobacter* and *Salinivibrio*), Firmicutes (*Bacillus*) and Alphaproteobacteria (*Aurantimonas* and *Roseovarius*) were identified. Almost a quarter of the strains obtained from four out of five media used were identified as *Salinivibrio costicola* One-third of the bacterial strains (originated mainly from the lake water) were identified as members of 10 different species of the genus *Halomonas*. The 16S rRNA genes from 82 bacterial and 95 archaeal clones were also phylogenetically analyzed. Bacterial clones were related to various genera of Gammaproteobacteria (*Alkalilimnicola, Alkalispirillum, Arhodomonas, Halomonas, Saccharospirillum*), Bacteroidetes (*Gracilimonas, Owenweeksia, Psychroflexus*) and Alphaproteobacteria (*Oceanicola, Rhodobaca, Roseovarius, Tropicimonas*). All the archaeal clones sequenced corresponded to a homologous cluster of *Halopelagius*.

## EFFECTS OF SURFACTANTS ON PRODUCTION OF PHYTASE FROM *THERMOMYCES LANUGINOSUS*

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Filamentous fungal fermentation is widely applied to commercially produce useful products such as enzymes, organic acids, antibiotics.Surfactants have been reported to have an effect on the growth rate and enzyme production of fungi. The addition of surfactants to culture media, in general, increased the enzyme yield, but their effects varied from organism to organism even from enzyme to enzyme. The mechanisms by which surfactants enhance extracellular enzyme production were reported to be increased cell membrane permeability through the change in lipid metabolism, which resulted in a higher release of the enzymes. Furthermore, it has been showed that the incorporation of surfactants inducing the formation of smaller pellets in submerged fermentation. Fungal growth in pellet form is favourable; it improves the culture rheology which results in better mass and oxygen transfer into the biomass and lower energy consumption for aeration and agitation. The main goal of

our work was to investigate the effect of various polyoxyethylene sorbitan esters (Tween) on growth of filamentous fungi. During the enzyme production the fermentation media was supplemented with 0.01% concentration of Tween 20, Tween 40, Tween 60, Tween 65, Tween 80, Tween 85. The phytase activity was highest at the 2nd or 3rd day of the fermentation (2300 U/L). Using Tween 20 or Tween 40 resulted in approximately 2-fold increase in phytase secretion comparing with controls. Tween 65 and Tween 85 have no positive effects or on the 3rd day resulted in a decrease of the production of phytase enzyme. Addition of Tween 20 and Tween 40 in the concentration range (0.1–1.5%) was also investigated. When the concentration of surfactant was increased above 0.5%, the stimulatory effect of the surfactant changed into an inhibitory effect of enzyme production. Best result were obtained when the fermentation media supplemented Tween 20 or Tween 40 at 0.025 % and 0,01 % concentration, respectively.

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## TRANSCRIPTION ANALYSIS OF *CHLAMYDIA TRACHOMATIS* D AND HERPES SIMPLEX-INFECTED HeLa CELLS

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*Chlamydia trachomatis* (*C. trachomatis*) is a Gram-negative, obligate intracellular bacterium that has a biphasic developmental cycle. Genital *C. trachomatis* infection is one of the most prevalent STDs and is the most frequently reported STD worlwide. Infection with *C. trachomatis* may result in urethritis, epididymitis, cervicitis, acute salpingitis, or other syndromes. However, the infection is often asymptomatic in women and could result in pelvic inflammatory diseases, one of the major causes of infertility. Herpes simplex virus type-2 (HSV-2) is an enveloped DNA virus of the viral family Herpesviridae. HSV-2 is the primary cause of genital herpes infection. After primary infection, HSV-2 can establish a latent, lifelong infection. Although most genital HSV-2 infections are clinically mild, HSV-2 can also be responsible for serious diseases, such as keratitis and meningitis. The co-infection with *C. trachomatis* and HSV-2 in vivo is common, but the effect of the two pathogens on the development of genital diseases has not been studied on molecular level yet. To investigate the co-infection, HeLa cells were infected with *C. trachomatis* D or HSV-2 or with both. Re-culturable *Chlamydia* titre was determined by indirect immunofluorescence. After 24 h incubation, total RNA was extracted from the infected cells. The RNA contents were determined and samples were subjected to microarray analysis. A part of the RNA was spared for RT-qPCR to validate the array results in respect of selected up-regulated genes. In comparison with the *C. trachomatis* infection alone, the co-infection with HSV-2 caused decrease in the titre of the culturable Chlamydiae. The DNA chip analysis clearly showed that the *C. trachomatis* and HSV-2 either alone or in combination had a significant impact on the gene expression of human epithelial cells. Among the up-regulated genes we found members of the inflammatory cascade, innate and adaptive immunity such as NFκB, STAT, GBP1, GBP5, GBP7, proteasome 8, proteasome 9 and CXCL10. Increased expression of host genes during co-infection with *C. trachomatis* D and HSV-2 might have an important role in the development of genital diseases. Our results help to understand the pathomechanism of the genital infections on molecular level.

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## COMPARATIVE DIVERSITY INVESTIGATIONS ON KARSTIC SOIL BACTERIAL COMMUNITIES OF THE TAPOLCA BASIN, HUNGARY

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The Tapolca karst is one of the karstic areas of Hungary that are most sensitive to anthropogenic impact. Microorganisms inhabiting karstic soils play an important role among the factors that influence the characteristics of epikarst systems. The goal of our research was to reveal and compare the phylogenetic diversity of bacterial communities of soil samples taken from an undisturbed and a polluted area of the Tapolca karst by applying cultivation dependent and independent methods. Altogether 200 strains isolated from nutrient, R2A and starch-casein agar were investigated. Two clone libraries with 96-96 members were created from community DNA of the soil samples. The phylogenetic identification of the representative strains and clones, using the NCBI and EzTaxon databases, was based on the 16S rRNA gene sequence analysis, following their grouping with the ARDRA method. The bacterial strains were identified as species from the phyla Actinobacteria, Firmicutes, Proteobacteria and Bacteroidetes. The distribution of the species within the phyla was very distinct between the sampling sites. Results of the cultivation showed that the bacterial community of the polluted area was more diverse than the community of the undisturbed area. The processed molecular clones showed very low sequence similarity with described bacterial species, most of them were affiliated with clone sequences deriving from soil and rhizosphere samples. Examined by the cloning method, the communities proved to be more diverse than observed by cultivation. Besides the taxons observed with the cultivation method, members of the phyla Acidobacteria, Chloroflexi, Cyanobacteria, Gemmatimonadetes and Verrucomicrobia, and the class Deltaproteobacteria were present in the samples.

## ANALYSIS OF *MUCOR CIRCINELLOIDES* CAROTENOID PRODUCING STRAINS TRANSFORMED WITH HOMOLOGOUS AND HETEROLOGOUS GENES

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Transformation systems resulting stable transformants are necessary for the functional analysis of genes and improvement of biotechnologically important strains. Transformation of zygomycetes with circular plasmids generally results in transformants harbouring the plasmids as autonomously replicating elements without any integration event; such transformants often prove to be unstable. *Mucor circinelloides* is a β-carotene producing zygomycete. The aim of the present study was to modify the carotenoid biosynthesis of *M. circinelloides* using both different autonomously replicating plasmids and integrative transformation systems. The fate of the transforming DNA and the carotenoid production of the transformants were analysed. Three structural genes of the isoprenoid biosynthesis, under the control of different promoter regions, were introduced into *M. circinelloides* on circular plasmids by PEG-mediated protoplast transformation to increase the β-carotene production. Co-transformations of these isoprenoid genes with the *crtW* gene of *Paracoccus* sp. N81106 strain were also carried out to achieve the conversion of β-carotene to its ketolated derivatives. Southern hybridization analysis showed that the transformants maintain the introduced

DNAs as autonomously replicating elements. Plasmid rearranges were also detected. In the co-transformants the copy number of the two plasmids was generally different and the copy numbers of the plasmids carrying the homologous genes were higher than those carrying the bacterial gene. All analysed transformants proved to be stable under selective and non-selective conditions. Increased copy number of the genes led to elevated relative transcription levels and carotenoid production in the transformants. Presence of new carotenoids in the *crtW*-expressing strains was also verified. An attempt to integrate the *crtW* gene into the *M. circinelloides* genome was also carried out. Different transformation methods were tested, i.e. transformation with linear fragments and REMI carried out via PEG-mediated protoplast transformation and the *Agrobacterium tumefaciens*-mediated transformation (ATMT). Integration of *crtW* into the *Mucor* genome was verified with Southern hybridization and inverse-PCR technique, while its copy number in the transformants was analysed with qPCR. With the exception of ATMT, all tested methods resulted in stable transformants. Several strains producing canthaxanthin and echinenone as the main carotenoids could be created.

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## KNOWLEDGE MINING FROM MICROBIOLOGICAL DATA – APPLICATION OF NETWORK SCIENCE IN FOOD MICROBIOLOGY

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Potentials of data mining is not sufficiently utilised in food microbiological practice. Publicly accessible sources can give first impression on trends and patterns that may coincide with intuition but may sometimes provide unforeseeable conclusions. The results can be, of course, biased, depending on the source of the data, the objective of the study and the resolution of the database. However, knowing the bias can also give means to correct it. Network science methods are useful where many data are available and those are systematically formatted, so that automated programs can browse them. The data can be summarised in tables, diagrams, networks. Network science methods are especially suitable to reveal patterns and trends behind links and relations shown by the data. Here examples are shown for simple web-based methods how to draw conclusions on the research interest and available data on the interactions between food-borne bacteria and moulds, as well as the food harbouring them. The global research interest is also demonstrated, and the nature of available data based on Google Scholar search as well as on a specific database on the microbial responses to food environments (ComBase). Data mining can be utilized in any organization that needs to find patterns or relationship in their data.
Results of data mining: forecasting what may happen in the future; clustering things into groups based on their attributes; associating what events are likely to occur together.

## RELATIONSHIP BETWEEN PHYLLOSPHERIC MICROBES OF RAGWEED (*AMBROSIA ELATIOR* L.) AND SOIL METAL CONTAMINATION

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Main focus of these studies are to assess the relationship between the cadmium, copper, nickel and zinc contents of soils at the uncultivated areas near the Nyiregyhaza site. The soil was assumed to contaminated by metals and we hypotehetised that the leaf surface are is showing those contaminations through the phyllospheric microorganisms of the ragweed. During growing and flowering seasons for five years, comparisons were made between upper and lower leaf surfaces microflora of ragweed plants grown in three areas: a relatively noncontaminated, a communal landfill and a complex contaminated with heavy metals. Results indicated that phyllospheric microbial communities were qualitatively and quantitatively related to metal content in plant leaf, leaf surfaces and metals contaminated ecosystems where the plants grown. Results showed that there was a high positive correlation between phyllospheric microbial population densities and Cd, Ni, and Zn content in ragweed leaves, while it was low in presence of Cu. Comparatively, the correlation was at highest in case of aerobic heterotrophic bacterial population densities and also at yeasts and the filamentous fungal communities. The most common metal resistant microorganisms were found, as *Pantoea agglomerans*, *Pseudomonas savastanoi*, *P. putida*, *Bacillus cereus*, *Corynebacterium striatum*, *Cryptococcus* and *Alternaria*. Further studies seem to be necessary to study the role of those phyllospheric microorganisms in the metal alleviation of ragweed.

## ETHANOL PRODUCTION FROM SECOND GENERATION FEEDSTOCKS

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World-wide, a strong drive is manifest towards increasing the production of bioethanol from biomass. Leading countries are Brazil and the USA, where bioethanol is produced from sucrose (sugarcane) and starch (corn), respectively. The general concern with these 'first generation' processes is that crops for biofuels will put high pressure on the availability of agricultural crop land that is needed for food. Alternative 'second generation' lignocellulosic feedstocks have to be used if bioethanol as a fuel is to considerably grow in a sustainable way. Preferably, the feedstocks are byproducts of existing industries that will not put an extra pressure on land use. Several challenges, however, face the development of industrial processes on the basis of second generation feedstocks. One important aspect relates to the hydrolysis of cellulose and hemicelluloses into monomeric sugars, whereas the utilization of xylose (resulting from hemicelluloses) is a key issue in the fermentation with yeasts [1]. In the last decade, concentrated efforts have been put in creating cheaper enzyme cocktails for the degradation of cellulose and hemicelluloses. These cocktails were mainly developed for improved cellulase activity, although they contain also some hemicellulase activity. To ferment the sugars and to produce ethanol, the yeast *Saccharomyces cerevisiae* is the organism of choice in the ethanol industry because of its high efficiency and conversion rate and its robustness in industrial

environments. However, this yeast is incapable of fermenting xylose. This has been overcome by insertion of a *Piromyces* xylose isomerase gene in *S. cerevisiae* and upregulation of the pentose phosphate pathway [2].
The presentation will deal with enzymological aspects of hydrolyzing hemicelluloses and mainly with the fermentation of xylose by engineered strains of *Saccharomyces cerevisiae*.

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## MINING FUNGAL BIODIVERSITY USING THE FUNG-GROWTH DATABASE

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Fungal genome sequences demonstrate the potential to utilize a variety of different carbon sources. Natural carbon sources for many fungi are based on plant biomass and often consist of polymeric compounds, such as polysaccharides. They cannot be taken up by the fungal cell and are extracellularly degraded by a complex mixture of enzymes. Plant polysaccharide degrading enzymes have been studied for decades due to their applications in food and feed, paper and pulp, beverages, detergents, textile and biofuels. These enzymes have been classified based on amino acid sequence modules (www. cazy.org). Based on the hypothesis that fungal genomes have evolved to suit their ecological niche, we have performed a comparative study using >100 fungal species. In this study we have compared growth profiles on 35different carbon sources (consisting of mono-, oligo- and polysaccharides, lignin, protein and crude plant biomass) to the CAZy annotation of the genomes to identify correlations between growth and genomic potential. Highlights of these comparisons will be presented as well as the importance of mining fungal biodiversity with respect to biomass utilisation for both fundamental research and biotechnology. The data from our study is accessible through a public database (www.fung-growth.org) that will also be presented in this presentation.

## SUBSTANTIAL CHANGES IN THE CLASSIFICATION OF YEASTS – THE NEW 5TH EDITION OF „THE YEASTS, A TAXONOMIC STUDY", 2011

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Most recently, the new 5th edition of „The Yeasts, a Taxonomic Study" has been published in three volumes edited by Kurtzman, Fell and Boekhout [1]. It has brought about substantial changes in the classification of yeasts since the previous 4th edition thirteen years ago in terms of both the numbers of approved yeast species and in their phylogenetic relations. The number of known yeast species has increased to 1500 in 148 genera, and in taxonomic terms these belong to two subphyla of Ascomycota, i.e. Taphrinomycotina and Saccharomycotina, as well as all three subphyla of Basidiomycota, i.e. Pucciniomycotina, Ustilaginomycotina and Agaricomycotina. In view of the enormous role of yeasts played in human affairs in both beneficial and harmful respects, it is timely to call the attention of changes in the nomenclature and classification of this group of fungi. Within the

limitation of time, however, a brief overview with few examples only can be presented. The current classification is based on molecular phylogenetic analysis of multigene sequences of regions of the small and large subunit of ribosomal RNA genes and certain other genes. Data on morphological and phenotypical characters, such as the cell wall composition, septal pores, coenzyme Q components, sexual structures, fermentation of, and growth on various substrates, etc) are now considered secondary features in determining the taxonomic relations and the identification of species. As a result, radical changes have been made in the classification of yeasts and a major revision of yeast taxonomy reflecting upon evolutionary relationships has taken shape with the inevitable consequences in nomenclature.
These will be illustrated by the examples of two major groups of yeasts with outstanding practical importance, the families of Saccharomycetaceae and Pichiaceae. Interested readers can find a more detailed review being published in the current volume of Acta Alimentaria [2].

[1] Kurtzman, C.P. et al. eds. (2011) The Yeasts, a Taxonomic Study. 5th edn., Elsevier, London.
[2] Deák, T., Péter, G. (2011) Acta Alimentaria, vol. 40 (submitted)

## PHYLOGENETIC ANALYSIS OF A NOSOCOMIAL TRANSMISSION OF HEPATITIS B VIRUS AT A PEDIATRIC HAEMATOLOGY WARD

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A nosocomial Hepatitis B virus (HBV) outbreak at a pediatric onco-haematology unit was investigated using molecular biological methods to determine the origin of the infections. The National Reference Laboratory of Hepatitis Viruses received seven HbsAg positive sera from patients and one from the brother of a patient. A fragment of the preS1/preS2/S genes from all samples was amplified, the PCR products were sequenced and a rooted phylogenetic tree was constructed. All nucleotide sequences from the different patients were very similar and 6 of the 8 sequences were totally identical, suggesting a common origin of the infections. These sequences were closely related to those amplified from a nosocomial HBV epidemic in another hospital in Hungary. The on-scene investigation revealed several malpractices. The two hospital departments had close connections and some of the patients were treated in both institutions. Present report underlines the importance of developing screening protocols for hepatitis viruses and that of the introduction of regular training programs for health care professionals in the field of hospital hygiene.

## NOVEL APPROACHES TO THE DIAGNOSIS OF TOXOPLASMOSIS: THE ROLE OF MOLECULAR METHODS

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Due to an incomparably wide array of hosts, which includes many species of birds and reptiles and all

warm-blooded animals including man, *Toxoplasma gondii* is one of the most successful parasites on Earth, infecting about one third of the human population worldwide. Controlled by the host immune response, *Toxoplasma* infection is infrequently clinically manifest in the immunocompetent, but is potentially serious in the fetus and the immunosuppressed. The fact that fetal infection is a consequence of maternal infection in pregnancy and opportunistic infection a consequence of reactivation of latent infection during immunosuppression, determines both the necessity of timely diagnosis and its reliance on the laboratory. Dating of *Toxoplasma* infection is in many cases feasible by serological screening, which includes quantitative determination of specific IgG (including IgG avidity), IgM and IgA antibodies, using various commercial assays (based on ELISA, IFAT or direct agglutination) available to most microbiological laboratories. However, doubtful cases, and particularly those of infection in pregnancy when antenatal diagnosis of fetal infection is warranted, require elaborate diagnostics, including isolation of the parasite and lately, detection of its DNA, from amniotic liquid or fetal blood. Whereas isolation of the parasite by xenodiagnosis takes weeks, and by tissue culture is not sensitive enough, detection of parasite DNA by classical or real-time PCR is quick and has proved most reliable. In addition, use of molecular methods has become the method of choice for the diagnosis of *Toxoplasma*-induced entities such as encephalitis and chorioretinitis, in which detection of parasite DNA in the cerebrospinal fluid and aquaeus humour, respectively, provides a rapid conclusive diagnosis. Another important use of molecular technologies involves genotypization of the isolates; the organism's highly clonal population structure, with archetypical strains (types I, II and III) mostly occurring in Europe and the US, and recombinant or atypical strains being increasingly isolated in South America and Africa, which may expand in the population leading to emerging diseases, is currently explored for clinical ramifications to treatment approaches in infection with different genotypes.

## BIOACTIVE METABOLITES IN WOOD EXTRACTS OF *ROBINIA PSEUDOACACIA* L. INHIBIT THE GROWTH OF *FUSARIUM PROLIFERATUM* AND VARIOUS BACTERIA

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*Robinia pseudoacacia* L. (black locust) though being a non-indigenous species in Hungary is a very aggressive and invasive woody plant that recently has gained significant importance as energy source in our country due to its fast growth. This aggressive growth is supposed to be the result of chemical elements as well besides physical and physiological characteristics. This idea gave the basis of researches that black locust has been recently subjected to the aim of which is to find new sources of environmentally sound chemicals, like pesticides and herbicides. We tested various wood extracts of *Robinia pseudoacacia* using agar plate disc diffusion method parallel with High Performance Liquid Chromatography (HPLC) measurements. Our results indicated that methanolic and phosphate buffer extracts of black locust wood had the highest bioactivity against *Fusarium proliferatum*, *Escherichia coli*, *Micrococcus luteus* and *Staphylococcus aureus*. Infection tests showed that *Fusarium proliferatum* treated with black locust extract had reduced ability to infect *Capsicum annuum* L. fruits as well. We were able to get good correlation between some particular components of the extracts and the rate of inhibition using HPLC.

## *ESCHERICHIA COLI* GENOME PLASTICITY: IMPLICATIONS FOR EVOLUTION OF BACTERIAL PATHOGENS AND STRAIN TYPING

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*Escherichia coli* exhibits considerable physiological and metabolic versatility and includes a variety of non-pathogenic, commensal variants, which belong to the normal gut flora of humans and many animals. Additionally, several pathogenic variants have been identified which cause various types of intestinal or extraintestinal infections in men and animals. In contrast to intestinal pathogenic *E. coli* (IPEC), which are obligate pathogens, extraintestinal pathogenic *E. coli* (ExPEC) are facultative pathogens which belong to the normal gut flora of a large fraction of the healthy population. Genome sequencing provided a wealth of insights into the genetics and biology of *E. coli*. Comparative genomics has been applied to study (i) genomic diversity, (ii) the mechanisms involved, and (iii) genomic regions that may support discrimination of different *E. coli* pathotypes. Horizontal gene transfer, recombination, DNA rearrangements and point mutations contribute to *E. coli* genome plasticity and results in the presence of distinct and variable "genomic islands" within the conserved *E. coli* "chromosomal backbone". Although ExPEC and IPEC are usually epidemiologically and phylogenetically distinct from commensal strains, certain ExPEC and commensal *E. coli* share large genomic fractions. Furthermore, IPEC variants may carry several ExPEC virulence markers or a combination of virulence genes of different diarrheagenic *E. coli* pathotypes. Consequently, strain typing based on the detection of a limited number of virulence/fitness-related genes may be ambiguous. Genomic variability can also be used to discriminate closely related variants, e.g. during outbreaks, as whole genome sequencing and comparative analysis can detect genetic diversity at a large scale with high resolution. *E. coli* population diversity and microevolution can thus be investigated. Against this background, we combine comparative and functional genomic as well as molecular epidemiological approaches to study *E. coli* adaptation to different niches and the evolution of pathogenic *E. coli* variants. Our results should improve our knowledge of *E. coli* pathogenicity and fitness factors, but also promote improved typing of bacterial variants.

## ISOLATION OF PANTON-VALENTINE LEUKOCIDIN TOXIN POSITIVE METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* STRAINS IN DEBRECEN

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Panton-Valentine Leukocidin (PVL) is a pore-forming cytotoxin that causes leukocyte destruction and tissue necrosis. It is produced by fewer than 5% of *Staphylococcus aureus* strains and is mainly associated with community acquired MRSA. PVL positive strains have an increased ability to cause spontaneous infection and recurrent disease as compared to toxin negative strains. Here we describe two cases of PVL toxin producing *S. aureus* infection. Our first patient was a 35 year-old breast feeding mother diagnosed with pyogen granuloma. Progression of her inflamed skin areas was noticed. The patient had wounds first on her left breast, left leg, and later on her right brest and arm.

Samples taken from her wounds, and abscess showed MSSA. Because about 25% of humans carry *S. aureus*, we took samples from the nostrils to find the source of infection and it yielded PVL positive MSSA. Nine months later her 34 year-old husband was also diagnosed with pyogen pustule on his lips and face. He had fever and elevated inflammatory parameters. Progression of the involved area was also observed. Samples taken from his wound and nose showed MSSA and both were PVL positive. Both patients received cefuroxim in combination with mupirocin and recovered after a few months. The isolates were identified by routine laboratory procedures. Clumping factor, tube coagulase test and PCR of the nucA gene were positive. Susceptibility tests to different antibiotics were performed using the disk diffusion method with Oxoid disks according to CLSI guidelines. Methicillin sensitivity was confirmed by PCR of absence of the mecA gene. PVL positivity was detected by coamplification of the lukS-PV and lukF-PV genes by PCR. Our further aims are to investigate the strains by multilocus sequence typing to confirm the genetic relationships between the strains. Most infections caused by PVL positive *S. aureus* are not dangerous. However, a correct diagnosis is important since recurrence of infection and spread between close contacts is a common feature. Nasal MSSA isolates that harbor the pvl genes may serve as an endogenous reservoir for skin and soft tissue infections. Rarely, PVL positive S. aureus causes severe and life-threatening infection such as pneumonia.
That is why the molecular testing of PVL is recommended in outpatients diagnosed with severe and/or recurrent skin and soft tissue infection caused by methicillin-sensitive *S. aureus*.

## ROSEMARY EXTRACT SHOWS DIFFERENT EFFECT ON THE GROWTH OF THE ENTEROHEMORRHAGIC *ESCHERICHIA COLI* STRAINS SAKAI AND EDL933 AND ON THE LABORATORY *ESCHERICHIA COLI* STRAIN MG1655

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Restrictive effect of the rosemary (*Rosmarinus officinalis*) oil was compared on the proliferation of two Enterohaemorrhagic *Eschericia coli* (EHEC) strains SAKAI, EDL933 and on the laboratory *Escherichia coli* strain MG1655. In the concentration range of 5-25ug/ml the effect of this herbal extract could be evaluated in liquid cultures. Proliferation of the investigetad two highly virulent EHEC strains could be inhibited while no restrictive effect could be observed in the case of the non pathogenic strain. To reveal those active components that are involved in this antimicrobial effect on the two EHEC strains, rosemary oil extract components were separated by Thin Layer Chromatography (TLC) and autobiography were carried out. Eucalyptol ($C_{10}H_{18}O$) proved to be the most effective component eliciting the largest clearing zone on the developed TLC plate, and its impact was confirmed also by employing a commercial eucalyptol standard. Identification of further active components are under way. We also carried out experiments to evaluate those phenotypic changes that can be in correlation with the active effect of this herbal extract. For this purpose a microchip electrophoretic system was used where changes in the protein profiles were tracked before and during herbal extract treatment.
Changes could be demonstrated on both total and outer membrane protein (OMP) levels. Our preliminary results point to the antibacterial impact of rosemary oil on two prototype EHEC strains but further investigations involving an extended number of EHEC and commensal *E. coli* strains are

necessary to elucidate the pathotype selectivity and practical applicability of this effect.

## STUDIES ON THE SAFETY AND IMMUNOGENICITY OF AVIAN INFLUENZA VECTOR VACCINES IN MUSCOVY AND PEKIN DUCKS

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The NOVADUCK project (EC-FP6) aims to develop and evaluate new avian influenza live vaccines for ducks based on live vectors and compatible with the DIVA strategy (**D**ifferentiating **I**nfected from **V**accinated **A**nimals). Here we report the results of safety and immunogenicity of 3 vaccination schemes involving Newcastle disease virus (NDV; paramyxovirus 1, APMV1) and fowlpox virus vectors harboring hemagglutinin (HA) gene from a highly pathogenic (HP) avian influenza (AI) H5N1 isolate, as well as inactivated vaccines. Immunogenicity of a heterologous prime boost vaccination scheme was compared to a homologous prime-boost in Muscovy ducks (*Carina moschata*) and in Pekin ducks (*Anas platyrhynchos*). Poxvirus-induced immunity was very low and transient. Significant increase of the immune response could be obtained by boosting the pox vector-induced primary response with an inactivated vaccine. This fowlpox/inactivated vaccination scheme was then shown to induce a good level of protection after H5N1 challenge in both Muscovy and Pekin ducks. However, the use of the inactivated vaccine suppressed the possibility to use the DIVA strategy based on commercially available NP-based ELISA. The NDV vector carrying the AI HA gene contains the HA protein in its envelope. The presence of this HA protein at the surface of the NDV vector may potentially modify its tissue tropism. That is why the tissue tropism of the NDV vector was compared to that of the parental NDV using quantitative real-time reverse transcriptase assay (qRRT-PCR) and indirect immunofluorescence assay (IFA). The first study performed in Pekin ducks showed no difference in tissue tropism. Similar data were obtained in Muscovy ducks.

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## IN MEMORIAM PROFESSOR IVÁN KÉTYI

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Professor Iván Kétyi, the internationally distinguished microbiologist and dedicated teacher of generations of medical students deceased on March 6th, 2011, at the age of 85. He was born in Pécs, Hungary, and except for a one-year fellowship in Copenhagen he remained confined to his home town throughout his entire life. His eminently expansive knowledge in the field of classical education rooted from the famous István Széchenyi Grammar School where leading Hungarian writers, architects, ethnographers, mathematicians and plant-breeders were tutorized. He received his medical

diploma in 1950, and joined his first and sole place of work at the University Medical School, Pécs as one of the earliest co-workers of Professor Károly Rauss, the founder of the Department of Microbiology. As a young associate he started his research activity on the subject of bacillary dysentery. That time shigellosis was a common enteric infection, and vaccine development was considered a health priority for military units. Beside the vaccine studies he exerted a pioneer work on Shigella genetics. His publications on plasmid mediated resistance, phage determined antigenic conversion and the development of Hfr derivatives constitute a fundamental contribution to the field of bacteriology. He was always altruistic to share his ideas with his colleagues, and readily handed down his knowledge to the upcoming generations. His engaging lectures and his special course on bacterial genetics were highly appreciated by the medical students, and attracted many of them to join the department or especially to his group. The monograph entitled "Microbial genetics" brought him the precious Niveau Prize. He was the co-editor of several editions of the student textbook "Medical microbiology". When Professor Rauss retired in 1975 he was appointed professor and head of the department. Owing to his humanism, wisdom, humor and understanding the period under his leadership was a golden era with broadening international relations and high publication activity. After resigning from the managerial function he continued to be active in science, and initiated new research projects on biofilm formation. Through several periods he was member of the Management Board of the Hungarian Society for Microbiology and the Editorial Board of Acta Microbiologica et Immunologica Hungarica. His death constitutes an irretrievable loss for his home University, for the Hungarian microbiologists, and for the whole scientific community.

## LOW CALCIUM RESPONSE PROTEIN E (LCRE) VACCINATION IS EFFECTIVE IN DNA PRIMING/PROTEIN BOOSTER FORM AGAINST *CHLAMYDOPHILA PNEUMONIAE* INFECTION IN BALB/C MICE

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*Chlamydophila pneumoniae* is an obligate intracellular human pathogen, which causes acute respiratory tract infections and can also cause chronic infections. Since antibiotics can not fully inhibit chlamydial growth and the protection induced by natural infection is incomplete, the development of an effective vaccine would be desirable to control the infections caused by this highly prevalent pathogen. Chlamydiae possess type III secretion system (TTSS), which allows them to secrete effector molecules into the inclusion membrane and the host cell cytosol. Components of TTSS are expected to be surface exposed and required for infection of host cells, thus, these are attractive vaccine candidates to investigate. Low calcium response protein E (LcrE) is a part of TTSS. Our aim was to evaluate in BALB/c mice the immunogenicity and protective effect of LcrE protein of *C. pneumoniae* applied as protein, DNA or combined immunization. The gene of LcrE in a 6His-tagged form was cloned from *C. pneumoniae* CWL029, expressed and purified from *Escherichia coli*, the gene was also cloned into a eukaryotic expression vector (pΔRC). Groups of BALB/c mice were immunized with 1) an intramuscular pΔRC inoculation then with purified LcrE protein subcutaneously; 2) two times with the recombinant plasmid expressing LcrE (pΔRCLcrE); 3) first with pΔRCLcrE inoculation then boosted with LcrE protein; 4) with pΔRC vector alone. LcrE-specific antibody response was induced by DNA immunization with a shift towards Th1 type pathway characterized by increased IgG2a production, and immunization with LcrE protein which generates an IgG1-dominated Th2 type response did not alter the DNA priming-polarized Th1 type response in DNA-primed and protein-boosted mice. DNA immunization given as priming and followed by a

protein booster significantly reduced the number of viable bacteria in the lungs after challenge with *C. pneumoniae* compared to the control group. These results emphasize the potential of DNA-prime/protein-boost vaccination over DNA/DNA and protein based vaccination in producing immunity against intracellular pathogens like *C. pneumoniae*.

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## CLIMATE CHANGE AND FOOD SAFETY

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Observations and modelling simulations reported by different scientific bodies, like i.e. the Intergovernmental Panel on Climate Change point convincingly to the facts of an on-going global climate change, becoming one of the greatest challenges of the 21sat century. Geographical shifts of climate regions and increasing frequency and intensity of weather extremities are parts of this situation. Climate change implications for food security and safety have been analysed timely by relevant UN and EU organisations, and a national collaboration of numerous Hungarian experts: the VAHAVA project. It has been established that the Carpathian Basin is one of the climatically vulnerable regions of Europe and the climate change shall affect our food security and may threaten the microbiological and chemical safety of our food, animal feeds and the drinking water. The increased opportunity for the occurrence of environmental contaminants and for the growth of food-borne pathogenic bacteria and toxigenic moulds, both pre- and postharvest, will be discussed. Adaptations strategies for addressing the observed and forecasted climate changes are of eminent importance.

A system-directed, multidisciplinary approach utilising network science to problems of food security and safety is required to assist the risk management of the complex interactions of relevant hazards.

## IDENTIFICATION OF A BETA-GALACTOSIDASE/LACTOSE PERMEASE GENE COUPLE AND THE CHARACTERISATION OF LACTOSE TRANSPORT IN *ASPERGILLUS NIDULANS*

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Lactose is intracellularly hydrolysed by *Aspergillus nidulans*. Classical mutation mapping data and the physical characteristics of the previously purified glycosyl hydrolase enabled the *in silico* identification of clustered, divergently transcribed intracellular *beta*-galactosidase (*bglD*) and putative lactose permease (*lapA*) genes. Phylogenic studies refuted the widespread misbelief that only lactose-utilising yeasts (a.o. *Kluyveromyces lactis*) are sources of *beta*-galactosidase with a neutral pH-

optimum. *bglD* and *lapA* were perfectly co-expressed in response to D-galactose, lactose or (at lower levels) to L-arabinose. However, no transcript was detectable in the co-presence of glucose and either of the above inducers. Interestingly, transcript analyses in CreA mutants showed derepression of both genes to a basal level under all non-inducing growth conditions, including on glucose, while full induction only occurred on lactose and galactose in the absence of glucose, indicating a prominent role for inducer exclusion in the system's regulation.
To confirm lactose permease function, the *lapA* gene was deleted. Unexpectedly, disruptants were still able to grow on liquid lactose medium, albeit at a much lower rate than wild type controls, and behaved normally on galactose. The effect of *lapA* deletion was exacerbated at lower lactose concentrations, suggesting the existence of a second uptake component of lower affinity.

## EXAMINATION OF THE OUTER MEMBRANE PROTEINS OF *PSEUDOMONAS* STRAINS TREATED WITH DIFFERENT METHODS

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The outer membrane (OM) is a typical component of the cell-wall of the Gram-negative bacteria. In it outer membrane proteins (OMP) can be found, which may have transport and receptor function and can play role in adhesion. Sometimes these proteins do not appear constitutively, but they are synthetized due to certain environmental or hostal conditions, their genetical information has often on extrachromosomal origin. Our aim was to examin the outer membrane proteins extracted from different *Pseudomonas* strains can occur in the natural environment and they play an important role in clinical work due to their ability of causing serious lifethretening infections. Their protein profile can rapidly change because of different environmental affecting their viability and virulence. This is a reason why the examination of their protein profile is so important. The advantages of the michrochip method are the increased sensitivity, easy management, rapidity, automation and low sample consumption (nanogramm). The instrument can detect the proteins labelled with fluorescent stain by a LIF (Laser Induced Fluorescence) detector in presence of sodium dodecil sulphate. The analysis of the outer membrane proteins by lab-on-a-chip, which is non-negligible in the diagnostics also, may replace other techniques just used before. The use of mass spectrometry (MALDI-TOF) applied recently more frequently, can provide a good alternative of LIF.

## HUMAN PAPILLOMAVIRUS 31 LCR (LONG CONTROL REGION) SEQUENCE VARIATION: PHYLOGENETIC AND FUNCTIONAL ANALYSIS

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About one-third of all HPVs (Human Papillomavirus) infect the anogenital tract. Cervical cancer, caused by infection with high-risk HPV types (HPV 16, 18, 31, 33, 35, etc.) is a major public health problem also in Hungary. All the genital HPV types are approximately 8 kb in lenght and their genome consist of early, late and noncoding regions. The LCR ( Long Control Region) of the viral genome contains regulatory elements, which bind many viral and cellular factors, so the LCR

modulates viral replication and viral gene transcription. We examined the functional significance of genomic sequence variation within the LCR of HPV 31. We examined clinical samples from women, who had colposcopical atypia in the cervix. First, the LCR (nt 7122-180) was amplified with PCR from 43 HPV 31 positive clinical samples . In order to evaluate nucleotide sequence variation, all the 43 HPV 31 LCR isolates were sequenced and a phylogenetic tree was constructed. Eight different variants were selected for further analysis. These LCR sequences were cloned into a luciferase reporter vector for functional studies. In order to study the transcriptional activity of the different HPV 31 LCR variants, the luciferase constructs were transfected into HeLa and C33-A cells and luciferase tests were performed. We found significant differences between the transcriptional activities of some HPV 31 LCR variants.
As the LCR region is governing the transcription of the E6 and E7 oncogenes, the differences in the activities of LCR variants may have a biological significance.

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## DETECTION OF HONEYBEE-PATHOGEN VIRUSES IN WASP (VESPIDAE) SAMPLES COLLECTED IN HUNGARY

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The honeybee (*Apis mellifera* L.) is a major pollinator of plants and is thus of global ecological significance. The intensifying international exchange of honeybee colonies and bee products has in the last decade increased interest in infectious diseases of bees. At present, at least 18 different viruses have been identified which are able to infect honeybees. Viruses are often present in colonies inapparently or they may not cause characteristic clinical signs. Although the parasitic mite Varroa destructor is suspected to have a special role in the manifestation and dissemination of viral diseases of bees, other species that have regular contact with bee colonies may can also serve as reservoirs. Wasp species are predators and food parasites of the honeybee; therefore they are often in close contact with them. The aim of the investigations were to assess the potential honeybee virus reservoir role of different wasp species. In our study, 56 samples of six different wasp species (Vespidae) collected in Hungary were investigated by reverse transcription-polymerase chain reaction (RT-PCR) in order to detect five honeybee viruses. Among the samples, 89% found to be infected with at least one virus: acute bee paralysis virus (ABPV) was detected in 45% of the investigated samples, 5% of the samples were infected with black queen cell virus (BQCV), 27% and 23% with deformed wing virus (DWV) and sacbrood virus (SBV) respectively. Chronic bee paralysis virus (CBPV) was not detected in the investigated samples. Simoultaneous infection with 2, 3 and 4 viruses occured in 7%, 16%, and 4% of the samples respectively. Bombus spp.) was previously described, by the results of our study the role of wasps in the maintenance and dissemination of honeybee viruses is supposed.

## DETECTING INTER- AND INTRASPECIFIC RECOMBINATION EVENTS IN POTYVIRUSES

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Recombination among co-infecting plant RNA viruses is a natural phenomenon that appears to have played a significant role in the speciation and evolution of many strains. It also has particular significance for the risk assessment of plants that have been genetically modified for disease resistance by incorporating viral sequences into plant genomes. In the world of RNA viruses the source of recombination during replication has a widely accepted model. By a process termed 'template switching', the viral replicase (an RNA dependent RNA polymerase) might switch from its viral RNA template to another viral RNA or to the transgenic mRNA and give rise to a recombinant RNA molecule. Recombination has played a significant role in the evolution of RNA viruses by producing genetic variation, reducing mutational load, and introducing new viruses (Worobey and Holmes, 1999). In the genus Potyvirus, recombination has been reported for a number of different species such as Plum pox virus (PPV) (Cervera et al., 1993), Potato virus Y (PVY) (Revers et al., 1993), Bean common mosaic virus (BCMV) (Revers et al., 1993), Yam mosaic virus (YMV) (Bousalem et al., 2000), Lettuce mosaic virus (LMV) (Krause-Sakate et al., 2003), and only one from the SCMV subgroup, the Sugarcane mosaic virus (SCMV) (Zhong et al., 2005). Several diferrent software packages exist for the detection of recombination between DNA and RNA sequences 'in silico'. As none of the statistical methods used by these softwares are completely reliable and optimal for detecting recombination under all conditions, we applied the PDM (Probabilistic Divergence Measure) method from the TOPALi software package and several other methods (RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan, Phylpro, LARD and 3Seq) from the RDP3 package. The TOPALi software found only one interspecific recombination event in the full length MDMV genomes, while the RDP3 package detected 4 breakpoints. The analysis of the SCMV subgroups led to the detection of a large number of recombination breakpoints with the RDP3 package but none were detected with the TOPALi software.

## LEISHMANIASIS: TOPICALITIES AND A REVIEW OF CASES DETECTED IN HUNGARY

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Leishmaniasis is a group of the protozoan diseases, transmitted by the bite of sandfly infected with *Leishmania* parasites. The infection of humans appears with multiple clinical manifestations including cutaneous (CL), mucocutaneous, diffuse and visceral (VL) leishmaniasis. The two forms of leishmaniasis present in Europe (VL and CL) are caused by *Leishmania infantum (L. infantum)*. Five haematophagous sandfly species are known in Europe as the vectors of *L. infantum*. Two of them are present in the western Mediterraneum from the Iberian to the Italian peninsula, while the further three species are distributed from Italy across Balkan to Turkey. CL is endemic in some areas of Greek, some autochthon cases occur in the southern parts of France, in Italy, and in Spain. VL is endemic in all countries bordering the Mediterranean Sea. In Europe, human VL is considered to be a rare disease, although its incidence increased significantly in the region during the 1990s, about 700 autochthonous cases have been reported each year from southern countries of Europe. As the climate of the temperate zone does not promote the spread of leishmaniasis-vectors, in the European countries with temperate climate only imported cases occur. The increasing number of imported cases of leishmaniasis seen in the Central European countries in the last few decades caused an increasing

healthcare problem in these countries, where leishmaniasis was uncommon previously. Except for a single infection in a small girl (without a travel history) reported by Makara, no human case was recorded in Hungary till the 1980's. In a three-year long period of the early 1980's, Várnai *et al*. reported 31 cases of CL among Hungarians returning from endemic areas. The first imported case of VL detected in Hungary was reported by Fried *et al* in 2003. The patient had already had a four months history of FUO (fever of unknown origin) when the diagnosis of leishmaniasis was made. The second imported case of VL was detected in Hungary in 2009 and reported by Péterfi *et al*. Detailed medical history revealed that the patient spent his holidays during the previous 3 years in Dalmatian territories (Dubrovnik, Makarska and Trogir) of Croatia. In the past two decades, VL incidence rates without HIV-1 co-infection have increased in Italy and France (including a four-fold increase in Alps-Maritimes), and new endemic areas have been detected where no previous autochthonous cases had been reported (e.g. in northern Italy, North Croatia, Switzerland and Germany). Climate-induced changes may increase the risk of emergence of new diseases including leishmaniasis in Central and Western Europe. In endemic countries, leishmaniasis is diagnosed quickly already in the early stage of the disease merely on the evidence of the clinical signs. In Hungary, where leishmaniasis is uncommon, establishing a correct diagnosis quickly can be a problem as the detailed medical history generally does not include traveling history.

## USING GRAPH-THEORETICAL METHODS FOR TARGET IDENTIFICATION IN MICROBIAL PROTEIN NETWORKS

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We introduce a novel mathematical method for identifying promising protein targets in microbial protein networks. Most network analysis methods return hub proteins, that is, large degree (or sometimes called 'large connectitvity') proteins as suggested drug targets. These hubs are functionally connected to numerous other proteins in the microorganism, and their inhibition may have significant off-target effects even in the host organisms (i.e., in humans), because of their universal biochemical importance. We show a method for identifying non-hub important nodes in metabolic bacterial protein networks, and demonstrate on examples of *Mycobacterium tuberculosis*, *Plasmodium falciparum* and MRSA *Staphylococcus aureus*, that some of the well known and well researched primary drug targets can be found this way. Additionally, there are a score of new, never examined non-hub proteins that may become promising novel drug target candidates.

## THE ROLE OF PGE2 IN THE VIRULENCE OF *CANDIDA PARAPSILOSIS*

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Prostaglandins are fatty acid metabolites build up of 20 carbon atoms. Mammalians produce immune response regulator prostaglandins from arachidonic acid by the contribution of *COX1* and *OLE2* deletion mutant through repeated application of a caSAT1 flipper KO cassette. We characterised the pseudohypha production, deletion mutant in comparison to that of the wild type strain and we found that mutant strain showed the same characteristics as the wild type. When prostaglandin $E_2$ ($PGE_2$)

production of *OLE2* deletion mutant and wild type was measured by HPLC analysis we found no $PGE_2$ production in case of the *OLE2* deleted mutant while wild type produced significant amount of $PGE_2$ from the supplemented arachidonic acid. According to mouse IP infection model there were no difference in the virulence properties of the *CpOle2* mutant strain compared to the wild type. We carried out killing assays on human PBMC-derived macrophage (PBMC-DM) cells, and the killing efficiencies showed an approximately 30% more effective killing of the mutant strain which indicates a setback in virulence. Phagocytosis assay were carried out by fluorescent microscopy with acridine-orange and crystal violet staining, and with flow cytometry, and these assays with human PBMC-DM also demonstrated reduced virulence of the *CpOLE2* deletion mutant compared to the wild type strain. According to these data we suggest that CpOle2 of *C. parapsilosis* is the exclusive enzyme of the first step of biosynthetic process of $PGE_2$ from arachidonic acid, and it is a virulence factor during human infections. After identifying fungal genera and species on the basis of ITS sequences, the phylogenetic tree was constructed. Our results show, that the orders Hypocreales, Pleosporales, Xylariales and Cantharellales are represented with most taxa. Several different genotypes of Fusarium and Alternaria were found in cherry tree tissues. We observed a sharp difference in the diversity of endomycota in different plant organs. The endophyte colonization rate strongly varied among the different rootstocks, but in endofungal diversity no significant differences were detected.

## EFFECT OF HUMAN PAPILLOMAVIRUS ONCOPROTEINS ON THE EXPRESSION OF INVOLUCRIN IN HUMAN KERATINOCYTES

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The productive phase of the human papillomavirus (HPV) life cycle is closely linked to keratinocyte differentiation. The E6 and E7 oncoproteins of high-risk HPVs are responsible for the transforming activity of the virus. Oncogenic HPV infection has been shown to hamper the normal differentiation of keratinocytes. Involucrin is found in the cytoplasm, cross linked to membrane proteins by keratinocyte transglutaminases and it is a precursor of the keratinocyte cornified envelope. It can be used as a marker for keratinocyte differentiation. The aim of this study was to investigate the effects of HPV16 E6 and E7 oncogenes on the expression of involucrin in 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, E7 or E6/E7 genes. These cells were induced to differentiate by culture in high calcium and serum containing medium for 24h. The expression level of involucrin and AP1 factors (c-jun, junB, junD, c-fos, FosB, Fra-1 and Fra-2) in differentiating and non-differentiating infected cells was estimated using real-time RT-PCR. The involucrin protein was detected by western blotting using monoclonal anti-human involucrin antibody. Primary human keratinocytes were co-transfected by luciferase reporter plasmids carrying different fragments of the involucrin promoter or AP1 factor binding sites along with vectors expressing HPV 16 E6 or E7 genes. After transfection, cells were either left untraeted or induced to differentiate by culture in high calcium and serum containing medium for 24h. Luciferase assay was used to measure the effect of the HPV oncogenes on involucrin promoter or AP1 reporter activity. The differentiation of keratinocytes by serum and high calcium significantly increased both the mRNA and the protein levels of IVL. The E6 and E7 oncoproteins of HPV16 together caused down-regulation of involucin mRNA and protein both in proliferating and in differentiating keratinocytes. In order to verify the effect of HPV oncogenes on the involucrin promoter, we made transient

transfection assays and found that the HPV E6 repressed involucrin promoter activity in proliferating keratinocytes. Both HPV oncoproteins caused a down-regulation of involucrin promoter activity in differentiating cells. The effect of HPV oncogenes was localized to the proximal region of the involucrin promoter (-231 from transcription start site). In this proximal region of the involucrin promoter, there are important transcription factor binding sites (AP1, C/EBP and Sp1). Therefore, we made transient transfection experiments with an AP1 reporter construct, and the results of these suggest that the HPV 16 oncoproteins may down-regulate transcription from the involucrin promoter through inhibiting the activity of AP1 transcription factors. These results suggest that the HPV16 oncoproteins decrease endogenous involucrin expression in keratinocyte cells through down-regulating involucrin promoter activity.

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## MYCOLOGICAL INVESTIGATION OF A GRAIN WAREHOUSE

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Grain industry workers are subjected to a high risk of respiratory diseases due to the large amount of dust generated during the work phases. Previous investigations performed in the grain warehouse of the Csepel Free Harbour revealed high concentrations of fungal spores, principally bunt (*Tilletia*) species in non-viable air samples, however, many observed spore types could have been identified by viable air sampling method only. Based on the size of the fungal elements, literature data and the symptoms occurring in the case of the colleagues performing the examination it was assumed that the small-sized fungal spores aerosolized during the movement of grain are capable of penetrating to the alveoli and causing farmer's lung disease. The aim of this study was to examine the fungi occurring in the air of a grain warehouse in order to assess the extent of health risk. Air sampling was performed by a viable Andersen-type air sampler (Lanzoni Co Ltd). During the sampling procedure, MEA and DG18 media were exposed to 100-100 liter of air at constant air stream of 28.3 liter per min. The equipment is capable of assorting the particles in air by their size and separate them according to the regional deposition of particles in the human lung. Fungal species isolated in the smallest size range can cause farmer's lung disease. The examined grain warehouse is a 13-floored relic building operating since 1928, where the storing in and out, aeration and - if necessary - fumigation of the crops is performed. The carriage (winter wheat, maize and sunflower seed) is layed up in open-top timber wall silos, thereby the building is single air spaced at each floor. Air samples were collected in replicates at 3rd and 5th floor as well as outdoors. Altogether, 36 air samples were collected during the study and a total number of 1811 and 1759 fungal colonies were isolated on MEA and DG18, respectively. Isolates were identified based on their morphological characters and where necessary, by the sequence analysis of the internal transcribed spacer (ITS) region. *Cladosporium*- and *Penicillium* species as well as *Aspergillus flavus* were occurring in all indoor samples. Indoor concentrations were higher in the case of these species than outdoor concentrations, suggesting that the fungal spores derived from the indoor space where the fungi were possibly introduced with mouldy crop and than dispersed into the air at high concentrations during the material movement processes. Besides the above mentioned species, *Alternaria* spp., *Aspergillus clavatus* and *Epicoccum nigrum* were also frequent. 10.8, 28.7 and 60.5% (MEA) as well as 9.2, 18.6 and 72.2% (DG18) of the total count was falling into the size ranges of >7 μm, 7-3.3 μm and <3.3 μm, respectively. In conclusion, fungal

spores capable of causing farmer's lung disease are present in high quantities in the air of the examined grain warehouse, thus in the absence of protection equipments the warehouse workers are subjected to a significant health risk.

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## DIVERSITY OF ENDOMYCOTA IN CHERRY GRAFTINGS

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Fungal endophytes have been defined as fungi colonizing healthy plant tissues without causing overt symptoms in or apparent injuries to the host. The plant may benefit from endophyte infection through improved drought and flooding tolerance, increased biomass production and improved herbivore and pathogen resistance. It is also believed that endophytes can live in plant tissues either as mutualists or antagonists, depending on the physiological status of the host. Moreover, both pathogenic and nonpathogenic fungi are routinely isolated from asymptomatic tissues, suggesting that the pathogens may either have a nonpathogenic lifestyle or remain dormant after infection until plant senescence. Identifying endophytic fungi living in a plant is possible by studying outgrowing colonies on fungal media or carrying out PCR directly from plant tissue. In spite of recent attempts, there is a lack of knowledge about the biological aspects of host plant-endophyte interaction and there are no specific estimates about the number of existing endophytes. In the present study, we describe the main taxa of endophytic fungi isolated from three different organs of cherry (*Prunus avium*). Samples were collected in three different seasons in the Corvinus University's experimental field in Budapest (Soroksár), Hungary. Leaf, twig and root samples were taken from the cherry cultivar 'Péter' grafted on 11 different rootstocks, among them 8 cultivars of *Prunus mahaleb*, a *P. avium*, a *P. fruticosa* and a *P. cerasus* x *P. canescens* interspecific hybrid. After surface sterilisation in 70% ethanol and 1.5% hypochlorite, samples were put on Potato Dextrose Agar medium and incubated at 22°C for 1-2 weeks. The outgrowing colonies were counted and morphologically classified. Monosporation and monohyphation technique was applied to generate axenic colonies of each individual fungus. DNA was extracted from mycelia of 43 selected colonies and ITS region was amplified by PCR. The applied primers were ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-CCTCCGCTTATTGATATGC-3'). After identifying fungal genera and species on the basis of ITS sequences, the phylogenetic tree was constructed.

Our results show, that the orders Hypocreales, Pleosporales, Xylariales and Cantharellales are represented with most taxa. Several different genotypes of *Fusarium* and *Alternaria* were found in cherry tree tissues. We observed a sharp difference in the diversity of endomycota in different plant organs. The endophyte colonization rate strongly varied among the different rootstocks, but in endofungal diversity no significant differences were detected.

## EFFECT OF INOCULUM'S AGE ON THE EFFICACY OF ESSENTIAL OILS

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In antimicrobial susceptibility testing young cultures, mainly in the mid-log growth phase, are used because of these cells have a very active metabolism and are more vulnerable than older cultures. In our experiments we investigated how can the age of bacterial inoculums influence the efficacy of essential oils (EOs) against spore-forming and non-spore-forming bacteria (*Bacillus cereus, B. subtilis* and *Serratia marcescens*). The agar diffusion method was used to test the antibacterial effect of clary sage, juniper, lemon and marjoram essential oil. Bacterial suspensions ($10^6$/ml) were made from agar slant cultures (24, 48, 72 and 96 h old) and 1 ml suspensions were spread on agar plates. After drying the plates holes (8 mm diameter) were bored with a sterile cork borer and filled with different concentrations of the EOs from 6,25 to 150 ul/ml. The diameter of inhibition zones decreased with decreasing EO concentrations and also decreased with increasing inoculum age. The effect of the positive antibacterial control, streptomycin at 100 or 1000 µg/ml showed no significant response to the different inoculum age. Our results suggest, that some defence mechanisms were induced in the cells of the mature biofilms on the agar slants providing decreased susceptibility also for the initiated culture to the EOs. In time kill experiments, the EOs were added in 10 µl/ml concentration to 24, 48, 72 and 96 h old cultures in liquid medium. Bacterial suspensions were treated for 3 h with the EOs then were immediately diluted to minimize the remaining EO activity. Survivals were determined by the agar count method. In many cases results showed no tendency except for marjoram oil, where the number of survivals increased until the culture aged reached 72 h, then no change (*B. subtilis*) or a sharp decrease (*B. cereus*) or no survivals were detected. It seems that the age of solid inoculum from agar slant affects the susceptibility of bacteria against EOs. Spore formation had no significant effect on the efficacy of the EOs.

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## PURIFICATION AND SOME BIOCHEMICAL PROPERTIES OF α-GALACTOSIDASE ENZYME FROM *BIFIDOBACTERIUM LACTIS* Bb-12

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Bifidobacteria are dominant members of the normal intestinal flora in humans and are thought to play an important role in the host's health. Some specific carbohydrates such as fructo-, xylo-, and galacto-oligosaccharides known as bifidogenic factors stimulate the growth and activity of these bifidobacteria. Galacto-oligosaccharides (GOS) containing galactose residues linked by α- or β-glycosidic bounds can be hydrolysed by galactosidases, thus bifidobacteria should synthesize these enzymes to utilise GOS. While much more information about β-galactosidase from bifidobacteria are available in literature, they are lacking of knowledge about the α-galactosidase enzyme that

hydrolyzes α-1,3, α-1,4 and α-1,6 galactosidase linkages found in α-GOS. These GOS are found in large quantities in some vegetables such as beans, can cause gastrointestinal disorders in sensitive individuals because monogastric mammals (such as humans) do not possess α-galactosidase enzyme in the gastrointestinal tract. In present research, purification and some biochemical properties of α-galactosidase enzyme from probiotic *Bifidobacterium lactis* Bb-12 strain, which has a long history in the food processing were studied. *Bifidobacterium lactis* Bb-12 was grown in TPY media containing 1% raffinose as carbon sources at 37°C for 24 hours. Fermentation was followed by the measurements of pH, determination of living cell number by plating methods and enzyme activity assay using artificial p-nitro-phenyl-α-D-galactopyranoside (p-NPG) substrate. Protein content was determined by Bradford method using Protein Kit from BioRad. The α-galactosidase enzyme was released from the cells by French Press homogenizer (Thermo Spectronic) at a pressure of 20000 psi. The crude extract containing α-galactosidase was purified chromatographically subjected onto columns with various resins. The final enzyme preparation was purified to give an overall yield of 15.27% of initial α-galactosidase activity that hydrolyzed p-NPG at a specific activity of 2.074 $Umg^{-1}$ protein. The α-galactosidase showed maximum activity at pH 6.5 and 45°C. Lower, higher pH values and higher temperatures rapidly reduced activity of the enzyme. $Hg^{2+}$, $Ag^{2+}$, $Co^{2+}$ was the strongest inhibitor and caused a 75%; 73% and 64% decrease in the α-galactosidase activity. The other tested ions also affected α-galactosidase activity although lower extent decreases in enzymatic activity. Examination of the enzyme stability indicated that the protein is stable in neutral pH values. Incubating α-galactosidase enzyme in pH 7.0 medium, half-life times at 40°C and 45°C were 1600 min and 400 min, respectively. Enzyme exhibited stability in range of pH from 6.0 up to 8.5. $K_m$ and $v_{max}$ of purified α-galactosidase for p-NPG were 19.36 mM and 9.5μM/s, respectively.
Preliminary results showed that this α-galactosidase may have galactosyl transferase activity, thus it can be used in the synthesis of novel galacto-oligosaccharides suitable as specific growth factors for probiotic bifidobacteria.

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## IS HIV-1 BECOMING MORE VIRULENT? A META-ANALYSIS OF TRENDS IN PROGNOSTIC MARKERS OF HIV-1 DISEASE PROGRESSION

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The potential for changing HIV-1 virulence in the human population has significant implications for the HIV/AIDS pandemic. Studies of trends in prognostic markers of HIV-1 infection as proxies for HIV-1 virulence have had conflicting results. We conducted a meta-analysis of studies examining changes in baseline CD4+ T cell counts and set point plasma viral RNA load over time, in order to establish whether these trends are consistent with changing HIV-1 virulence. We searched PubMed for studies of trends in HIV-1 prognostic markers, and supplemented findings with publications

referenced in epidemiological or virulence studies. In two cohorts, we performed new analyses of original data to obtain updated estimates of virulence trends until the present time. Using random-effects meta-analysis, we estimated summary effect sizes for trends in HIV-1 plasma viral loads and CD4+ T cell counts. We next conducted meta-regression analyses of study-level covariates versus effect size magnitude. We identified 12 independent studies of trends in baseline CD4+ T cell counts (21,052 total individuals), and eight studies of trends in set point viral loads (10,785 total individuals), spanning the years 1984-2010. Baseline CD4+ T cell counts showed a summary trend of decreasing cell counts (effect = -4.93 cells/μL /year, 95% C.I. -6.53 to -3.3), with indications of a decelerating trend in recent years. Set point viral loads showed a summary trend of increasing plasma viral RNA loads over the documented time span of the epidemic (effect = +0.013 log10 copies/mL/year, 95% C.I. -0.001 to 0.03). Trends in prognostic markers are consistent with increased virulence of HIV-1, which may reflect viral evolution and adaption in the human population. In analyses of summary trends estimated for each year with represented studies, trends of increasing virulence appear to be slowing. Further studies are needed to determine if the observed trends are occurring in other populations and locales.

## HERPESVIRAL ACTIVITY IN APICAL PERIODONTITIS

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Apical periodontitis, the inflammation of the apical area of the tooth, is characterized by a polymicrobial infestation, with a dominance of opportunistic Gram-negative bacteria. Nevertheless, a pathogenic role of human herpesviruses such as Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV) has been implicated recently. The aims of this study were to determine the prevalence, activity and disease association of EBV, HCMV and HHV-6 in apical periodontitis. 40 samples with apical periodontitis (17 symptomatic and 23 asymptomatic) and 40 healthy pulp controls were collected. EBV, HCMV and HHV-6 prevalences were measured by PCR detection of the viral DNA and viral activity was tested by reverse transcription PCR amplification of viral mRNA. EBV DNA and EBNA-2 mRNA were found in apical periodontitis lesions at significantly ($p<0.0001$) higher frequencies (72.5% and 50%, respectively) than in controls (both 2.5%). Occurrence of HCMV infection was rare in both apical lesions (10%) and controls (0%). Presence of EBV DNA in apical lesions was significantly associated with large (≥5mm) lesion size ($p=0.02$). Symptomatic manifestation was significantly associated with the co-occurrence (OR=8.80, $CI_{95\%}$: 1.69-45.76) of EBNA-2 mRNA and large lesion size. HHV-6 DNA was observed in significantly higher frequencies in apical periodontitis samples than in controls (20% vs. 2.5%, $p=0.03$). Further classification of apical lesions revealed that subtype B of HHV-6 was significantly associated with large sized and symptomatic lesions ($p<0.01$). Our findings suggest that EBV and HHV-6B infections are frequent events in apical periodontitis, especially in large sized and symptomatic lesions. This study showed that symptomatic manifestation was likely to occur if a large sized apical periodontitis lesion is aggravated with active EBV infection.

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## IN MEMORIAM DR BÉLA LÁNYI

MÁRIA HERPAY

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The history of his life-work is the history of the epidemiological-clinical bacteriology of his epoch at the same time. Dr. Béla Lányi finished his academic studies at the Medical University of Budapest, in 1951. Following the four-month work in the clinical laboratory of the County Hospital in Székesfehérvár, he began his career at the Department of Bacteriology in the National Institute of Public Health where he worked for 45 years and acted as the Head of Department since 1984. From 1953, he gradually developed the production of immune sera for the serological diagnostics of *Salmonella, Shigella* and *Escherichia coli* bacteria and organized the up to date sera and diagnostics producing laboratory to supply the KÖJÁL/ÁNTSZ (Station of Public Health and Epidemiology/ National Public Health and Medical Officer Service) laboratory network. From the start, his duties included the improvement and quality control of culture media produced for the KÖJÁL/ÁNTSZ laboratory network, the control of trade media, diagnostic antimicrobial disks and other diagnostic products, and also the microbiological standard evaluation of antibiotics. He was the Founding Head of the Hungarian National Collection of Medical Bacteria (HNCMB) (1962) that he made internationally recognized. With his unswerving efforts, he created the conditions of external and internal quality control for bacteriological laboratories and elaborated its efficient control system. For the KÖJÁL/ÁNTSZ laboratories (from 1960), he organized the examination and evaluation of unknown test materials (collaborative studies) and the local surveys combined with the processing of test materials from 1982. As the Chair of Microbiological Expert Committee, he extended the advisory and control activities to the medical bacteriological laboratories operating in the preventive-curative and university institutes, from 1984. In the seventies, he had the unified, IT data supply and evaluation system introduced for the epidemiological bacteriological laboratories. With the regular evaluation of the annual work of KÖJÁL/ÁNTSZ laboratories (so-called “White Note-books”, he made a substantial contribution to the improvement of level of diagnostic work. As the result of his editorial activity, the Methodological Guide (1969) – summing up the national methodological procedures based on standard methods – and the medical-bacteriological diagnostic handbook with the title of Epidemiological and Clinical-bacteriological Methodology Guide (1980) were published. He had undying merits in medical education and further education, and in the field of training for hygienists. He achieved important accomplishments in the research of serological and biochemical features of *Pseudomonas aeruginosa* and related bacteria, and in the studies of their public health significance. One of his most important works was the *Pseudomonas* antigen scheme – accepted also by the National Committee of Nomenclature. He identified the antigen relationship of members in *P. aeruginosa* and *Enterobacteriaceae* family. He studied the pathogenicity of serogroup *E. coli* O124, the aetiological agent of infant and childhood gastroenteritis, the occurrence of *Salmonella* infections, and the serology and pathogenicity of bacteria belonging to *Proteus* group. He worked out new, practical procedures for the identification of bacteria and for the determination of their antibiotic sensitivity. About 300 references were made to his 94 publications and chapters. He developed significant, international scientific collaboration for the study of immune chemical structure of *P. aeruginosa* O antigens and for the determination of antigen structure of a facultative pathogen *Pseudomonas* group. In 1969, with his dissertation *Pseudomonas aeruginosa* he became the holder of candidate’s degree in medical science. For his thesis with the title of ‘Serological, hygienic and clinical characterization of *Pseudomonas aeruginosa*, he obtained the Academic Degree in Medicine of the Hungarian Academy of Sciences in 1980. He was the Editor (from 1971) and Editor-in Chief (from 1976) of the journal *Acta Microbiologica.* He was the Editor-in Chief of Alpe-Adria Microbiology Journal (from 1992).

He was the leading member in the Hungarian Society of Microbiology, Chair of the Bacteriological

Section, President of the Microbiological Expert Committee governing the inspection of medical-microbiological laboratories, Examiner of the National Qualification Committee, member of the Public Health Department of University of Postgraduate Medical Education, and member of the Committee for Microbiology, Epidemiology and Vaccine, member of the Board of Infectious and Tropical Diseases, member in the Working Committee for the Microbiological Code Review and Cross-checking of Charges of the Ministry of Welfare, member of the Committee of General Microbiology of the Hungarian Academy of Sciences, member of the Board for the Hungarian National Foundation of Genital-urinary Medicine, leading member of the Alpe-Adriatic Microbiological Group, member of the Clinical-Epidemiological Committee in the National Institute of Traumatology, and the representative of the Hungarian Society of Microbiology in the International Committee for Bacteriological Nomenclature and Taxonomy. He had a considerable work in the institution of the Board of Microbiology and was its President until his death.

## ENTEROAGGREGATIVE SHIGA TOXIN PRODUCING *ESCHERICHIA COLI* O104:H4 OUTBREAK IN GERMANY AND ITS CONSEQUENCES FOR EUROPE AND HUNGARY

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The *Escherichia coli* strain causing a large outbreak of haemolytic uraemic syndrome (HUS) and bloody diarrhoea in Germany in May and June 2011 possesses an unusual combination of pathogenic features typical of enteroaggregative *E. coli* together with the capacity to produce Shiga toxin. It has shown a number of striking features: an unusually large proportion of HUS cases as compared with diarrhoea cases. Furthermore, whereas usually HUS triggered by Shiga toxin-producing *Escherichia coli* (STEC) infection predominantly affects young children, the great majority of cases in this outbreak are adults and two thirds are women. Over 95% of STEC cases have been reported from Germany and the vast majority of cases reside in or have a history of recent travel to northern Germany. The epidemic agent was an *E. coli* strain of rare serotype O104:H4, with production of Shiga toxin 2 (*stx2a*) gen. Moreover, it was further atypical in that it lacked the attaching/effacing pathogenicity island of virulent STEC strains, as indicated by negative PCR results for the intimin (*eae*) and haemolysin (*hly*) genes. In addition the epidemic agent was PCR-positive for the *aggR* gene, which is typical of enteroaggregative *Escherichia coli* (EAggEC). The outbreak strain is indeed a typical EAggEC strain that has acquired the bacteriophage encoding Stx/VT. EAggEC is a common pathogen causing diarrhoea in travellers and persistent diarrhoea in infants and young children living in countries with poor sanitation. In contrast to STEC strains that have an animal reservoir, mostly ruminants, EAggEC strains have a human reservoir. Little is known about the pathogenic role and epidemiological features of infections caused by strains of the hybrid EAggEC STEC pathotype. To the best of our knowledge, this unusual combination of virulence factors of STEC and EAggEC has rarely been described in humans. The microbiology findings and technical recommendation presented were immediately shared by the authors through EU and international public health and food safety laboratory alert networks. The National Center for Epidemiology (NCE) enhanced epidemiological and microbiological surveillance for case findings in Hungary. The National Reference Laboratory inside the NCE worked out a simple diagnostic screening tool for primary culture and applied it together with molecular detection of specific pathogen markers.

## THE ROLE OF INTESTINAL MICROBES IN THE EVOLUTION OF BLIND MOLE-RATS (RODENTIA: SPALACINAE)

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Although intestinal microbial communities have been investigated for a long time, it has been only recently proven that intestinal microbes have an important role during the evolution of mammals. But it is still questionable if the feeding strategies or the taxonomic relationships were the most important factors during the coevolution of microbes and mammals. Eurasian blind mole-rats (Subfamily: Spalacinae) are strictly vegetarian rodents, which are extremely adapted to subterranean lifestyle and regarded as an evolutionary model for active speciation and adaptive radiation. They live in and are adapted to a variety of habitats (temperate steppes to deserts) characterized by different vegetation. Cytogenetic and molecular biological mechanisms of speciation in mole rats were studied in detail, but, the role of microbial communities in the speciation process has not been investigated yet. In this study fecal and cecal bacterial communities were investigated. Fecal samples were collected from two individuals (male and female) of the Judean mole rat *Nannospalax* (*ehrenbergi*) judaei during two experimental periods, when they were fed two different diets (tubers with high starch content and fresh green shoots). For bacterial community analysis from fecal samples community DNA was isolated and PCR-DGGE fingerprint analyses were performed. Samples were ordinated and grouped based on the fingerprints and bands were excised, reamplified and sequenced for identification of key members of the community. At the same time, we investigated the microbial communities in the appendices of 9 different species (17 individuals in total) collected from locations which covered the whole distribution area of the subfamily. Communities were compared using PCR-DGGE fingerprinting. Both fecal and cecal communities were dominated by different members of phyla Bacteroidetes and Firmicutes. Significantly different communities were detected in the different feeding regimes. It indicates that intestinal bacterial communities of mole-rats quickly adapt to the changing food resources. Multivariate statistical analyses of bacterial community fingerprint derived from the cecum of 9 mole-rat species resulted in groups of samples identical to host taxonomic groups. Moreover, the dendrogram topology deriving from cluster analysis of the microbial communities was similar to the phylogenetic positions of mole-rat species based on mtDNA sequences and karyotypes. It suggests co-evolutionary changes of bacterial communities during the adaptive radiation and active speciation of Eurasian mole-rats.

## MULTIPLEX DETECTION OF HUMAN PATHOGENS IN INTENSIVE CARE UNITS

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The most frequently found causative agents in systematic infections in ICUs are the bacterial infections; however, invasive fungal infections have emerged as major causes of morbidity and mortality among critically ill patients. Early detection and adequate treatment of infections are critical

for successful outcomes for patients with systemic infections. The use of PCR is significantly more sensitive than the use of conventional blood culturing techniques for the detection of bacteraemia and candidaemia in seriously ill patients. Application of blood cultures are time consuming and in the case of fungi often yield false-negative results due to low sensitivity. Our aim was to find a rapid molecular diagnostic tool to detect and to differentiate the most commonly encountered pathogen bacteria and fungi in intensive care units (ICU) with a single test procedure. Our idea was to separate these pathogens with the help of the melting point analysis. The identification and the differentiation took place in one real-time PCR reaction We used two specific primer pair to differentiate the fungal and bacterial amplicons by capillary Real-Time PCR**.** As regard to fungi it was easy to separate each species with melting point analysis due to the extreme variability of the amplified region. In order to detect and differentiate Gram-positive (G+) and Gram-negative (G-) bacteria we used a G+ and a G- specific F-RET probes which are the recommended probes to use with the LightCycler (LC) system. Thus we used the upper channels (640 and 705 nm) of the LC for the bacterial detection while the fungal detection happened on the lower one (580 nm). The melting point analysis following the amplification was appropriate to discriminate the 7 clinically most significant *Candida* species. We could also differentiate the G+ and G- bacteria in the same tube by the analysis of the melting temperature of the probes. We found an easy-to-perform and rapid diagnostic tool to detect the clinically most relevant *Candida* species, G+ and G- bacteria in a single tube. The sensitivity of the PCR reaction is suitable for the detection of the blood-stream infection. This method is not adaptable as a general identification method but useful when some of the fungal species are expectable from clinical samples. Our further aim is to improve the reliability of the reaction with internal control.

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## THE EXTENT OF FITNESS COST ASSOCIATED WITH RESISTANCE TO FLUOROQUINOLONES IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IS DIVERSE ACROSS STRAINS WHICH MAY GOVERN CAPACITY TO DISSEMINATE IN THE HOSPITAL SETTING AND COULD DETERMINE CLONAL DYNAMIC

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The factors responsible for the existence of clonality in methicillin-resistant *Staphylococcus aureus* (MRSA) remain obscure. The contribution of fluoroquinolone resistance to the emergence of MRSA clones has been investigated. Resistance to ciprofloxacin has been induced in strains of community-acquired (CA) MRSA and the associated fitness cost measured in a propagation assay. The fitness cost observed in the fluoroquinolone resistant mutants proved diverse and was more a function of mutations in the *gyrA, gyrB, grlA* and *grlB* genes than that of the MIC values. Silent mutations in the grlB gene profoundly impacted viability. In addition, the viability of two MRSA strains from the Hungarian clone (ST 239) was significantly more compromised by resistance to fluoroquinolones than that of isolates from the New York-Japan (ST5) and South-German (ST 228) clones. This could account for the sudden clonal shift observed in Hungary about ten years ago when strains of the Hungarian clone were almost completely replaced by those of the two big international clones. Moreover, two strains from the EMRSA-15 (ST22) clone showed less fitness cost than even some of

the New York-Japan and South-German strains which could explain the recent rise in the incidence of the EMRSA-15 clone in Hungary. We conclude that fitness cost associated with the development of resistance to fluoroquinolones exerts strong influence on the clonality of MRSA.

## CELL LENGTH GROWTH PATTERNS AND SIZE CONTROL IN FISSION YEAST MUTANTS

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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. Length growth patterns may therefore indicate connections between volume changes and cell cycle events. A size control acts in every cell cycle to keep the size of the cells constant in consecutive generations. With further analyses of growth patterns we are able to estimate the position of this size control. When a cdc mutant is shifted up to the restrictive temperature, division stops but growth continues and produces oversize cells. If the culture is then shifted down to the permissive temperature, the cells go through a series of rapid synchronous divisions as they slowly return to normal size. We refer to such a block and release experiment as induction synchrony (IS). 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 (RCP)) 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, like Akaike Information Criterion (AIC), which are suitable for comparing differently parameterised models. We have analysed the length growth pattern of ~470 fission yeast cells (60 wild type, 60 *wee1*Δ, 60 *cdc2-3w*, 60 *cdc2-3w cdc25*Δ double, 60 *cdc2-3w cdc25*Δ *pyp3*Δ triple mutants and 170 *cdc2-33* IS). 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 model 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 bilinear patterns, the slopes of the two linear segments were also compared with t-test. In wild type, *wee1*Δ and *cdc2-3w* mutant cultures, more than 2/3 of the cells showed a bilinear pattern, while in the case of *cdc2-3w cdc25*Δ double and *cdc2-3w cdc25*Δ *pyp3*Δ triple mutants, the ratio of bilinear cells was slightly above 50%, moreover in the case of the *cdc2-33* IS the ratio was only about 40%. In wild type and *wee1*Δ mutant cells, size control seems to act before the RCP, while in *cdc2-3w* mutant cells the size control seems to act both before and after RCP, furthermore in the double and the triple mutants, size control seems to act after the RCP. In the case of the *cdc2-33* IS strain we found that the smaller cells showed a size control up to a breakpoint whereas the larger cells lack such a size compensation. Studying the *cdc2-33* IS indicates that the larger cells have a tendency to grow linearly with a higher probability than smaller cells.

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## SESQUITERPENE EMISSION OF FUNGI BASED ON PURE CULTURE EXPERIMENTS

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The role of different biogenic sesquiterpenes (SQTs) in the formation of secondary organic aerosol is significant. In the present study, we investigated if the contribution of SQTs emitted by fungi is considerable compared to the total emission from vegetation. The importance of the question is stressed by the prevalence of fungi in the upper layers of soils. The SQT emission of the pure culture of eight fungal strains grown on malt extract agar was measured in an aseptic flow-through apparatus designed for solid phase microextraction in our laboratory. The tested fungal strains generally emitted more than twenty different molecules of SQTs; still 49 were done by *Trichoderma harzianum* Mt29. All of the tested strains emitted the secondary metabolite SQTs in a remarkable amount, 41 ng $h^{-1}$ $g^{-1}$ as the maximum by *Fusarium verticillioides* Zt05 and 1-2 ng $h^{-1}$ $g^{-1}$ as the minimal value by *Aspergillus versicolor* Lf43.
Since SQTs are secondary fungal metabolites and fungi live in soil in huge quantities, the in situ measurement of SQT emission of soils was essential. Although soil is a physically and chemically complex system and fungal mycelia are incorporated in colloid particles of the soil, we were able to demonstrate that SQTs can emanate from the soil directly to the atmosphere. Considering the mass of the experimental mycelia and the average level of natural fungal biomass in soil, we conclude that the amount of SQTs emitted by fungi can be comparable to that emitted by vegetation.

## THE ROLE OF *CANDIDA PARAPSILOSIS* SECRETED ASPARTYL PROTEASE 1 IN HOST-PATHOGEN INTERACTIONS

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Infections caused by *Candida* species manifest in a number of diseases, including candidemia, vulvovaginal candidiasis, endocarditis and peritonitis. *Candida albicans* and *Candida parapsilosis* are two of the leading *Candida* species causing infections worldwide. Candidal disease is facilitated through a number of virulence factors including adherence to host cells, biofilm formation and secretion of hydrolytic enzymes, such as proteases, phospholipases, and lipases. *Candida parapsilosis* has three secreted aspartyl proteinase genes: *SAPP1, SAPP2, SAPP3*, where *SAPP1* has the highest expression level upon infection. A duplicated region containing *SAPP1* ORF sequence was identified by our in silico analysis and *SAPP1a* and *SAPP1b* genes were distinguished. Systematic deletion of the two genes resulted a *SAPP1* minus deleted mutant. We examined the growth rate and pseudohyphal formation of the mutant strains and wlid type in different media and temperatures, and found no difference between them. Real-time PCR analysis revealed high overexpression level of *SAPP2* in *SAPP1* mutant strain in inducing medium, that can be due to a compensatory mechanism. The loss of *Sapp1p* and the elevated level of *Sapp2p* was confirmed using HPLC. To investigate host-pathogen interactions we applied infection systems using peripherial blood mononuclear cells (PBMC) and PBMC derived macrophages (PBMC-DM). We peformed killing experiments using

peripherial blood mononuclear cells (PBMC) and PBMC derived macrophages. Our experiments showed elevated killing efficiency in *SAPP1* mutant strain compared to the wild type. In addition, the damage of the host cells was decreased after co-cultivation with the mutant strain compared to the wild type. According to these results elevated phagocytotic level and increased number of phago-lysosomes could be observed in case of the *SAPP1* mutant strain. To investigate the effect of the complement system, we cultivated our strains in human serum with or without heat inactivation and CFU determination was performed after 8, 12, 24, 48 hours of incubation.
Our results showed decreased level of living cell number in case of *SAPP1* mutant strain cultivated in human serum without heat inactivation.Taken together, our results suggest, that *SAPP1* has an important role in virulence of *C. parapsilosis* infections.

## HIGH-THROUGHPUT SEQUENCING PROVIDES INSIGHTS INTO GENOMIC PLASTICITY AND PATHOGENICITY OF *PROPIONIBACTERIUM ACNES*

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*Propionibacterium acnes* (opportunistic anaerobic Gram-positive bacterium that forms part of the normal human cutaneous microbiota and is associated with several diseases such as acne vulgaris, corneal ulcers, endocarditis, sarcoidosis, synovitis, hyperostosis and osteitis (SAPHO) syndrome. Acne vulgaris is a chronic inflammatory disease of the pilosebaceous follicle, affecting >70% of adolescents and young adults. Inflammation in acne is associated, in part, with abnormal ductal colonization by phylogenetic clusters (IA, IB, II and III). Importantly, the majority of facial isolates belongs to IA and IB strains. In order to identify novel virulence factors, we have determined the genomes of >20 strains using next-generation sequencing (NGS) technology. Isolates, including non-acne derived strains were selected to represent all phylogenetic clusters and predominant sequence types. Our analysis highlighted numerous variations, such as SNP/MNPs and small indels, when comparing the genomes of strains belonging to different phylogenetic groups. In addition, we have determined numerous gene clusters that are uniquely characterizing phylogenetic groups and sequence types, which may possibly encode for pathogenicity islands. Notably, enzymes that are potentially involved in degrading skin-derived substances, such as lipases, proved highly variable.

## EVALUATION OF A POTENTIAL MYCORRHIZA INOCULUM ON SALT-AFFECTED CONTRASTING SOILS AND WHEAT HOST

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The using of commercial arbuscular mycorrhiza fungal (AMF) inoculums become more important nowadays. The fungi can provide beneficial effects mainly among environmental stress-conditions, such as the salinity. The mycorrhiza-wheat symbiosis was studied on three characteristic soil-types at lower and higher salt-stressed conditions by using a potential AMF product. Characteristic Hungarian

soils (calcareous chernozem, brown forest and meadow soil), with differences in several properties (pH, compaction, H%) were used in a pot experiment (V= 1kg) with common wheat (Tricium aestivum L.) in three replicates. The soils in the pots were inoculated with AMYKOR mycorrhizal product (contains: *Glomus intraradices)*. The plants were irrigated two times a week in three different doses (control, D1: 0,0%; D2: 0,5% and D3: 1,0% of salt-solution) until the end of the 11th week then the salt-solution was increased twofold. Harvest time the total amount of NaCl was 8750 kg/ha in the case of the 0,5% salt-solution and 17500 kg/ha in the case of the 1,0% salt-solution. The plant roots were stained with aniline blue and AMF colonization assessed by 5-class system of Trouvelot-method (1986). The input salt-solution increased the NaCl content all the soil-types, irrespectively from the soil characteristics. However the ratio accumulated salts was rather variable in the wheat biomass. $Na^+$ uptake of the wheat was multiplied by 37 at the first and 55 at the second salt level compare to the control on the brown forest soil. The mycorrhiza colonization (M%) increased of the D3 and especially D2 treated plants compare the control of the chernozem soil. The biggest M% values had the control of the brown forest soil and the D3 of the meadow. Mycorrhiza inoculums could provide further beneficial effect beyond the indigenous fungi mainly on the chernozem soil.

## INVOLVEMENT OF INTRA- AND EXTRACELLULAR BETA-GALACTOSIDASES IN LACTOSE CATABOLISM IN *PENICILLIUM CHRYSOGENUM*

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*Penicillium chrysogenum* is an economically important fungus used as industrial producer of penicillin. Apart from traits related to penicillin biosynthesis, general aspects of its carbon metabolism have received little attention. We investigated the catabolism of lactose, an abundant component of the diary residue whey that has been used extensively in pencillin fermentation, comparing the type strain NRRL 1951 as a wild-type reference with the industrial penicillin-producer ASP-78. Both strains grew similarly on lactose as the sole carbon source under batch conditions, consuming 15 g/L lactose in about 84 hours. The time-profile of sugar depletion concurred with the presence of both intra- and extracellular beta-1,4-D-galactosidase (bGal) activities, measured as ortho-nitrophenyl beta-D-galactopyranoside (ONPG) hydrolases. Upon growth on commonly occuring monosaccharides, such as D-glucose, D-fructose, D-xylose or D-galactose, as well as on glycerol, neither extra- nor intracellular bGal could be detected. However, L-arabinose induced ONPG-hydrolysing activity to about half the values measured with lactose-grown material. Similar results were obtained with mycelia pregrown on glycerol and transferred to fresh minimal medium with the various carbon sources tested. The measured bGal activities were similar for the two investigated strains. In silico analysis of the genome sequences revealed that *P. chrysogenum* features at least five putative bGal-encoding genes at the annotated loci *Pc22g14540, Pc12g11750, Pc16g12750, Pc14g01510* and *Pc06g00600*. The first two proteins appear to be orthologs of the Aspergillus nidulans intracellular family 2 glycosyl hydrolases specified by neighboring loci *AN3201* and *AN3200* that cluster with a lactose permease-encoding gene (*AN3199*). The latter three *P. chrysogenum* proteins feature an N-terminal secretion signal and appear distinct paralogs related to the extracellular bGal from *Aspergillus niger*, *LacA*, a family 35 glycosyl hydrolase. Transcript analysis of *Pc22g14540* and *Pc12g11750* showed that they were expressed exclusively in response to

lactose but completely repressed on the mixed growth substrate glucose/lactose. *Pc16g12750* was seemingly co-expressed with the two putative intracellular *bGal* genes, while its two paralog genes were apparently not transcribed under any condition tested. This expression profile is distinct from those in other ascomycetes, like *Trichoderma reesei* or *A. nidulans*, where *bGal* genes are induced by the monosaccharides D-galactose and/or L-arabinose. Our results indicate that the L-arabinose-induced ONPG-hydrolase observed in *P. chrysogenum* may correspond to a side activity of glycosyl hydrolases that are not classed in families 2 or 35. Nevertheless, it is likely that various of three transcribed *bGal* genes described here are involved in lactose catabolism in *P. chrysogenum*.

## CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE STRAINS IN HUNGARY - WHAT HAPPENED FROM THE FIRST ISOLATION IN 2008 TO OCTOBER 2010

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Carbapenem resistance among Enterobacteriaceae strains due to the production of carbapenemase enzyme is a growing clinical problem all over the world. Based on the mechanism, carbapenem-hydrolysing b-lactamases can be either serin-type b-lactamases (such as *Klebsiella pneumoniae* carbapenemases=KPCs) or metallo-b-lactamases (MBLs). Since the carbapenemase-producing strains usually show multidrug- or extensively drug-resistant phenotype, antibiotic therapy of infections caused by such strains is strongly limited. After the detection of the first carbapenemase-producing Enterobacteriaceae isolate in Hungary in October 2008, several carbapenem non-susceptible isolates were submitted to the National Center for Epidemiology to confirm the suspected carbapenemase production. The aim of our work was the complex characterization of these carbapenemase-producing Enterobacteriaceae isolates. The putative production of a carbapenemase was tested by modified Hodge test. The minimum inhibitory concentrations of carbapenems and other antibiotics used in therapy were determined by Etest. The presence of carbapenemase- and extended spectrum b-lactamase (ESBL) encoding genes was verified using PCR followed by sequencing. In some cases, the possible clonal relationships were investigated by pulsed field gel electrophoresis (PFGE) analysis and multilocus sequence typing (MLST). Between October 2008 and October 2010, we established 75 carbapenemase-producers among carbapenem non-susceptible isolates belonging to Enterobacteriaceae family. of these, 11 *Klebsiella pneumoniae* isolates produced KPC-enzyme, and 64 strains (40 *Enterobacter cloacae*, 19 *Serratia marcescens*, 2 *Citrobacter freundii*, 1-1 *K. pneumoniae, K. oxytoca* and *Escherichia coli*) produced VIM-type MBL. All the KPC-producing isolates and half of the MBL-producing isolates proved to be ESBL-producers, as well. Clonal relationship was proved between the 9 KPC-2-producers isolated in 2009. These strains belonged to the internationally successful KPC-producing ST258 *K. pneumoniae* clone. The KPC-producers

isolated in 2010 (n=2) appeared sporadically. The $bla_{VIM-4}$ gene detected among the MBL-producing isolates was part of a class-1 integron in all cases. In many isolates, the structure of the detected integron was found to be identical with an integron previously characterized in Hungary from *Pseudomonas aeruginosa* isolates and an *Aeromonas hydrophila* isolate. This might indicate the common origin of these integrons. It is important to emphasize that the VIM-4–producing *Enterobacter cloacae* isolates (n=35) caused an outbreak in a neonatal intensive care unit in Budapest in 2010, carried the same integron, as well.
Our results highlight the fact that carbapenemase-producing Enterobacteriaceae strains have emerged in our country. The increasing number of these strains demonstrates the need of a continuous surveillance and forceful activities of infection control in Hungary.

## FUNCTIONAL PROBIOTICS, SUPPLEMENTED WITH ANTIOXIDANTS

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Functional foods for specified health uses are produced for the maintenance of health in the digestive trackt and for the treatment of some metabolic diseases. Probiotic bacteria, for example Bifidobacteria and some intestinal Lactobacilli have positive health effects for many reasons. They are very useful in conseqence of the fermentation processes and metabolites, such as lactic acid and acetic acid. These compounds decrease the pH value in the ladge intestine and therefore reduce the number of harmful bacteria. Moreover, the probiotic species are able to produce bacteriocins against pathogen microbes. The aim of our experiments was to make probiotic yoghurts, based on lactose-hydrolysed milk and commersial cow's milk by *Bifidobacterium breve* and *Lactobacillus paracasei*. After the fermentation these probiotic yoghurts were completed with 10% of special jam, which was made from fruit of black elder. (This fruit is rich in polyphenol compounds, antocyans and vitamins.) New types of starters and this supplementation of yoghurts with special fruit jam, give a good possibility to produce new fermented milk products, which are rich in antioxidants.)The fermentation were run at 45°C until complete coagulation.The titritable acidity and viable cell count of probiotic bacteria were obtained during the fermentation. The organoleptic characters of new probiotic yoghurts were compared with each other by Kramer method.(The supplemented yoghurts were better, then thouse, which were made without supplementation.) It was given to differentiate the our samples from each-other and commersial yoghurts by measuring of an electric tongue.

## GENOTYPING OF HUNGARIAN FOWL ADENOVIRUS ISOLATES AND OTHER BIRD ADENOVIRUSES FROM CENTRAL EUROPE

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Typing of different avian adenoviruses (AdVs) was performed by PCR and sequencing. The subject viruses included fowl adenovirus (FAdV) strains isolated from poultry in Hungary recently, as well as AdVs found by PCR screening in randomly collected avian samples. In many cases, the assumed

relation between certain FAdV types and different disease manifestations in poultry is not convincingly proven. For example, in the gizzard erosion of chickens, FAdV-1 is detected almost invariably, but not every strain of this type will induce the disease. Wild birds are barely screened for the presence of AdVs thus the prevalence and the diversity of AdVs infecting wild birds are almost unknown. This makes the risk evaluation of potential new FAdV hazards unreliable. The classical serological typing of FAdVs is laborious, time consuming and requires the full collection of reference strains and antisera. Genetic typing by PCR and sequencing seems to be a straightforward alternative. While the criteria of species demarcation are well determined, the rules for type assignment are yet precarious especially because of the genetic variability of FAdVs. The samples originated from Hungarian diagnostic institutes, from the veterinary faculty in Budapest or from field veterinarians. PCRs targeting the gene of the viral DNA-dependent DNA polymerase or the hexon were conducted, and the resulting PCR products were sequenced. Distance matrix analyses of the newly determined nucleotide and deduced amino acid sequences were performed to reconstruct phylogenetic trees. The results revealed a stunning diversity of AdVs in wild birds. Putative AdVs representing three genera (Avi-, At- and Siadenovirus) were identified. The recent Hungarian FAdV isolates were most frequently typed as members of species Fowl adenovirus D or E. FAdV-1-like types (from species FAdV-A) were also found. However, the presence of a type belonging to species FAdV-B was demonstrated for the very first time in Hungary.

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## REDUCTION OF HIV INFECTIVITY IN VITRO BY MODIFYING CD4 BINDING SITE WITH THYOLATED NUCLEOTIDES

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The aim of this study was the analysis of binding properties of different HIV isolates, and evaluating the anti-HIV effect in vitro of a chemically modified thyolated pyrimidine nucleotides termed UDs and derivates. The initial molecular events of HIV infection, determined by gp120 and gp41 surface glycoproteins, as well as cellular receptors 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. HIV-1 glycoprotein genes were amplified and cloned into the eukaryotic expression vector pCI resulting in a set of env expression plasmids. The other proviral plasmids carry HIV gag-pol genes and firefly luciferase, Egfp or lacZ respectively as reporter genes. Pseudovirions were produced by transfecting both pEnv pGag plasmids (with reporter genes) into HEK293T cells. In transfected cells phenotypical mixtures of constructs (pseudoviruses) form, which are infectious but replication incompetent. To examine transfection efficiency at the time of pseudovirion harvest, expression of the reporter genes was observed upon luciferase activity or detecting eGFP expression. Human and animal cell lines, expressing CD4 and CCR5 or CXCR4 selectively or both, have been used for the determination of pseudovirion tropism. UD29 (Suligivir) compound in concentrations 2.5-40 microgram/ml were added prior and at the time of infection of cells with HIV pseudovirions (moi: 2). HIV p24 antigen expression was quantitatively determined by Vidas Elisa. Antiviral effect of UD29 were also tested in a syntitium assay on MT-2 lymphoid cell lines , and also in an in vitro MAGI (multinuclear activation galactosidase indicator) assay on

HeLaCD4+β-gal cells. Preliminary results with UD29 showed a prominent antiviral effect in the concentration range used.
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.

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## CHARLES JÁRMAI, TUMOUR VIRUS RESEARCHER, FORERUNNER TO INVENTION OF THE ENZYME REVERSE TRANSCRIPTASE

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*Ch. Jármai* (1887-1941), professor of pathology (1918-1941) at the Veterinary Faculty in Budapest (Hungary) developed his institute into a well-known tumour-virus research center. Along a series of investigations on *fowl leukosis*, he examined the filterability, size, infectiosity and antigenicity of the agent as well as the problems of susceptibility, resistance and immunity. He successfully propagated the virus in embryonated eggs and made a trial with tissue- (cover glass) culture, although without success. On the basis of his immunological and biochemical investigations he was convinced that the agent of fowl leukosis must be an *enzyme* (1931). After his theoretical analysis concerning the genesis of tumour-viruses he studied the *papillomatosis* of calf and tried to immunize the animals with phenol-vaccine against this disease.
At the same time by describing the occurrence of *infectious feline gastroenteritis*, he became the first who reported on the presence of *parvovirus* infections of cats in Hungary

## HOW TO KNOCKOUT GENES FROM A GENETICALLY INTRACTABLE OBLIGATE INTRACELLULAR PATHOGEN OF HUMANS: *CHLAMYDIA TRACHOMATIS*

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*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen that infects hundreds of millions of individuals globally causing blinding trachoma and sexually transmitted disease. More effective chlamydial control measures are needed but progress towards this end has been severely hampered by the lack of a tenable chlamydial genetic system. Here, we describe a reverse genetic approach to create isogenic *C. trachomatis* mutants. *C. trachomatis* was subjected to low-level ethyl methanesulfonate mutagenesis to generate chlamydiae that contained less then one mutation per genome. Mutagenized organisms were expanded in small sub-populations that were screened for mutations by digesting denatured and re-annealed PCR amplicons of the target gene with the mismatch specific endonuclease CEL I. Sub-populations with mutations were then sequenced for the

target region and plaque cloned if the desired mutation was detected. We demonstrate the utility of this approach by isolating a tryptophan synthase gene (*trpB*) null mutant that was otherwise isogenic to its parental clone as shown by *de novo* genome sequencing. The mutant was incapable of avoiding the anti-microbial effect of interferon-γ induced tryptophan starvation.
The ability to genetically manipulate chlamydiae is a major advancement that will enhance our understanding of chlamydial pathogenesis and accelerate the development of new anti-chlamydial therapeutic control measures. Additionally, this strategy could be applied to other medically important bacterial pathogens with no or difficult genetic systems.

## EFFECT OF ESSENTIAL OILS AND THEIR MAIN COMPONENTS ON BIOFILM FORMING ABILITY OF FOOD-RELATED MICROORGANISMS

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Biofilm forming on the surfaces of foods and food industrial equipment is a serious problem because of microbial films are more resistant to antimicrobial agents than planktonic cells. Biofilm forming is controlled by several environmental factors and cell-cell communication (quorum sensing, QS) may play also a role. Several essential oils (EOs) are known to have good antimicrobial properties. Since the cell membrane is the main target of EOs, it is possible that the damaged membrane lost its ability to attach on different surfaces, thus EOs are able to reduce biofilm formation. It was reported, that in some cases EOs are also able to inhibit QS. In our study, we investigated the anti-biofilm-forming and anti-quorum-sensing capacity of four EOs (clary sage, juniper, lemon and marjoram) and their main components (linalool, α-pinene, limonene and terpinen-4-ol). The inhibition of biofilm formation of Bacillus cereus and Pichia anomala was measured by the crystal violet staining method. Quorum sensing inhibition was determined by measuring the colourless zones of on petri plate spread *Chromobacterium violaceum*, developed around paper discs containing the investigated EOs and their main components. The colouration of the bacterium is under QS control. Essential oils reduced the biofilm formation of the bacterium and yeast at varying degrees. In the case of *B. cereus* best results was achieved with marjoram and lemon EO causing 71% and 61% reduction of biofilm formation. The highest biofilm reduction of *P. anomala* was shown by juniper and lemon at a degree of 51% and 32%, respectively.
All components showed better biofilm formation reduction capacity than the parent EOs, reaching as high as 99% reduction (linalool for *B. cereus*). In the QS inhibition experiments juniper and limonene had no effect, while lemon and α-pinene showed concentration-dependent inhibition of QS.

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## INCIDENCE OF HAEMOLYSIN GENES IN DIFFERENT PATHOGENIC *ESCHERICHIA COLI* ISOLATES OF HUMAN ORIGIN

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Haemolysins are virulence factors produced not only by *Escherichia coli*, but also by other taxa. *E. coli* strains can express alpha-haemolysin (HlyA), cryptic haemolysin (HlyE, SheA or ClyA), haemolysin F (HlyF), and two kinds of enterohaemolysins - one being similar to alpha-haemolysin (Ehx) and the other is associated with a temperate phage (Ehly).
In our study we investigated the occurrence of haemolysin genes in different pathogenic *Escherichia coli* strains. One hundred ExPEC, 60 ETEC, 80 EIEC, 72 ECOR, 40 EHEC, and 50 commensal faecal *E. coli* strains were examined for the presence of genes of alpha-hemolysin, cryptic haemolysin, haemolysin F and enterohaemolysins by PCR amplification. In the investigated isolates the occurrence of *hly*A and *hlyF* in ExPEC strains was significantly higher than in intestinal *E. coli* strains. *HlyA* was present in 2 EIEC, and in 2 normal faecal strains. *Ehx* gene was detected only in EHEC strains. *Ehly* was found only in 1 ETEC strain. *HlyF* gene is not present in EHEC, EIEC, and normal faecal strains but occurred in 1 ETEC strain and also in 2 ECOR strains. *ClyA* occurs significantly more frequently in intestinal strains. Our results suggest that haemolysin F similarly to alpha-haemolysin may play a selective role in ExPEC virulence.

## VIROCLIME – THE EFFECT OF CLIMATE CHANGE ON WATERBORNE VIRUSES

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VIROCLIME is a 3 year FP7 project funded by the European Union. It aims to assess the effect of climate change on the trasport and survival of human pathogen viruses in aquatic environments by applying hydrological models. The models use empirical viral prevalence data from 5 sites liable to climate change (extreme weather events) in Spain, Sweden, Greece, Brazil and Hungary. Surface waters and potential pollution sources (raw sewage and treated effluent) are monitored for potentially pathogenic viruses and feacal indicator bacteria in the 18-months surveillance case studies. The viral load of surface waters, the survival and seasonal variability of viruses is estimated. Hydrological and meteorological conditions are also recorded for model construction. The model will predict the alterations of virus titer and persistence as well as the associated risks as a result of climate change. In the first year of the project methods for detection and source tracking for viruses were developed and optimized. A direct flocculation method was implemented for the concentration of the water samples, which is more reproducible and more work - and cost efficient than the previous adsorption-elution methods. Nucleic acid extraction was performed using QIAamp Viral RNA kit. Real-time PCR for quantification of 3 human viruses (adenovirus, norovirus genogroup I and II) was optimized. Hungarian case study site is a section on Tisza between Szolnok and Tiszakécske. Samples are collected bi-weekly since January 2011. To date, adenoviruses were present in every river water sample ($10^3$-$10^5$ genome copies/L). The norovirus titers were in a wider range ($10^0$-$10^6$ genome copies/L). Viral titers varied with both the time and the location of the sampling. Waste water samples were less variable, raw sewage contained $10^6$-$10^7$ GC/L adenovirus and $10^5$-$10^8$ GC/L

norovirus. Sewage treatment reduced the viral titers by 1 to 2 log orders, it was more efficient for norovirus elimination probably due to their RNA genome. The fecal indicator counts showed the expected contamination levels, waste water treatment reduced the bacterial counts by 2-3 logs. There was no clear trend of correlation with the physico-chemical characteristics of the water or the meteorological conditions. Statistical analysis will reveal the associations with the environmental parameters upon the completion of the sampling.

## CHARACTERISATION OF *BORDETELLA BRONCHISEPTICA* STRAINS ISOLATED FROM PET ANIMALS

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*Bordetella bronchiseptica* is a widespread Gram-negative bacterium that causes respiratory tract infections of various mammals and humans as well. It is the etiological agent of atrophic rhinitis in pigs, kennel cough in dogs, bronchopneumonia in cats, rabbits, and guinea pigs. In many species *B. bronchiseptica* appears occasionally, the impact of zoonotic risk of this pathogen is remarkable. The incidence rate of The aim of our work was to investigate the prevalence of *flaA* (partially region of flagellin coding gene) and Nasal swabs were collected from dogs (68), rabbits (34), cats (4) and guinea pig (1) showing symptoms of upper respiratory tract infections. Swabs were inoculated on MacConkey agar, and suspect bacteria were sub-cultured on Columbia agar plates with 5% sheep blood. Since 2009, altogether 38 (10 from dogs, 10 from rabbits, 3 from guinea pigs, and 2 from cats). The isolates were identified in traditional biochemical tests (oxidase, catalase, urease, nitrate and indole tests, glucose, lactose and sucrose utilisation) and Virulence factor coding genes were analysed by PCR-RFLP (polymerase chain reaction followed restriction fragment length polymorphism analysis). For the HincII and cyaA analysis SalI restriction enzymes were used. All *flaA* gene, but the 2151 base pairs length PCR product of cyaA. Strains were grouped to three types (A, B, C) based on PCR-RFLP patterns on . All strains originated from dogs proved to be typed A while strains from cats and guinea pigs were classified as type C. The PCR-RFLP analysis of strains originated from rabbits showed them to belong to either type A or B (40% and 60%, respectively).

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## DEVELOPMENT OF A TAQMAN REAL-TIME RT-PCR ASSAY TO DIFFERENTIATE BETWEEN CLADES OF HIGHLY PATHOGENIC H5N1 VIRUSES CIRCULATING IN VIETNAM

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The highly pathogenic avian influenza (HPAI) H5N1 virus, which is panzootic in poultry, continues

to spread and poses a major challenge to animal and human health. The HPAI H5N1 can be divided into several clades based on phylogeny and nucleotide identity of the hemagglutinin (HA) gene. To date, the HAs of H5N1 viruses from at least seven clades (Clade 1, 2.3.2, 2.3.4, 3, 5, 7, 8) have been identified in Vietnam. Over the past three years, 4 clades have been characterized– Clade 1, 2.3.2, 2.3.4 and 7 – with clade 1 and 2.3.4 considered the dominant strains infecting poultry and humans in Vietnam. Although viral RNA sequencing and phylogenetic analysis are the gold standard for determination of HPAI H5N1 clades, many laboratories have limited ability to sequence viruses for clade-determination. We developed, therefore, a versatile, sensitive TaqMan-based real-time RT-PCR assay to differentiate between each of the four H5N1 clades known to be present in Vietnam. Our assay was validated after testing with circulating clades from Vietnam and other countries using two different commercially available real time RT-PCR kits and varying levels of RNA concentrations. All clade-specific primer/probe combinations were specific for viruses within the homologous clade and did not produce false positive results (a cycle threshold (Ct) value cutoff of 35 is recommended as the limit of detection). Reactions resulting in Ct values higher than 35 were repeated. The Ct value range using clade-specific 1, 2.3.2, 2.3.4 and 7 primers/probes were demonstrated to be on average the same as the reference CDC universal influenza A matrix gene real-time RT-PCR assay. Our assay will allow the user to predict the H5N1 clade of the virus in question prior to confirmatory HA gene sequencing. In addition, the results will allow the laboratory to obtain preliminary molecular epidemiological results pertaining to specific outbreak investigations and/or other surveillance activities. The assay is recommended for use only after the laboratory has obtained a positive influenza A matrix gene and H5 HA gene real time RT-PCR results; our assay is not a substitute for standard diagnostic testing. Finally, the results from this assay may be helpful to identify novel clades of H5N1 in Vietnam, since a positive H5 HA real-time assay with subsequent negative results using each of the four clade-specific primers/probes might indicate significant genetic drift of currently circulating viruses or emergence of a novel clade.

## THE ROLE OF XLR1 IN PENTOSE CATABOLISM AND PATHOGENICITY IN THE RICE BLAST FUNGUS *MAGNAPORTHE ORYZAE*

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*Magnaporthe oryzae* is a plant pathogenic ascomycete fungus that infects grass and cereal species. The plant cell wall of grasses consists of polysaccharides such as arabinoxylan. The fungus produces extracellular enzymes to degrade these polysaccharides and to release pentoses that can be used as a carbon source. So far, little is known about the regulation of pentose release and catabolism in *M. oryzae*. In *Aspergillus niger*, D-xylose release and utilization is controlled by the transcriptional activator XlnR. A deletion strain for the *M. oryzae* ortholog of *xlnR* (*xlr1*) was constructed to study the role of this regulator in *M. oryzae* during saprobic growth and infection of barley. Growth of the Δ*xlr1* strain was impaired on D-xylose and reduced on beechwood xylan. A reduction in mRNA levels of the pentose catabolic pathway (PCP) genes was observed in the Δ*xlr1* strain on D-xylose. These data indicate that Xlr1 is the transcriptional activator of the PCP during growth on D-xylose in *M. oryzae*. In addition we will present data that demonstrates a role for *Xlr1* in pathogenicity.

## EXAMINATION OF THE GENETIC BACKGROUND OF FUMONISIN AND OCHRATOXIN PRODUCTION IN *ASPERGILLUS NIGER* AND *A. AWAMORI*

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Fumonisins are carcinogenic mycotoxins which were originally identified in *Fusarium verticillioides*. According to recent findings, fumonisins are also produced by some black *Aspergillus* species including and are able to produce fumonisins in high quantities on agar media with low water activities. Raisins are excellent substrates for fumonisin production for black Aspergilli. On the other hand, these species are also able to produce another harmful mycotoxin, ochratoxins. The aim of our studies was to examine the diversity of some of the genes taking part in fumonisin and ochratoxin production of these fungi. The mycotoxin biosynthetic genes targeted included *FUM1* encoding an polyketide synthase and *FUM8* encoding an α-oxoamine synthase gene taking part on fumonisin biosynthesis, and a chloroperoxidase gene presumably taking part in ochratoxin biosynthesis in black Aspergilli. Primers were designed for regions of these genes, and the amplified products were subjected to sequence analysis. Results of the phylogenetic analyses revealed that none of these genomic regions could be used for distinguishing fumonisin or ochratoxin procuders from non-producers, since amplifications were successful in some mycotoxin nonproducing isolates too. Besides, there was no strict correlation between the phylogenetic trees based on sequences of partial calmodulin gene and either *FUM1* or *FUM8* sequences of black Aspergilli. Presumably balancing selection could be responsible for maintaining sequence polymorphisms within the fumonisin gene cluster in black Aspergilli, similarly to that observed previously in the trichothecene biosynthesis gene cluster of the *Fusarium graminearum* species complex. On the other hand, sequences of a chloroperoxidase gene were found to be useful for species delineation. Further studies are in progress involving more isolates and gene sequences (e.g. *FUM13, FUM19*) to prove these observations.

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## A CASE REPORT OF DISAPPEARING HEPATITIS B SURFACE ANTIGENEMIA (HBSAG) WITH PERSISTING DNAEMIA DURING ACUTE HEPATIC FAILURE TREATED BY PLASMAPHERESIS

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Here, we report a case of acute hepatic failure transferred from another hospital to our academic departmental ICU by the beginning of the weekend duty for plasmapheresis in agreement with the national hepatic transplantation center. We performed plasmapheresis with the transfusion of human plasma. Patient care in the preceding hospital included ordering hepatitis serology on a routine base, which meant that the results were not expected until the week after. Thus, after the initiation of the plaspapheresis, the duty service of the academic microbiology lab was asked to resolve whether Hepatitis B or C were involved. Although the technical validation of the HBsAg test revealed negative result somewhat below the cut-off value (0,11 RLU compared to 0,13 RLU), the presence of

anti-HBc antibodies raised the possibility of false negative result due to possible interference by plasma transfusion and consecutive plasmapheresis. Later, the lab results of the pre-treatment serum sample indeed verified a high level of HBsAg and an in-treatment serum sample revealed that HBV DNA was maintained. In conclusion, the loss of hepatic function probably suspended the production of progeny virions and envelopes, while the neutralizing antibodies transferred by plasma transfusion and the plasmapheretic treatment plausibly removed the circulating HBsAg.
This case highlights the impact of laboratory testing prior to treatment and the impact of HBV DNA test in case pre-treatment testing is not available.

## THE ANTIFUNGAL PROTEIN, PAF MAY INVOLVE IN THE PROGRAMMED CELL DEATH OF THE PRODUCER *PENICILLIUM CHRSOGENUM* FILAMENTOUS FUNGUS

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Apoptotic cell death is a vital process in multicellular organisms: it normally occurs during development and is associated with maintenance of cell homeostasis, elimination of damaged cells, response to infectious agents, aging and differentiation, as well as the adaptive responses of cells to biotic and abiotic stresses. Conditions that induce fungal apoptosis include various types of stress, such UV or oxidative stress, treatment with broad-spectrum elements such as salts and acids or challenge with specific compounds including antifungal agents. PAF produced by a filamentous fungus, Penicillium chrysogenum is a small molecular mass antifungal protein which triggers ROS mediated apoptotic cell death accompanied by hyperpolarization of the plasma membrane, phosphatidylserine externalization and accumulation of DNA-strand breaks in *Aspergillus nidulans* [1]. In this work we also managed to prove that PAF participates in the fine-tuning of cell death processes in the producer *P. chrysogenum* strain by detecting the exposure of phosphatidylserine on the protoplast surface using Annexin V assay. Deletion of the *paf* gene in this fungus resulted in decreased apoptotic rate compared to the wild type parental strain [2]. Furthermore, *P. chrysogenum* Δ*paf* protoplasts were sensitive to PAF treatment in contrast with the wild type protoplasts. Most likely, adaptation mechanisms to combat the cytotoxicity of PAF were not operating in the Δ*paf* cells. PAF may give its producing organism an ecological advantage when *P. chrysogenum* competes for nutrients with other microorganisms in its habitats.

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## INVESTIGATIONS ON THE HAEMOLYTIC ACTIVITY AND MATRIX PROTEIN BINDING CAPACITY OF ASYMPTOMATIC BACTERIURIA *ESCHERICHIA COLI* ISOLATES

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Asymptomatic bacteriuria (ABU) is a condition when a significant number of bacteria is present in the urine accompanied by no overt clinical symptoms. Predisposing factors like female gender, pregnancy, indwelling catheters, elderly and diabetes mellitus facilitate the development of this condition. Numerous papers have been published on the virulence factor status of ABU isolates including alpha-haemolysin (HlyA) but no information is available on haemolysin F (*HlyF*) and silent haemolysin (*SheA*) production, or on interactions of ABU strains with extracellular matrix proteins. In this report we give an account of our preliminary studies on the occurrencee of the above characters in seven Escherichia coli ABU isolates (six from diabetic children and one from a healthy adult). The genetic background for *HlyF* and *SheA* was searched for by PCR using specific primer pairs. Simultaneously the alpha-haemolytic (*HlyA*) status of the strains was also assessed. In the matrix protein binding experiments collagen types I and IV, fibronectin and laminin were applied. Bacterial attachment to matrix protein coated microtitre plates was evaluated by taking colony counts after detachment of adhered bacteria. Out of the seven strains four ones generated amplificates for *hlyF* and two ones for *sheA*, but none of them was positive for both. Three strains demonstrated the presence of *hlyA*, and two of them possessed also with *hlyF*. None of the strains harboured determinants for all the three types of haemolysins. The investigated strains presented with only a moderate skill to interact with matrix proteins. Only a single isolate exhibited a remarkable adhesive capacity to collagen type IV, and another one to fibronectin. These preliminary data show that ABU strains may possess with genes for such minor virulence factors like haemolysin F and silent haemolysin. Further studies will elucidate if these haemolysins appear phenotypically, and if they have a virulence role in animal models. Similarly to their known low cell attachment capacity the ABU *E. coli* strains seem to have a low affinity to attach to extracellular matrix compounds.
This attribute may also explain the observation that asymptomatic bacteriuria does not elicit appreciable inflammatory response in the host.

## INDUSTRIAL MICROBIOLOGY FOR THE PRODUCTION OF BIOHYDROGEN AND BIOGAS

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*Thiocapsa roseopersicina* BBS is a purple sulfur phototrophic bacterium belonging to the Chromatiaaceae family. Its natural habitat is the illuminated anaerobic zone of sea water, but survives in a variety of growth conditions. Five sets of [NiFe] hydrogenase structural genes have been identified in this bacterium, at least four of them code for active hydrogenases. The various [NiFe] hydrogenases differ in their intracellular localization, subunit composition, stability and catalytic properties. It is reasonable to assume distinct functions for each hydrogenase. The roles of the hydrogenases in the complex network of the bioenergetic processes have been studied using molecular biological, biochemical and physiological approaches. The genome sequencing of *T. roseopersicina* is at its last stage. The intricate relationship between hydrogenases and metabolic pathways are being mapped. *T. roseopersicina* has the capability to produce hydrogen in vivo both by its nitrogenase enzyme and using the Hox1 hydrogenase enzyme. Significant amount of hydrogen

production was achieved by this phototrophic strain using both systems after modification of the assembly apparatus of the hydrogenase enzymes. The results help in designing robust catalysts for biohydrogen production. The various hydrogenases link bioenergetic processes, such as nitrogen fixation, synthesis and utilization of storage materials, sulfur metabolism, light energy conversion in *T. roseopersicina*. Biogas is a renewable energy carrier and the production of biogas is associated with double benefits: elimination of environmental pollution problems is coupled with the generation of useful energy. The utilization of the digestion effluent as fertilizer for agricultural application facilitates nutrient recovery and eliminates the need for artificial fertilizers, the production of which is a highly energy demanding process.

Biogas technologies commonly apply natural anaerobic consortia of microbes. This is partly due to the fact that, from a microbiological aspect, this is a very complicated and complex system. Moreover, the population dynamics of the natural ecosystems could not be properly studied before the introduction of molecular biological techniques and high throughput sequencing methods. In natural ecosystems a very low partial pressure of $H_2$ is maintained, which may be a limiting factor for the methanogens. We demonstrated earlier that reductant accessibility is indeed a limiting factor in biogas production and presented data supporting the hypothesis that the introduction of $H_2$-producing bacteria into the natural biogas-generating consortium effectively increases biogas production both in batch fermentations and in a scale-up anaerobic digestor. Systematic, continuous experiments were conducted in 5 litre fermentors, constructed for biogas research on a laboratory scale. These devices model the real-life, large scale biogas production plants much better than the routinely used batch systems, and the first results are reported here. *Caldicellulosiruptor saccharolyticus* is a good $H_2$ producer and the beneficial effect of adding this strain to the biogas-producing system has been demonstrated. Anaerobic digestion of slaughterhouse waste presents a specific task being rich in proteins. Efficient degradation of blood containing samples were observed using a specially adapted microbiological consortium. Contrary to the findings published earlier ammonia did not inhibit the biogas process at concentrations lower than10 g N/dm$^3$.

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## SOIL DEPENDENT EFFICIENCY OF PLANT-MICROBE (WHEAT-MYCORRHIZA) INTERACTION AT INCREASING DOSES OF NITROGEN FERTILIZER

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Effect of high nitrogen fertilizer doses was studied in mycorrhiza inoculated soils on winter wheat (*Triticum aestivum lupus*) test plant in a greenhouse pot experiment. Our aims were to know, how the existing mutualistic connection between the host plants and the fungi will change by the applied fertilizer rates in three characteristic soil-types (the calcareous chernozem, the brown forest and the meadow). The soils were inoculated with AMYKOR commertialized microbial product, which contains propagules of *Glomus intraradices* mycorrhiza fungi. Different doses of $Ca(NO_3)_2$ were used such as the 0, 220, and 880 mg N in each pots, applied in the irrigation water twice a week.. Alltogether 11 treatments were used till the first sampling. After that, to the second sampling, the

applied nitrogen approximately double amounts, 420 mg N/pot (as 351 kg N/ha), and 1680 mg N/pot (as 1403 kg N/ha). Root samples were collected and stained by aniline-blue as it described by Trouvelot et al. (1986). Mycorrhizal colonization intensity (M%) and arbusculum richness (A%) were assessed. Significantly increased mycorrhizal colonization was found at the brown forest and meadow soils, but at the calcareous chernozem soil these values showed somehow a decreased tendency. More particularly at the highest N-rates, a reduced arbusculum richness was found, except the brown forest soil were a significantly higher A% values could be developed at the 1403 kg N/ha doses. Significantly increased N-content was found in the test plants, (DC25 phenological phase) grown in the forest soil (P=1%), and in the meadow soil (P=10%). Beside well-studied phosphorous nutrients, the nitrogen levels can be also crucial parameters in a soil-dependent way at mycorrhiza application.

## OCCURRENCE OF *TRICHODERMA PLEUROTICOLA* IN SHIITAKE CULTIVATION MATERIAL, *AGARICUS* COMPOST AND THE NATURAL SUBSTRATE OF WILD-GROWING *AGARICUS* SPECIES

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*T. pleuroticola* is a *Trichoderma* species from the Lixii-Catoptron clade of the genus *Trichoderma*, which – along with the genetically closely related species *T. pleurotum* – was found to be associated with the green mould infections in oyster mushroom production. *T. pleuroticola* has been isolated from oyster mushroom cultivation substrates in a series of countries including South Korea, Italy, Hungary and Romania, from soil and wood in Canada, the United States, Iran, New Zealand and Hungary, from growing substrates and surface of the basidiomes of wild growing oyster mushrooms as well as from the surface of insects collected in oyster mushroom growing facilities. During a large scale sampling project aimed at the investigation of the diversity of *Trichoderma* species associated with mushrooms, a total number of 150 *Trichoderma* strains were isolated from different mushroom-related artificial and natural habitats, including shiitake cultivation substrate from Hungary, *Agaricus* compost samples from Bosnia and Iran as well as the natural environment of wild-growing *Agaricus* species (*A. bisporus, A. bitorquis, A. praequalesquamosus* and *A. sylvicola*) collected in Hungary. The isolates were identified by the sequence analysis of the internal transcribed spacer (ITS) region with the aid of the barcoding program TrichOKey 2.0 (www.isth.info). The only species that could be isolated from the substrate sample derived from shiitake cultivation was *T. pleuroticola*. This is especially interesting as the sample derived from the experimental shiitake cultivation of an oyster mushroom farm where the most frequently isolated green mould agent in oyster mushroom cultivation is not *T. pleuroticola* but the closely related species *T. pleurotum*. In the examined *Agaricus* compost sample from Bosnia, the three species detected were *T. harzianum, T. atroviride* and *T. pleuroticola*. *T. pleuroticola* could be also isolated from 1 out of 5 examined *Agaricus* compost samples collected in Iran. Besides 9 other *Trichoderma* species (*T. atroviride, T. cerinum, T. citrinoviride, T. hamatum, T. harzianum, T. koningii, T. koningiopsis, T. virens* and *T. velutinum*) found to be present in the natural environment of wild-growing *Agaricus* species, *T. pleuroticola* could also be isolated from wild-growing *A. praeclaresquamosus*. *T. aggressivum* could not be found either in the examined compost samples or in the natural environment of wild-growing *Agaricus* spp. The results of this

study provide important data about the distribution of *T. pleuroticola*, and suggest in agreement with the results of previously performed dual culture experiments that this species is not just an oyster mushroom pathogenic green mould, but a mushroom-related *Trichoderma* species with a wide host range, which should therefore be considered as a general agent of mushroom green mold diseases.

## IN SILICO ANALYSIS OF FUNGAL PDR-TYPE ABC TRANSPORTERS

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ATP-binding cassette (ABC) transporters are integral membrane proteins which can mediate transport of natural toxic compounds over biological membranes. Typical ABC transporters of fungi contain two intracytoplasmic regions which both harbor nucleotide binding folds (NBF) and two hydrophobic regions, both with six transmembrane domains ($TMD_6$). Based on the topology of NBF and $TMD_6$, ABC transporters can be classified to pleiotropic drug resistance proteins (PDR, $[NBF\text{-}TMD_6]_2$), multidrug resistance proteins (MDR, $[TMD_6\text{-}NBF]_2$), half-sized transporters ($TMD_6$-NBF or NBF-$TMD_6$) that are The aim of this study was the Phytophthora species from the Oomycota. Within the Hypocreales, the 3 examined saprophytic and mycoparasitic *T. atroviride* and *Haematonectria* (*haematococca* could be characterized with a larger set (21) of these transporter proteins. One of the transporter genes of *Agaricus bisporus, Laccaria bicolor, Pleurotus ostreatus* and *Botrytis cinerea* BMR1 protein - which is playing a role in extruding iprobenfos and polyoxin - are present in both *P. ostreatus*. Half-sized PDR-type ABC transporters were also detected in a series of fungi, including *Venturia inaequalis* proved to be a half-sized transporter in contrast to the full sized orthologs in other Sordarialean fungi. Our results enable the functional prediction of a number of PDR-type ABC transporters based on their homologies with transporters of known function.

## SPATIAL AND SEASONAL DIVERSITY OF WATER MICROBIOTA OF LAKE HÉVÍZ REVEALED BY DENATURING GRADIENT GEL ELECTROPHORESIS

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Lake Hévíz is the largest thermal spa lake of Central Europe. The water of the lake originates from two crater springs with different temperatures (26°C and 41°C). Thanks to a special thermoregulatory mechanism, the water temperature never falls below 22°C. The lake water harbors particular bacterial communities which may contribute to the curative effect. The aim of the present study was to gain information about the horizontal, vertical and temporal variability of the aquatic bacterial communities. Water samples were collected in November 2009, April, July and October 2010. On the first occasion, samples were collected vertically per meter from the water surface to 29 m depth above the crater. In 2010 water samples were taken 1 m below the water surface at 14 different locations. Denaturing Gradient Gel Electrophoresis (DGGE) based on 16S rRNA gene was performed with Bacteria and Cyanobacteria specific primers.

According to the results, no differences were found in the structure of bacterial communities inhabiting the different water depths above the crater probably due to the strong stirring effect of the water stream. Comparing the bacterial and cyanobacterial communities taken at different sampling

times, seasonal and spatial alternations in microbial community structures were observed on the basis of the band numbers and intensities. In April and July bacterial communities from near the crater and from the shore could be separated from each other. The least spatial differences among the bacterial communities from different sampling sites were detected in October. DGGE patterns obtained by the analysis of Cyanobacteria showed that samples taken near the crater in July clearly separated from the others. Sequences, derived from the dominant DGGE bands, were affiliated with members of class α-, β- and γ-Proteobacteria and phylum Actinobacteria.

## SCREENING OF ZEARALENONE DEGRADING MICROBES BY YEAST OESTROGEN REPORTER SYSTEM *BLYES

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Zearalenone is produced by a number of species of *Fusarium.* The main concern is that zearalenone is a naturally occurring oestrogen that is well recognised as causing hormonal effects in humans and livestock animal. Zearalenone has been shown to occur in almost every agricultural product, grains, corn products, and in animal feed products. Thus new monitoring and elimination methods are essential. For toxin degradation several techniques exist, however, nowadays biodegradation process come into prominence. Nevertheless, biodegradation of zearalenone by microbes and their potential application may cause an unexpected risk as well. Biotransformation of zearalenone could produce harmful by-products necessitating the need for monitoring of potentially harmful effects. Biological effect monitoring of biotransformation processes have an increasing importance; especially since 2010, when the European Food Safety Authority (EFSA) reported that for substances for reduction of the contamination of feed by mycotoxins the combined effects of both the additive and the resulting metabolite(s)/degradation products(s) on the safety for the target animal needs to be examined in appropriate toxicological studies. For monitoring biodegradation of zearalenone, bioluminescence based yeast bioreporter system (BLYES) was adopted. In the BLYES system, presence of endocrine disrupting chemicals (EDC) result an increased bioluminescence of the tester strain. On the other hand the *BLYR tester strain monitors the cytotoxic effect of the tested sample, where decreased biolumescence demonstrated the associated cytotoxic effect. The aim of this study was to screen microbes for their zearalenone degrading potential, and to select microbes whose activities do not create toxic or endocrine disrupting metabolites. Bioluminescent based bioreporters were successfully adopted to monitor toxin degradation; moreover, the results of zearalenone biodegradation experiments were confirmed by parallel chemical analytical and ELISA tests. By the use of this complex evaluating system the most appropriate microbes with minimal toxic and oestrogenic cleave products could be selected. The most promising strains belong to *Streptomyces* and *Rhodococcus* genera. Our findings underline the necessity of such biotests, since remaining oestrogen effect may occur even after substantial toxin degradation.

We concluded that the adopted combined profiling method is cost effective, prompt and reliable tool for screening zealarenon degrading microbes. Moreover the use of this monitoring system would be appropriate for elucidating the metabolism of zearalenone (e.g. screening of thousands of clones originated from transposon and UV mutagenesis clone libraries).

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## TOTAL COLONYFORMING UNIT NUMBER IN CONNECTION WITH THERMOSTAT TIME AND TEMPERATURE

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In the pharmaceutical industry the pharmacopoeias guide is most important direction. For different tests the pharmacopoeias specify different time for thermostat time. In the everyday practice the test time is a common problem. The client needs the result in short time, but the test need much longer. Are there any difference between the 3rd day and the 5th day CFU results? We made tests for clear up the real connection between the growths of some different representative bacteria and fungi (by the European Pharmacopoeia) and the thermostat time for the real total microbiological contamination number (Robustness of the test for time). We applied other parameters according to Pharmacopoeia's in this examination. Are there any differences between the results when the temperature is in the Pharmacopoeia's tolerance and out of it? We made some representative tests for different temperature in and out of the Pharmacopoeia's tolerance (Robustness of the test for temperature). The aims of these challenges are to study the changes of CFU number by these parameters modification, to collect theoretical data. In the everyday practice we always use the Pharmacopoeia's prescriptions.

## SUSCEPTIBILITY OF THREE HUMAN PATHOGENIC *BIPOLARIS* SPECIES TO CURRENTLY USED ANTIFUNGAL AGENTS

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Members of the genus *Bipolaris* (Ascomycota, Euascomycetes, Pleosporales, Pleosporaceae) are imperfect, filamentous fungi (their perfect forms belong to the genus *Cochliobolus*). They are known as plant pathogenic organisms, which cause diseases mainly in gramineaceous hosts. Three closely related species, *B. australiensis, B. hawaiiensis and B. spicifera*, are also known as agents of human and animal phaeohyphomycoses (infections caused by melanin producing fungi). They are able to infect both immunocompetent and immunocompromised hosts; their clinical manifestations may include invasive sinusitis, keratitis, endophthalmitis, endocarditis, osteomyelitis and cutaneous and pulmonary infections. After *Fusarium* and *Aspergillus* species, these fungi are the third most common causes of ocular fungal infections, which are increasingly recognized as important causes of serious vision loss and blindness worldwide. Limited data are available on the antimicrobial susceptibility of human pathogenic *Bipolaris* species.

Because of the different sensitivity of the strains to the antifungal drugs, the selection of the adequate agent is important in the clinical practise. In this study, almost 40 isolate of human keratomycoses against currently used antifungal drugs, such as amphotericin B and different azoles (miconazole, ketoconazole, itraconazole, fluconazole, clotrimazole, and econazole) were examined in microdilution tests. Combinations of these antifungals with so called non-antifungal drugs were also tested by a checkerboard titration method. The minimal inhibitory contcentrations ($MIC_{90}$) were determined for all of the tested drugs. With the exception of fluconazole, all tested compounds proved to be effective

against *Bipolaris* species, but significant intraspecific variability was observed. The effective dosage of the antifungals could be reduced combining them with other drugs. Mostly, additive interactions were detected, but, in some cases, synergism was also observed.

## CARBON ASSIMILATION SPECTRUM OF HUMAN PATHOGENIC *BIPOLARIS* SPECIES

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The ascomycetous genus *Bipolaris* contains dematiaceous, filamentous fungi. They have economic importance as plant pathogens causing diseases of graminaceous hosts. Three species have also been reported from human and animal phaeohyphomycoses. *Bipolaris hawaiiensis, B. spicifera* and *B. australiensis* are able to cause infections in both immunocompetent and immunocompromised hosts with diverse clinical manifestations, such as invasive sinusitis, keratitis, endophthalmitis and endocarditis. The identification of the opportunistic pathogen species (based on the examination of the conidial and plate morphology, including colour, shape, size, numbers and distribution of conidial septa) has special diagnostic importance. However, identification of the species within the genus is relatively difficult due to the possible inconsistencies among the isolates, variable cultural conditions, or loss of ability to produce conidia. Therefore, it is important to give an exact description of the morphological and physiological features of the genus under different cultivation conditions. In this study, utilization of 64 compounds as sole carbon source were analyzed in conventional utilization test and rapid strip tests developed for clinical diagnosis (Api 50 CH, Biomérieux). The differently used compounds were identified and their effects on the morphologic features of the strains were recorded. Differences in the growth or in the morphology (shape, colour and size) among the studied strains were observed in case of 29 compounds. Within the involved species, high intraspecific variability was detected. Several carbon sources (such as D-galactose, D-lixose, protocatechuic acid and glycerine) proved to be suitable for the differentiation of these species. Based on our results, the carbon source assimilation tests can help in the precise identification of the *Bipolaris* isolates, thereby facilitating the selection of the appropriate treatment in the clinical practice.

## NEW OPPORTUNITIES FOR MYCOTOXIN MONITORING AND DEGRADATION BASED ON THE RESULTS OF THE MYCOSTOP RESEARCH PROGRAM

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Mycotoxin contaminations cause a worldwide problem, that is present in Hungary as well, including economic and health risk. The MYCOSTOP (NKTH-NTP/A3) project which started in 2009 has two

main focuses including the research and development of mycotoxin monitoring and mycotoxin biodegradation. In the monitoring part of the research the aflatoxin B1 (AFB1) production of Aspergillus strains isolated from grain cops at Hungarian sites was for the first time confirmed by immunochemical (ELISA) and analitical (HPLC-FLD, HPLC-MS) methods besides a genotoxicicity test (SOS-Chromotest). For monitoring biological effects of mycotoxins SOS-Chromotest that indicates genotoxicity, a cytotoxicity test that uses *Aliivibrio fischeri* as a test-organism, and a yeast based oestrogen-reporter system for measuring endocrine disrupting compounds were adopted. These biotests are not suited for direct toxin measurements; however, they seemed to be appropriate tools for analysing biodegradation experiments. Within the biodegradation research programme 215 microorganisms were identified by molecular taxonomic tools and their mycotoxin degrading capacity regarding five mycotoxins (Aflatoxin B1, Ochratoxin, Zearalenone, T2-toxin, Deoxinivalenol) was analysed. Among these microbes the degradation potential of members of the genera *Rhodococcus* and *Pseudomonas* was outstanding. By performing a combined toxicity profiling method three strains were successfully detected which could significantly eliminate AFB1 without forming genotoxic and/or cytotoxic metabolites. By using these strains the development of bio-filters suited to Zealarenone and AFB1 elimination is well-founded. *Rhodococcus pyridinivorans* strain AK37 significantly degraded AFB1 and Zearalenone. This strain was involved in a detoxification experiment that was adapted to the feeding of rats (Zearalenone) and broiler chickens (AFB1). Significant biodegradation of zearalenone was detected and the long term experiment demonstrating aflatoxin B1 degradation efficiency is still in progress. As *Rhodococcus pyridinivorans* AK37 was selected for practical applications de novo genome project of this strain was successfully carried out for facilitating research on AFB1 and Zearalenone metabolism.

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## ENTOMOPATHOGEN EFFICIENCY OF *BACILLUS THURINGIENSIS* TYPE STRAINS

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Among the entomopathogenic microorganisms the *Bacillus* genus has particular importance in the biological pest control. Numerous *B. thuringiensis* subspecies provide valuable source for the development of effective biopesticides against the larvae of certain pests causing considerable economic losses in both agricultural and horticultural production, e.g. Western corn rootworm.We have studied the larvicide effect of *B. thuringiensis* type strains potentially toxic for root damaging insect larvae in laboratory tests and determined their larvicidal spectrum. As type strains, *B. thuringiensis* NCAIM B.01292T (ref5), *B. thuringiensis* ssp. *kurstaki* NCAIM B.01262 (ref1), *B. thuringiensis* ssp. *israelensis* B.01289 (ref2), *B. thuringiensis* ssp. *kumamotoensis* DSMZ 6070 (ref3) and *B. thuringiensis* ssp. *kurstaki* ATCC 33679 (ref6) strains were used. Larvicidal effect was studied on the larvae of 3 insect species, Western corn rootworm (*Diabrotica virgifera virgifera*), mealworm (*Tenebrio molitor)* L1 and housefly (*Musca domestica*). In the first phase of efficiency studies we assessed the mortality of insect larvae treated with different degree dilutions of spore+toxin crystal preparations of type strains. In case of strains inducing larval mortality, the second phase aimed at distinguishing the factors influencing the entomotoxic effect (e.g. fermentation media) and

determining the time the toxic effect is manifested. The preparations isolated from strains ref1 and ref3 on 3 different fermentative media exerted a strong larvicide effect, inducing 100% mortality in housefly larvae. The effect of 16-, 32- and 64-fold dilutions of spore+toxin crystal preparations on mealworm was also studied, observing mean values of larval mortality between 0 and 30%. Strains ref1 and ref3 induced similar mortality indices, even at 64-fold dilutions. Analysis of the fermentation samples of these strains showed that mean larval mortality values were in negative correlation with the degree of dilution. The larvicide effect of certain type strains was also investigated via the histopathological examination of treated and deceased and non-treated mealworm larvae. Findings included an expressed damage in the small and large intestines of treated larvae, referring to the toxic effect of treatments. In case of western corn rootworm larve, either hatched from eggs, or collected, high mortality was experienced in the control experiments also, which questioned the reliability of experimental conclusions, urging the improvement of experimental design.

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## EFFECTS OF THE PRO/ALA SUBSTITUTIONS ON THE STRUCTURAL FEATURES OF INDOLICIDIN

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Indolicidin (H-ILPWKWPWWPWRR-$NH_2$) is an antimicrobial peptide (AMP), which was first purified from the cytoplasmic granules of bovine neutrophils. This AMP possesses a broad spectrum of antibacterial and antifungal activities, nevertheless, it exhibits hemolytic and antiviral effects too. Indolicidin contains three Pro amino acids, and in accordance with the cis-trans isomerism about the Xaa-Pro peptide bonds, this peptide exists as an equilibrium mixture of eight different stereoisomeric forms. The results obtained from our previous study revealed that the stereoisomers of indolicidin could be characterized by typical conformational features, pointing out that the Pro residues play an important role in the determination of the three-dimensional structure of this AMP. For the indolicidin analogs containing one or three Ala amino acids, other earlier studies led to the observation that the substitution of Pro amino acids by Ala residues induced changes in the antimicrobial activity of these peptides. Thus, the aim of this theoretical study was to investigate how the Ala-substitutions affect the structural and conformational properties of parent peptide. For the indolicidin derivatives, in which the Pro amino acids were systematically replaced by Ala residues, molecular dynamics (MD) simulations were performed. Due to the substitutions of Pro amino acids, the numbers of stereoisomers of the Ala-containing analogs were lower compared to that of indolicidin, and all stereoisomeric forms of these derivatives were modeled, respectively. Based on the MD calculations, the appearance of different conformational features was studied, and their alterations were examined as a function of time. In the case of each stereoisomer of indolicidin analogs containing one or more Ala amino acids, the presence of various secondary structural elements (i.e. β-turn and helical structures) was studied. Additionally, the occurrence of different stabilizing intramolecular interactions (i.e. H-bonds; aromatic-aromatic, proline-aromatic and cation $\pi$ interplays) was investigated. Data derived from the above-mentioned analyses of MD trajectories indicated that the stereoisomeric forms of the Ala-containing derivatives of indolicidin showed also characteristic conformational features. Comparing the results of present MD calculations, regarding the indolicidin analogs, with those of our previous MD simulations, concerning the parent peptide, it could be concluded that the Ala-substitutions affect the structural properties of indolicidin. All data

obtained by the MD calculations mentioned above revealed that the appearance of secondary structural elements and intramolecular interactions proved to be dependent not only on the cis-trans isomerism about the Xaa-Pro peptide bonds, but also on the Pro/Ala substitutions.

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## STUDYING THE CHARACTERISTIC CONFORMATIONAL PATTERNS OF TRITRPTICIN BY MOLECULAR DYNAMICS METHODS

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Tritrpticin is a Trp- and Arg-rich antimicrobial tridecapeptide, which possesses a remarkable primary structure with a high content of aromatic (i.e. **Trp** and **Phe**) and basic (i.e. Arg) amino acids (i.e. H-VRR**F**P**WWW**P**F**LRR-OH). This peptide was identified in the porcine bone marrow, and it shows a broad spectrum of antimicrobial activity against Gram(+) and Gram(-) bacteria, as well as fungi, however, it exhibits also hemolytic effect. Beside the aromatic and basic residues, tritrpticin contains two Pro amino acids, thus, four distinct stereoisomeric forms could be distinguished for this peptide, according to the cis-trans isomerism about the Xaa-Pro peptide bonds. In order to study the characteristic conformational patterns of tritrpticin, molecular dynamics (MD) calculations were carried out on its stereoisomers, applying the following two methods: simulated annealing-molecular dynamics, and MD simulations with random starting velocities. On the basis of these MD calculations, the appearance and alterations of different structural properties were investigated as a function of simulation time, and they were compared to one another, considering all stereoisomeric forms of tritrpticin. In the case of stereoisomers, the occurrence of various turn structures (i.e. types I, III and VI β-turns) was examined, and typical turn conformations were identified, which were found to be characteristic to certain tetrapeptide units of the sequences of stereoisomeric forms. Nevertheless, the presence of two types of intramolecular interactions (i.e. i←i+3 H-bonds and proline-aromatic interplays) was studied, which played a relevant role in determining and stabilizing the different turn structures mentioned above. Among them, the i←i+3 H-bonds evolved between the backbone NH donor and CO acceptor groups contributed to the structural stabilization of types I and III β-turns. The other type of interactions, namely, the proline-aromatic interplays formed between the neighboring Pro and aromatic residues, produced a stabilizing effect on the structure of type VI β-turns. The results obtained from the MD simulations indicated that the stereoisomeric forms of tritrpticin could be characterized by typical conformational patterns, with regard to their turn conformations, as well as to the appearing intramolecular interactions. Nevertheless, it is worthwhile to mention that both conformational similarities and dissimilarities could be detected, taking into account all the stereoisomers. On the whole, it could be concluded that the appearance of various structural and conformational features of the stereoisomeric forms depended on the cis or trans nature of two Xaa-Pro peptide bonds of tritrpticin.

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## PRODUCTION OF DGGS INDUCED PROTEASE ENZYME PRODUCING FERMENTATION CULTURES

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Dried distillers grains and solubles (DDGS), a byproduct of bioethanol production, has a relatively high fibre content, which limits its usability in animal feeding, usually it is fed only to ruminants. Increasing DDGS production has entailed the rising demand for a technology that would make it digestible for monogastric animals, primarily poultry [1]. DDGS contains approx. 30% crude protein, 11% crude fibre and 9% crude fat [1]. Compared to maize, the digestibility of proteins in DDGS is lower. During the drying process, a part of the proteins become indigestible due to the Maillard reaction occuring at high temperature, in which glucose forms an insoluble compound with certain amino acids. The amount of unavailable protein can be reduced with the use of protease producing microorganisms. Yeast (*Pichia stipitis*), a filamentous fungus (*Thermomyces lanuginosus*) and a Gram positive bacterial species (*Bacillus licheniformis*) were cultured on media containing low quality, dark brown DDGS in different ratios (4%, 8% and 16%). The cultures were incubated for 24 and 48 hours, respectively. The supernatant of samples were then measured for protease activity. In the following, the strains were cultured on their optimal culture media, supplemented with 4% DDGS. The cultures were incubated for 24 hours at optimal temperature, then the protease activity of the supernatant was determined. Results indicate that DDGS is an appropriate source of nutrients for all of the microbial strains used in the experiment. Enzyme production reached its maximum after 24 hours of incubation. Highest protease activity was measured in *Thermomyces lanuginosus* and *Pichia stipitis* cultures containing 16% DDGS (1.12 U/ml and 0.96 U/ml, respectively). DDGS supplementation of the optimal culture media did not increase protease activity significantly.

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## BIOCONVERSION OF WDG VIA SEMI-SOLID ANAEROBIC FERMENTATION BY LACTOBACILLI (*LACTOBACILLUS LACTIS*), YEASTS (*PICHIA STIPITIS, SACCHAROMYCES CEREVISIAE*) AND FILAMENTOUS FUNGI (*THERMOMYCES LANUGINOSUS*)

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The use of wet distillers grains, the byproduct of – usually corn-based – bioethanol production, in animal feeding is limited. Due to its high moisture content and relatively high pH-value (pH=5), the shelf life of WDG is short and its high fibre content can cause digestive disorders. Via microbial bioconversion, the fibre content can be reduced and by inducing fermentation the pH value can be lowered to increase shelf life. In our experiments, wet distillers grains was exposed to fermentation by various microorganisms in anaerobic conditions on non-sterile semi-solid culture media. A water activity reducing substance (ALM) was mixed to the substrate in a 20% rate. The media was then inoculated with selected and own-developed Anareobic cultures were incubated for 8 weeks at room temperature. Samples were examined for crude fat, crude protein and crude fibre content, the proportions of fibre fractions and protease enzyme activiy. All inoculated microbial strains were successfully reisolated from semi-solid cultures at appropriate cell counts. In the cultures inoculated

with *P. stipitis* and the combination of the four strains, respectively, fermentation had started, resulting in acetic acid production, as confirmed by the acidification of cultures (pH=4.5). The highest protease activity values were also linked to these cultures (1.92 U/g). Inoculation with yeasts, filamentous fungi or bacterial strains did not reduce the protein content of the distillers grains substrate. Inoculation with the combination of *P. stipitis* - *S. cerevisiae* - *L. lactis*, and *T. lanuginosus* - *L. lactis*, respectively, has decreased the proportion of fibre fractions. In the cultures inoculated with *P. stipitis*, crude fibre content decreased by 13%, and the proportions of NDF, ADF and ADL fractions have decreased by 30%, 18% and 18%, respectively. The combination of *T. lanuginosus* and *L. lactis* have reduced the amount of crude fibre by 16%, meaning 18%, 21% and 22% reduction in the proportion of NDF, ADF and ADL fractions, respectively. Based on the rate of fibre degradation, the decrease in the cellulose and hemicellulose content could be calculated. In the cultures inoculated with *P. stipitis* the cellulose content decreased by 18% and hemicellulose content decreased by 42%. The combination of *S. cerevisiae* and *L. lactis* has reduced cellulose and hemicellulose content by 28% and 30% respectively. In *T. lanuginosus* - *L. lactis* cultures a 21% and 14% decrease was observed in the cellulose and hemicellulose content, respectively. The WDG cultures inoculated with the combination of all four microbial strains could be stored for more than 3 months without any signs of decomposition and used in animal feeding.

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## EVALUATION OF EFFECT OF SECRETED ASPARTYL PROTEINASE 2 FROM *CANDIDA ALBICANS* ON MACROPHAGES ACTIVITY AGAINST BLASTOCONIDIA IN-VITRO

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*Candida albicans* is a fungus that alters into a pathogenic organism under appropriate conditions and results in fungal infection. Secreted aspartic proteinase (Sap) activity is considered an important virulence factor in such infections. We examined the in vitro effect of Sap2 *Candida albicans* on NO production by murine peritoneal macrophages. The methylotrophic yeast *Pichia pastoris* was chosen as an expression system for preparing substantial amounts of Sap2 isoenzyme (the most important Sap in pathogeneses). In this study, Sap2 was produced as a high-level expression and active recombinant enzyme without any post-translation change. Then, the effect of the produced protein on macrophage activity (ingestion, Nitric oxide release) was evaluated against blastoconidia in Balb/c macrophages. In order to examine the effect of Sap2 on macrophage ingestion, $10^6$ log-phase candida blastoconidia were added to $2\times10^5$ mouse peritoneal macrophages. To swallowing assessment, the supernatant from macrophage lysate inoculated on SCC medium and colonies were counted. The result of candida colony number showed that the ingestion of macrophage treatment with Sap2 was 68% less than that of the negative control (group untreated with Sap2). We examined the in vitro effect of *Candida albicans* Sap2 on NO production by macrophages. Nitric oxide assay was also performed using the grass method. Sap2 of *Candida albicans* also inhibited NO production. Our results suggest that Sap2 may evade host defense mechanism(s) through NO production suppression—mediated mechanism of stimulated macrophages.

The present work assessed the ability of Sap2 to inhibit the macrophage in response to the ingestion and NO production of *C. albicans* blastoconidia.

## GENOTOXICOLOGICAL ASPECTS OF UV-INDUCED PHOTOLYSIS AND ITS COMBINATION WITH OZONATION IN THE TRANSFORMATION OF PHENYLUREA HERBICIDES, ANALYZED BY AMES TEST

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The Ames genotoxicity test employs several histidine auxotrophic strains of *Salmonella typhimurium*, which have been selected on the bases of their sensitivity to distinct types of mutagens. Theses reverse mutation tests are performed by mixing the test substance solution and the tester strain together in a rich liquid medium, which contains only small amounts of histidine. Histidine permits the inoculated test organism to undergo a limited number of divisions, but it is insufficient to permit normal growth. However, if the strain undergoes a reverse mutation, (spontaneous, or induced by the test substance or a positive control material) the organism no longer requires histidine to grow and can produce visible revertant colonies after spreading the treated cell suspension on the surface of minimal medium. In our investigation, we observed the usefulness of a simple Ames test system without S-9 metabolic activation for the genotoxicological measurement of water samples containing distinct phenylurea herbicides before and after UV-light induced photolysis treatments. The tester strains were selected on that manner, that they allow the detection of various types of mutagens. The tester *Salmonella typhimurium* strains employed were TA98, TA1537 and TA1535. Strains TA98 and TA1537 detect mutagens responsible for frameshift mutations, while TA1535 detects compounds causing base substitution mutations. Forty six herbicide containing samples, which were treated with UV-light, in the presence or in the absence of oxygen or ozone, were investigated for genotoxicity with the four Ames tester Salmonella strains. In the case of diuron and fenuron, frequently intensive positive results were obtained with the frameshift mutation detecting strains.

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## IDENTIFICATION AND CHARACTERIZATION OF RED YEAST STRAINS USING SOME MOLECULAR TECHNIQUES

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Red yeasts are known by their unusually composed and rigid cell wall which makes cell dissintegration and intracellular molecule isolation extremely difficult. Thus, characterization of red yeast genome and identification of these strains by molecular methods is complicated by problems with DNA isolation. In this work non-isolation DGGE analysis (denaturation gradient gel

electrophoresis) of red yeast DNA was compared with karyotype analysis by PFGE (pulsed field gel electrophoresis). Six red yeast strains (*Sporobolomyces roseus, Rhodotorula glutinis, Rhodotorula mucilaginosa, Rhodotorula aurantiaca, Cystofilobasidium capitatum*) were enrolled into this comparative study. Cells were cultivated at 28°C at permanent shaking and lighting for 50 and 80 hours. After cell collection yeast DNA for DGGE analysis was isolated using Ultraclean Microbial DNA Kit (Quiagen). Genes of 26S ribosome subunit were amplified using nested PCR with NL1 and NL4 primers in 1st step and LS2 and reverse NL1 primers in 2nd step. PCR conditions were as follows: 1st PCR - initial denaturation 95°C 1 min, 30 cycles – denaturation 95°C 30 s, annealing 55-68°C 30 s, extension 72°C 60 s, cooling 4-10°C hold; 2nd PCR – initial denaturation 95°C, 5 min, 30 cycles – denaturation 95°C 1 min, primer annealing 52-65°C 2 min, extension 72°C 2 min. PCR products of 1st PCR (about 600 bp) and 2nd PCR (final products – about 280 bp) were analyzed using 1.8% agarose electrophoresis (80 V, 3 hours, TAE buffer). DGGE was performed in 8% polyacrylamide gel (19:1), denaturation gradient 30 – 45% was used for red yeast strains, 30 – 50% for real samples. Separation was performed at 120 V and 60°C for 4 hours. Visualisation was done by silver staining. PFGE analysis was performed using DNA isolated by specific procedure in presence of low melting agarose. Yeast cells were disrupted by combination of enzyme (lyticase, proteinase K), detergent (SDS) and mechanical lysis (glass beads, 100 um). PFGE was performed in 1% agarose gel and 0.08 mol/L TBE buffer. Pulses were set as follows: 150 s /15 hours, 200 s/20 hours, 250 s/20 hours and 300 s/ 20 hours. Yeast DNA was visualized by ethidium bromide. Karyotypes obtained by PFGE exhibited characteristic patterns and enabled yeast identification according to genus and species. Substantially more similar chromosome sets to *Rhodotorula* were obtained in *Cystofilobasidium* samples than in all *Sporobolomyces* strains.
PFGE is suitable to identification of red yeasts in pure single culture. DGGE patterns of all red yeast strains were very similar, no identification according to genus was possible, better resolution was obtained in 30 - 45% gradient. DGGE can be used mainly for group identification of red yeasts in mixed natural samples and in special environments.

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## MELANOMA CELL DERIVED EXOSOMES TRANSFORM IMMUN CELL FUNCTIONS IN VITRO

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Previous studies showed that tumor cells release microvesicles (exosomes) with a size of 20-100 nm that contain molecular markers characteristic of their plasma membranes. Because exosomes carry surface proteins associated with antigen presentation and apoptosis, they may affect anti-tumoral immune responses. However, the immunomodulatory functions of tumor cell derived exosomes are poorly understood. Both activatory and inhibitory effects were reported depending on the exosome phenotype and tumor stage. In the present study we demonstrate that B16F1 melanoma cell derived exosomes (mcd-exosomes) affect immune cell functions in vitro. We found that bone marrow derived

dendritic cells exposed to mcd-exosomes enhanced CD4+ T cell proliferation in an allogeneic system. In addition we detected that mature macrophages stimulated with mcd-exosomes exhibited an increased level of NF-κB activity, suggesting that mcd-exosomes activate the immune system. However exosome-treated macrophages secreted lower amounts of inflammatory cytokines and chemokines to mediating anti-cancer immune responses. This study illustrates that soluble and cellular responses to exosomes can lead to opposing immune status. We also found that mcd-exosomes carry many miRNAs. Our findings suggest that the tumor derived exosomes could contribute to tumor escape using different pathways.

## HEPATOCYTE-TARGETED TRANSGENE EXPRESSION BY INTEGRASE-DEFECTIVE LENTIVIRAL VECTORS INDUCES ANTIGEN-SPECIFIC IMMUNE TOLERANCE IN MICE WITH LOW GENOTOXIC RISK.

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Integrating lentiviral vectors are attractive tools for liver-directed gene therapy [1, 2] because of their capacity for stable gene expression and the lack of preexisting immunity in most human subjects. However, the use of integrating vectors may raise some concerns about the potential risk of insertional mutagenesis [3]. In the present work we investigated liver gene transfer using integrase-defective lentiviral vectors (IDLVs) [4] in conjunction with miRNA-mediated post-transcriptional regulation [5]. Hepatocyte-targeted expression using these IDLVs resulted in sustained and robust induction of immune tolerance to both intracellular (GFP) and secreted proteins (coagulation factor IX, FIX), despite the reduced transgene expression levels in comparison with the non-mutated, integrase-competent vector. IDLV-mediated and hepatocyte-targeted FIX expression prevented the induction of neutralizing antibodies to FIX even after antigen rechallenge in hemophilia B mice and accounted for relatively prolonged, 6 months, therapeutic FIX expression levels. Upon the delivery of intracellular model antigens, hepatocyte-targeted IDLVs induced transgene-specific regulatory T cells that contributed to the observed immune tolerance. Deep sequencing of IDLV-transduced livers showed only rare genomic integrations that had no preference for gene coding regions and occurred mostly by a mechanism inconsistent with residual integrase activity. Conclusion: IDLVs provide an attractive platform for the tolerogenic expression of intracellular or secreted proteins in the liver with a substantially reduced risk of insertional mutagenesis.

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## THE LATENT AND INHERITED HUMAN HERPESVIRUS-6A AND 6B IS A POSSIBLE CAUSE OF A SUBGROUP OF CHRONIC FATIGUE SYNDROME CASES

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Previous research by our lab and others has definitively illustrated the integration of human herpesvirus-6 (HHV-6) into host cell chromosomes *in vitro* and *in vivo*, and the viral genome can be inherited through the germ line (iHHV-6). Amplification and sequencing of the HHV-6A and more recently HHV-6B viral-chromosome junction identified that the right direct repeat ($DR_R$) integrated into the telomere directly adjacent to the subtelomere of the chromosome. A series of mapping data show the presence of tandem array of telomere repeats [$(TTAGGG)_n$] at the end of the virus-invaded chromosome. Therefore, the structure of the latent HHV-6 is as follows: chromosome-subtelomere-$(TTAGGG)_{5-41}$-$DR_R$-$U_L$-$DR_L$-$(TTAGGG)_n$. Here we also show the induction of integrated latent HHV-6 with trichostatin-A or TPA lead to the excision of the integrated genome and rolling-circle replication and concatamer formation. Reactivation from the peripheral blood of an iHHV-6 Chronic Fatigue Syndrome patient also resulted in the production of an infectious, replication competent virus. Sequencing results indicate that the reactivated virus is colinear to, but distinctly different from that of the prototype U1102 strain. There is a high level of sequence conservation in the central unique region of the reactivated HHV-6 genome, while significant variability is noted within the direct repeats. These data provide further evidence that iHHV-6 can reactivated from its integrated form. Taken together, the data suggest that HHV-6 is unique among human herpesviruses: it specifically and efficiently integrates into telomeres of chromosomes during latency, while at the same time the chromosome's telomere remains stably intact during viral integration. Finally, this is the first demonstration of reactivation of inherited integrated viral genome from cells of a patient suffering from Chronic Fatigue Syndrome raising the possibility that iHHV-6 is one of the possible underlying causes of this debilitating disease.

## DOMINANCY OF N/ST15 PULSOTYPE OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES IN THE UNIVERSITY HOSPITAL OF PÉCS BETWEEN 2004-2009

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*K. pneumoniae* is one of the most common nosocomial Gram-negative pathogens. The increasing prevalence of ESBL production and the appearance of carbapenemase genes among *K. pneumoniae* strains is a major health concern. In our previous work we revealed the presence of one large, genetically closely related cluster of ESBL positive K. pneumoniae strains in the University Hospital

of Pécs in the investigated period (2004-2009). Aim of the present study was to identify any underlying microbial characteristic that could be associated with the dominancy of this cluster. According to PFGE 68% (71/104) of the strains belonged to the N/ST15 pulsotype. Three further clonal groups have been identified: R/ST147 (5/104, 4,8%) and two smaller local ones (Pécs-I 10/104, 9,6% and Pécs-II 4/104 3,8%). The antimicrobial suspectibility testing showed the majority of the strains being resistant to gentamycin (86,5%), ciprofloxacin (90,4%) chloramphenicol (80,8%) and tetracycline (75,7%) while resistance against amikacin (10,6%) and trimethoprim/sulfamethoxazole (24%) remained relatively low. All of the strains belonging to the N pulsotype were resistant to ciprofloxacin (71/71 100%), and also larger portion of them were not susceptible to chloramphenicol (69/71 97,2%) and tetracycline (56/71 78,9%) than the non-N isolates (5/33 15,2% and 9/33 27,3%). We have identified one strain belonging to N/ST15 pulsotype being resistant to imipenem. In this case the presence of VIM carbapenemase on type 1 integron has been confirmed with PCR. Screening for integrons was performed with multiplex PCR. There have been significantly ($\chi^2$, $p<0,05$) more non-N pulsotypes (25/33 75,8%) isolates carrying type 1 integrase gene than N pulsotypes (5/71 7%). There was one strain harbouring type 2 integrase located on a 9,435 MDa large plasmid. Regarding the virulence determinants, significantly ($\chi^2$, $p<0,05$) more isolates belonging to the N pulsotype were positive for enterobactin (69/71 97,2%), biofilm (69/71 97,2%) and type 3 fimbria (65/71 95,8%) production than non-N pulsotype strains (25/33 75,8%; 27/33 81,8%; 16/33 48,5%). There was no significant difference in the aspects of hypermucoviscosity phenotype, rmpA, magA, k2A and aerobactin production. This study has shown the advantage of N/ST15 pulsotype over the other ESBL producing *K. pneumoniae* strains in the case of biofilm, type 3 fimbria, enterobactin production and antibiotic resistance, although the prevalence of type I integrase was lower in this group. It is likely that not only microbial features itself are responsible for the dominance of the N/ST15 pulsotype, environmental factors may also have impact on the spreading of ESBL positive *K. pneumoniae* strains.

## MOLECULAR DETECTION AND TYPING OF *TREPONEMA PALLIDUM* SSP. *PALLIDUM* IN CLINICAL SAMPLES BASED ON SEQUENCING OF *TP0136*, *TP0548* AND 23S rRNA GENES

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Syphilis, caused by the spirochete *Treponema pallidum* subsp. *pallidum* (TPA), is a sexually transmitted infectious disease with worldwide occurrence. The bacterium TPA cannot be cultivated in vitro, therefore laboratory diagnostics is traditionally made by microscopy and serology testing, which do not provide epidemiological data. Molecular detection of treponemes could type the clinical strains and provide the antibiotic susceptibility information.

During the years 2004-2010, 294 patients (415 samples) were tested for the presence of treponemal DNA. Primary screening of clinical specimens included nested PCR detection of two TPA specific loci (tmpC and polA genes). Out of 294 patients, 91 patients were PCR positive. PCR positive patients were more often in the primary stage of syphilis ($p=0.0003$) compared to the control (PCR negative) group of syphilis seropositive patients. Treponemal DNA was detected in 4 types of clinical material: genitoanal, pharyngeal and skin swabs (75 samples), whole blood samples (42),

cerebrospinal fluid (1) and blood serum (1). Molecular typing method was based on amplification and sequencing of two sequentially variable genes including TP0136 and TP0548 together with the 23S rRNA gene, where the mutation in position A2058G or A2059G causes the macrolide resistance. Out of 91 patients with PCR positive samples, 49 patients were completely typed (sequences of TP0136, TP0548 and 23S rRNA genes were determined), 15 patients were partially typed (only two out of 3 loci were detected). Nine different genotypes among the 64 completely or partially typed patients were found. 35.6% of treponemal strains were resistant to macrolide antibiotics. Identified subtypes of TPA strains were further typed with the CDC typing system for TPA treponemes (comprising analysis of the arp and tpr genes). The obtained unique TP0136 and TP0548 sequences were found to combine independently with CDC subtypes indicating their potential for more detailed genetic characterization of TPA-containing clinical samples.

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## THE TRANSCRIPTIONAL PATTERN OF *CHLAMYDIA MURIDARUM*'S PLASMID GENES

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The Chlamydiae is a phylum of intracellular bacteria with unique phylogenetics. *Chlamydia trachomatis* (*C. trachomatis*) is the major human etiological agent of several diseases with importance in public health including trachoma and a variety of sexually transmitted diseases which have marked risks for blindness and infertility, respectively. *Chlamydia muridarum* (*C. muridarum*) is a mouse-adapted species, its genome exhibits high similarity to the *C. trachomatis* genome in terms of gene content and chromosomal synteny. Infection of mice with *C. muridarum* provides a useful model of the *C. trachomatis* infection in humans. A fundamental ambiguity of *C. muridarum* biology is association of a cryptic 7.5-kb plasmid of unknown function. The strong selection to maintain the plasmid by Chlamydia strains implies its importance in the pathogenesis of infection or disease. Our aim was to describe the transcriptional pattern of *C. muridarum*'s plasmid genes in *C. muridarum* infected mice. During our *in vivo* experiment BALB/C female mice were inoculated intranasally with $1x10^3$ IFU/mouse *C. muridarum*. Mice were sacrificed at different time points after inoculation. Lungs were removed, homogenized and total RNA was extracted from half of the lungs to determine the expression of different plasmid genes by RT-qPCR. The supernatants of the other part of the lung homogenates were subjected to quantitation of recoverable *C. muridarum*. Serial dilutions of the lung supernatants were placed onto HeLa monolayers and after a 48-h culture, the cells were fixed and stained with monoclonal anti-Chlamydia LPS antibody and FITC-labeled anti-mouse IgG. The number of *C. muridarum* inclusions were counted under UV microscope, and the titer was expressed as IFU/ml. To investigate the expression of *C. muridarum* plasmid genes *in vitro*, different mouse cell lines (macrophage-like J774A, mouse fibroblast L929 and epithelial BM-12) were infected with *C. muridarum* and total RNA was extracted from the samples and the expression of different plasmid genes was analyzed. In our experiment the infectious bacterial titers increased to color:black;mso-fareast language:HU">7,99x10$^3$ IFU/lung by day one. The peak titer of *C. muridarum* was at $5,08x10^5$ IFU/lung on day 7, and the titer decreased to $2,60x10^5$ IFU/lung on day 14 after infection. The *C. muridarum*-specific IgG antibody was tested in indirect immunfluorescence test. The *C.*

*muridarum*-specific antibody titer was the highest 2 weeks after infection and declined slowly by week 4 and 8. The increased expression of different plasmid genes was observed on day 7. Later the expression of the individual genes showed different kinetics. The expression of the TCA1, TCA2, TCA3, TCA6 and TCA7 was 3-5 folds higher on day 7 compared to the expression detected on day 1 and increased further 5-7 folds on day 14. Interestingly the expression of the TCA4 and TCA5, which are closely related, was similar on day 7 and 14; 3 and 3 folds respectively.
The results of our *in vivo* experiments suggest that the Chlamydia's plasmid genes might have a role during Chlamydial infection.

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## NEW WAYS IN CLINICAL MICROBIOLOGY: RAPID DETECTION of RESISTANT ORGANISMS WITH MALDI-TOF MS

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The current increase in the number of resistant microorganisms causing severe infections and spreading in both the hospital setting and in the community necessitates their rapid detection in routine clinical microbiology practice. Various attempts have been made to reduce the time needed for the phenotypic detection of the resistance of bacteria or fungi, or to apply molecular methods to demonstrate the presence of special resistance genes directly in the specimen or in the cultured microorganism. The recent developments in mass spectrometry relate to the analysis of proteins and more recently the DNA of microorganisms are opening up new vistas in diagnostic microbiology. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry allows the rapid identification and in some cases the sub-typing of pathogenic and non-pathogenic bacteria and fungi grown on solid media, requiring only a small amount of biological material. The very simple sample preparation and the highly accurate measurement, with low costs per analysis, make the method ideal for routine laboratory practice. MALDI-TOF MS has been utilized in different ways for the detection of resistant microorganisms. In early studies, involving the use of intact cell mass spectrometry, methicillin-sensitive and methicillin-resistant *S. aureus* strains were distinguished via various cell wall proteome-related structure alterations. However, recent studies based on the detection of differences in the ribosomal proteins did not confirm those findings. A MALDI-TOF MS-based mini-sequencing method was used with success for the rifampin and isoniazide resistance in M. tuberculosis, and penicillin, tetracycline and fluoroquinolone resistance in *N. gonorrhoeae*. A definitive "proteome shift" has been found in *C. albicans*, corresponding to its fluconazole MIC measured by conventional methods. Another approach is to register certain markers of the metabolic response of bacterial cells to antibiotic treatment by mass-spectrometric profiling. After ampicillin, tetracycline and ceftriaxone treatment, both minor and significant changes in the MS profile of *E. coli* have been observed. These changes depended on the duration of exposure and also on the antibiotic concentration. A different approach to the detection of beta-lactam-resistant strains is to culture the isolates in the presence of the antibiotics and measure the supernatants by MALDI-TOF MS after a 3-hour incubation. Evaluation of the ratio of the sum of the areas of the non-hydrolyzed forms and the sum of the enzymatically converted forms of the antibiotics may furnish a simple method for the detection of resistant bacteria. During a recent Europe-wide study on the antibiotic resistance of *Bacteroides* strains (carried out by the ESCMID Study Group on Anaerobic Infections), *cfiA*-positive

(Division II) and *cfiA*-negative (Division I) *B. fragilis* strains could be distinguished clearly by means of MALDI-TOF MS. The chromosomally determined *cfiA* gene is responsible for the carbapenem resistance in *B. fragilis*, which leads to resistance to all beta-lactam antibiotics. The rapid detection of the presence of this gene by separating the two Divisions with the simultaneous identification of *B. fragilis* can help prevent treatment failure in cases where *B. fragilis* is involved.

## *HMG-CoA* REDUCTASE GENES OF THE CAROTENOID PRODUCING FUNGUS, *MUCOR CIRCINELLOIDES*

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*Mucor circinelloides* is a β-carotene accumulating filametous fungus. Carotenoids are isoprenoid chemical compounds. Their biosynthesis branches from the common acetate-mevalonate pathway. A rate-limiting step of this pathway is the conversion of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate catalyzed by the HMG-CoA reductase enzyme. The *M. circinelloides* genome contains three HMG-CoA reductase genes (*hmgR*). In this study, expression of these *hmgR* genes and their role in the carotenoid biosynthesis was examined. Transcription levels were analysed by the quantitative real-time PCR method. Expression of the *hmgR* genes cultivating the fungus on several carbon sources, which were previosuly found to be stimulative for the carotene production, were tested. Effects of different light conditions, aerobic/anerobic growth and salt concentration in the medium were also investigated. In these studies, *hmgR1* showed a constitutively low and *hmgR2* a constitutively high transcription level. Our results suggest that *hmgR2* may play an important role in the general metabolism of isoprenoid compounds, while *hmgR3* may be necessary to the sensing of the oxygen concentration of the environment. Elevation of the copy number of the three *hmgR* genes in homologous genetic transformation experiments led to an increased β-carotene production and a decreased sensitivity to statins (inhibitors of the HMG-CoA reductases) in case of all three genes albeit the extent of the increment was different.

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## WHERE IS THE UNSEEN FUNGAL DIVERSITY HIDING? A STUDY OF *MORTIERELLA* REVEALS A HIGH CONTRIBUTION OF TYPE STRAIN SEQUENCING TO THE IDENTIFIABILITY OF ENVIRONMENTAL SEQUENCES.

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Patterns of unseen fungal diversity have been of central interest in mycology with highly discordant estimates so far. Recent burst in the application of 454-sequencing techniques intensified the debate about the proportion of undescribed fungal species and the power of 454-based approaches to recover them. Here, using in silico techniques, we modeled the effects of increasing the efforts of type-strain sequencing to the success of identification of unidentified environmental sequences present in

GenBank. Surprisingly, our principal finding is that the number of described species in Mortierella matches the number of species estimated via modeling surprisingly well, which contradicts recent claims that only 3 - 10% of all fungal species have already been described. Our finding can be generalized to other fungal groups too, since *Mortierella* does not constitute a particularly well-studied group, rather, it belongs to the under-investigated ones. We suggest that recently accepted figures about unseen fungal diversity highly overestimate the number of undescribed species and that vast majority of taxa have already been described.

## INVESTIGATION OF THE NITRIFICATION AND DENITRIFICATION PROCESSES FACILITATED BY MICROORGANISMS IN DRINKING WATER NETWORKS

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The accepted limit value of the ammonia and nitrite is 0.2mg/L and 0.1mg/L respectively, and of the nitrate 50mg/L in drinking water networks. Eliminating ammonia is important because nitrification generates nitrite and nitrate, which causes public health problems. The detection and quantitative determination was carried out of the microorganisms involved in nitrification and denitrification processes, in five drinking water networks. The microbes were analyzed by molecular methods as Polymerase Chain Reaction (PCR) - for the gene of *16S rRNA, amoA, NirS and NirK* - and Terminal Restriction Fragment Length Polymorphism (T-RFLP) and Denaturing Gradient Gelelctrophoresis (DGGE). The organism was enumerated by most probable number (MPN) analysis. Each network had different pattern of the community structure, wells of the individual networks also had different pattern by principal component analyzes. The wells had significant diversity, which decreased in the consecutive points of the networks. The heterotrophic plate count increased in the consecutive points of the drinking water networks. The number of the ammonia-oxidizing bacteria (AOB) was low and they were detected only a few sampling points. Increasing number the nitrite-oxidizing bacteria (NOB) was detected all of the sampling points. The plate count of both groups increased in the end points of the networks, in line with increasing concentration of nitrite and nitrate. *Nitrospira moscoviensis* and *Nitrobacter vulgaris* were the determining species of the NOB. The T-RFLP method confirmed the presence of the two genuses and other members of these were detected in some samples such as Candidatus *Nitrospira defluvii* and *Nitrospira* sp. Denitrifying bacteria was detected all of the sampling points, there was significant differences in the plate count in respect of each network. The total inorganic nitrogen was decreased by the activity of the denitrifying bacteria in two drinking water networks; while in the remaining networks the presence of the denitrifying bacteria was only potentially. Four drinking water networks were disinfected by chlorine-dioxid. Its concentration was decreased in line with increasing bacterial plate count. The disinfectant may have played role the decreasing diversity in the networks, and the inhibition of the denitrifying bacteria in three of the drinking water networks.

## MOLECULAR CHARACTERIZATION OF FLUOROQUINOLONE-RESISTANCE IN A MULTI-DRUG-RESISTANT *SALMONELLA ENTERICA* SEROGROUP C2 HUMAN ISOLATE

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Fluoroquinolone-resistance in *Salmonella* is a concern that has arisen a decade ago among medical and veterinarian researchers. The most common mechanisms involved and described are mutations in the quinolone-resistance determining regions (QRDRs) of the target genes (mainly in the *gyrA* which encodes DNA gyrase) and plasmid-mediated resistance (mainly associated with qnr genes). A multi-drug-resistant clinical isolate of Salmonella enterica serogroup C2 associated with food-borne infection was assessed for the molecular basis of quinolone-resistance using PCR sequencing for the *gyrA* gene and triplex-PCR for qnr genes (*qnrA, qnrB, qnrS*). The strain presented a double mutation in the *gyrA* gene, S83F and D87G. No qnr-like genes were detected. S83F and D87G are among the mutations rather frequently encountered in *gyrA*, but strains exhibiting both mutations have been seldom signaled in *Salmonella* serogroup C2. The emergence of quinolone resistance in the most common *Salmonella* serotypes worldwide involved in clinical pathology is a serious public health concern, but rare strains of *Salmonella* as that described by us might as well be implicated in the spread of resistance and thus must not be ignored.

## EFFECT OF HIGH-PRESSURE TREATMENT ON THE MICRO-ORGANISMS LIQUID WHOLE EGG

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Several researches are going on today to replace the widespread traditional liquid egg pasteurization technologies (60 – 65 °C, 5 – 10 minutes). One such procedure includes treatment of liquid egg products at high hydrostatic pressure. Application of this technology allows better preservation of natural characteristics of many raw materials used in the food industry with the same anti-microbial efficacy as with heat treatment. In this work our purpose was to investigate the effect of treatment at high hydrostatic pressure not significantly deteriorating the organoleptic and calorimetric properties (treating pressure below 500 MPa) on the physical and microbiological characteristics of whole liquid egg. In our tests we artificially infected the liquid whole egg samples with *Salmonella Enteritidis, Listeria monocytogenes*, and *Staphylococcus aureus* bacteria, and then treated the samples in „Food Lab900" high hydrostatic pressure instrument (S-FL-850-9-W, STANSTED Ltd., UK) for 3 - 17 minutes at 200 - 400 MPa (by using central complex rotation design - 17 tests). Subsequently, the change of the viable cell count of the specific bacteria by sampling every five minutes for a period of 20 minutes while maintaining the samples at a specific temperature. In addition to the samples infected with various bacteria, non-infected samples were also treated in each test and the change in viable cell count, colour (Minolta CR-200), calorimetric (Setaram MicroDSC III) and rheological properties (Brookfield, LFRA 4500 Texture Analyser) of the samples upon the effect of the treatment. In summary, it can be concluded that in each test of our investigations the viable cell count of *S. Enteritidis* critical for egg products is reduced significantly (at a level of 5 magnitudes or above) while the reduction of the total viable cell count was around 2 magnitudes. Based on our results microbial destruction, reduction of enthalpy caused by the treatment at high hydrostatic pressure and colour change are primarily affected by the pressure level, while the changes in rheological properties are also significantly affected by the duration of high-pressure treatment ($p<0.05$).

## HEAT-RESISTANCE OF *SALMONELLA ENTERITIDIS*, *ESCHERICHIA COLI*, *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* MICROBES IN LIQUID WHOLE EGG

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There are numerous investigations going on recently for bringing about generally accepted pasteurization processes. One of these procedures may be the solution that the eggs stored refrigerated are broken, boxed and the resulting whole liquid egg are heat-treated on 50–55°C in the box at least during 3 hours. However it has to be considered that the heating up of the liquid egg of 4°C packed in a 100–2000 ml packaging to 55–55°C lasts a rather long time, during which the heat-resistance of the included bacteria may increase due to the heat-shock. In our assays the liquid egg samples were infected with *Escherichia coli, Salmonella Enteritidis, Listeria monocytogenes*, and *Staphylococcus aureus* bacteria, then with linear heating (using central complex rotation design) they were heated to a temperature of 50.1–54.9°C with a rate of 1.0–5.1°C per minute. Thenceforward, maintaining the samples on the specific temperature, during 20 minutes the alteration of the live germ count of the specific bacteria was examined by sampling in every 5 minutes. Examining the decimal reduction time of several bacteria, it may be concluded that neither the applied temperature, nor the heating rate had identical influence on the decimal reduction time of the several bacteria. In case of *E. coli* exclusively the heating rate had significant influence ($p<0.15$) on the D-value, while on the decimal reduction time of *S. Enteritidis* and *S. aureus* the temperature ($p<0.05$) and the speed of heating up ($p<0.05$ and 0.01) had demonstrable influence on different levels of significance. Our measurements have shown that both the temperature and the speed of heating up had significant influence ($p<0.01$) on the thermal destruction of *L. monocytogenes*.

## *CANDIDA* INFECTIONS TRIGGER OVEREXPRESSION OF TNFRSF9 (A MEMBER OF TNF RECEPTOR SUPERFAMILY) CO-STIMULATORY MOLECULE IN MOUSE AND HUMAN MACROPHAGES

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Rising number of *Candida parapsilosis* infections among neonates and older people show, that the significance of this pathogen has increased continously during the last decade, which makes the study of host-pathogen interactions more and more important. To examine this interaction between the pathogen and the host, we used *C. parapsilosis* wild type strain and J774.2 mouse cell line. The performed microarray analysis revealed a significant elevation of a mRNS of a certain receptor called TNFRSF9 (also known as 4-1BB, CD137 and ILA). Validation of this data using qRT-PCR showed over a 50-fold overexpression in the case of infection comparing to the control. We also examined the amount of the receptor in the cell membrane using FACS analysis. This experiment demonstrated elevated level of the protein in the cell membrane as well. We also analysed whether this response is specific to *C. parpasilosis* or is shared by members of the *Candida* genus, therefore other seven *Candida* sepecies were included to our experiments. The results showed elevated level of TNFRSF9 mRNA in each of *Candida* species, however members of the parapsilosis „sensu lato" group (*C.*

*parapsilosis, C. metapsilosis, C. orthopsilosis*) increased the TNFRSF9 mRNA level more significantly, than those of other *Candida* species we used. Based on the results from murine cell-lines, we examined the response of the human PBMC derived macrophages upon *C. parapsilosis* infection, and found the 4-1BB response similarly to that of the mouse macrophages.

## ENHANCE EFFICIENCY OF ETHANOL PRODUCTION BY FERMETATION WITH MIXED CULTURES AND IMMOBILIZED CELLS

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Modern civilization is heavily dependent on the consumption of fossil energy sources. Moreover, the scale and intensive use of this causes many problems especially for the environment and the economy. It is therefore increasingly urgent to replace the depleting energy sources with environmentally friendlier, renewable energy sources. The most dominant biofuel produced today is bioethanol, mainly from sugar and starch based biomass. The currently dominating crops and the land on which they are produced are also potential resources for food production, and it is well recognized that the use of edible crops for biofuel production may threaten food security. Thus, development and production of second generation biofuel are become to front. One of promising raw materials is Jerusalem artichoke (JA), which is favourable for cultivation and nutritive properties. Main goal of this work is enhance Due to lack of inulinase activity in *Saccharomyces cerevisiae* and weak capacity of fermentation of *Kluyveromyces* species, monoculture fermentation of JA substrate were inefficiently with bioconversion rate lower than 70 %. Screening of different *Kluyveromyces* species for co-fermentation of ethanol with *S. cerevisiae* was carried out and *Kl. marxianus* Y.00959 strain was selected for further studies. Effects of the extract content in the mash and initial cell numbers on co-fermentation with mixed culture were investigated using response surface methodology. Optimal extract content and cultured cell numbers were determined to be 25 % and 210 OD600xml, respectively. Naturally, dry matter of Jerusalem artichoke extract varies from 15 % up to 20 %, thus only 200 OD600xml of cell numbers should be enough to initiate co-fermentation of ethanol. The efficiency of ethanol fermentation was also studied using immobilized cell technology, where *S. cerevisiae* cells were immobilized on the surface of Siran SIKUG 035/xx/300/A carrier. Bioreactor was engineered by adding different microcapillars to improve the diffusion of substrate and products as well as block formations of gas-microfilms. Jerusalem artichoke substrates with different concentration and flow rates were used to model operation of the engineered reactor. Optimal dilution rate was 0.23/h in the case of 13% extract content. The productivity of ethanol was about 13 g/Lh. At least 6 hours residence time was needed to reach 80% of bioconversion. Km and column capacity were 2.12 w/v % and 9.25 g/Lh, respectively. These results of both fermentation technologies are promising for development of technology for production of bioethnaol based on Jerusalem artichoke as raw material. The use of mixed culture increases efficiency of bioconversion, while application of immobilised cell bioreactor, the fermentation time may be shortened significantly and the productivity was multiple compared to batch fermentation. Scaling-up studies are needed in order to apply these technologies in semi-pilot or pilot scale.

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## EPSTEIN BARR VIRUS - CLINIC AND DIAGNOSTICS - TIPS AND PROBLEM SOLVING

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Epstein-Barr virus (EBV) is an ubiquitous human herpesvirus which persists latently for life in the memory B-lymphocytes and is periodically shed in the saliva of healthy virus carriers. Infectious mononucleosis and a wide spectrum of malignant diseases is associated with EBV. In immune suppressed or transplant patients, the timely diagnosis of EBV-associated lymphoproliferative disease is of utmost importance. While the serologic testing of antibody patterns remains the mainstay in diagnosing acute and past infectious mononucleosis, nucleic acid amplification techniques on blood or tissue samples are instrumental in diagnosing proliferative EBV-disease. In addition, EBER in situ hybridization and analysis of B-cell receptor clonality may be helpful. Functional T-cell assays are under development and may become a future diagnostic standard.

## GROWTH INHIBITION OF DERMATOPHYTE FUNGI WITH DIFFERENT STATIN DRUGS

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Dermatophytosis is the most frequent mycotic disease world-wide. Dermatophyte strains can cause severe and chronic infections, which treatments are difficult and prolonged. The investigation of antifungal activities of non-antifungal drugs, which can be used to substitute or supplement the presently applied antifungal agents, is in the forefront in the recent years. Statins are used for hyperlipidemia control and protection from cardiovascular events by reducing the level of cholesterol in the human blood. However, recent studies revealed that statins exert substantial growth-inhibitory effects on different yeasts, ascomycetes and zygomycetes. In the present study, the *in vitro* antifungal activities of different statins (lovastatin, fluvastatin, simvastatin, rosuvastatin, atorvastatin and pravastatin) have been studied against four dermatophyte fungi: *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum gypseum* and *M. canis*. The antifungal susceptibilities were determined using a broth microdilution method, which was performed in accordance with Clinical and Laboratory Standards Institute guidelines. Our results proved that statins also exhibit potent antifungal activities against dermatophytes. The investigated fungi showed different degrees of sensitivity to the statins: *T. mentagrophytes* and *M. canis* were the most sensitive species, while *M. gypseum* was the less sensitive one. Fluvastatin and simvastatin generally had higher activity than the other statins. Lovastatin, rosuvastatin and atorvastatin also had significant growth-inhibitory effect, while pravastatin had slight antifungal activity. Investigations are also under way to examine the interactions between the statins and different antifungal agents (such as terbinafine, griseofulvin and some azole and polyen antifungals) against these dermatophyte fungi. Positive interactions (addition or synergism) could be observed in several cases; however the best results could be reached with those combinations, which contained fluvastatin, simvastatin or atorvastatin. These observations suggest that fungal colonization could be affected by statin therapy and these compounds may be used also as antifungal agents in the future.

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## MOLECULAR PHYLOGENY OF MORTIERELLALES

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Mortierellales constitutes one of the largest groups of zygomycetes. Among them, some species have biotechnological significance as producers of polyunsaturated fatty acids, while others have been applied in various regio- and stereospecific biotransformations. One species, *Mortierella wolfii*, is reported as animal pathogen being casual agent of bovine mycotic abortion, pneumonia and systemic mycosis. The aim of the present study was to infer a comprehensive phylogeny from nuclear ribosomal sequence data. Therefore, the complete ITS region and the LSU and SSU genes were sequenced in a large number of type and reference strains of the genera *Mortierella, Dissophora, Gamsiella* and *Lobosporangium*. Using these data, phylogenetic analyses involving several partitioned and mixture models were performed. The resulting phylogeny substantially deviates from the traditional, morphology-based division of the order raising the need for a new classification of Mortierellales. Although Mortierellales proved to be monophyletic, the genera *Dissophora, Lobosporangium* and *Gamsiella* were found to be nested within the genus *Mortierella* indicating the paraphyly of the latter genus. *Dissophora* and *Gamsiella* (together with some *Mortierella* species) formed a common subclade. *Mortierella longicollis* was found in a basal position forming a sister group of the core Mucorales. It was also observed that phenotypic traits of mortierellalean fungi strongly depend on the culturing conditions. The need to standardized descriptions for the reporting of the phenotype in taxonomic studies will also be discussed.

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## HUMAN AND ANIMAL MODELS TO STUDY TRANSACTIVATION OF HIV BY HETEROLOGOUS VIRUSES

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Since the discovery of HIV-1 there has been a great deal of interest in identifying cofactors that might accelarate its biological effects. Beside inherent factors and environmental agents several heterologous viruses have been identified as AIDS promoting cofactors. They can transactivate HIV-1 in the same cells after a simultaneous infection. Alternatively, mediators released from cells infected by a heterologous virus can stimulate another cells carrying HIV-1. In both ways, nuclear transcriptional factors upregulate LTR sequences of HIV-1 in synergism with tat driven activation. HHV-6 is a predominantly T cell tropic virus with unique immunomodulatory activities and a wide range of transactivating potential. Its variant A targets the suppression of cellular immunity. In doubly infected CD4+ immune cells, products of DR7, U3, U16/17, U18, U19, U27, U86/87, U89 HHV-6A

genes activate HIV-1 LTR, while the product of U94 might inhibit it. HHV-6A elicits CD4 receptor expression of different CD4 negative lymphocytes extending the range of susceptible host cells. Supernatant samples obtained at time of HHV-6A early gene expression increased the production of HIV-1 in another lymphocyte cultures. Several proinflammatory cytokines, e.g. TNF-alpha, IFN-gamma, IL-1beta, IL-6 mediate HIV-1 transcellular transactivation. Concomitant infection by HHV-6A and HIV-1 results in increased HIV-1 load, Th1 to Th2 shift in the cytokine pattern, synergistic destruction of lymph nodes, multiorgan failure, accelarated AIDS progression and premature death. HHV-6B infection primarily weakens humoral immunity, and its biological effects do not affect HIV-1. HHV-7 closely related to HHV-6 utilizes CD4 molecules as receptors, and as such, interferes with HIV-1 dissemination in the body. Its effect on the immune system is self-limiting, and can be additive to immune suppression induced by HIV-1 in vivo. A major hindrance to elucudating the in vivo role played by HHV-6A in AIDS has been the lack of a reliable animal model system. The availability of pig-tailed macaques whose T cells are highly susceptible to HHV-6A and SIV infection has become an ideal model. In coinfected animals, a progressive loss of CD4+ T cells, higher plasma viremia, more severe lymph node destruction, earlier onset of clinical signs were seen than in singly infected animals. Feline immune deficiency virus (FIV) has a pathogenesis similar to that of HIV-1 infection, thus it might prove to be another ideal model for AIDS cofactor studies. So far, only a limited number of experiments has been conducted. Coinfection of FIV infected cats with feline herpesvirus type 1, feline adenovirus, feline leukemia virus induces T cell deficiency aggravating feline AIDS. Toxoplasma gondii and Listeria monocytogenes disrupt normal Th1 cytokine synergism contributing to the loss of cellular immunity in FIV positive animals. These different systems clearly show that the progression of feline AIDS is facilitated by a wide array of microbes.
The array of microbes acting as transactivators and causing opportunistic infections are overlapping. Antimicrobial therapy, therefore, might inhibit their both effects.

## IN VITRO INVESTIGATION OF THE ROLE p63 PLAYS IN VESICULAR STOMATITIS VIRUS (VSV) AND HERPES SIMPLEX VIRUS (HSV) INFECTIONS

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p63 is instrumental in the development and maintenance of stratified epithelial tissues and their derivatives. p63 is a transcription factor belonging to the p53 family. Six major p63 isoforms exist, including two variants at the amino terminus (TA and deltaN) and three at the carboxy terminus (alpha, beta and gamma). The TAp63 isoforms, possessing the transactivation domain, are capable of inducing apoptosis. The deltaNp63 isoforms, lacking the transactivation domain, exert dominant-negative activities, act as transcriptional repressors and inhibit apoptosis. Stress signals that alter the expression pattern of p63 have the potential to cause profound alterations in the viability of epithelial tissues. The epithelia of the skin and eye may function as entry sites for several microorganisms. However, the effects of viral infections on the expression pattern of p63 have not yet been elucidated. Thus, we set out to investigate the effects of HSV and VSV infections on the expression levels of p63. HaCaT keratinocyte cell line was infected with VSV, HSV-1 or HSV-2 at various multiplicities. Virus replication was measured by immunofluorescence assays, plaque titrations and Western blot analyses. Cell viabilities were determined by MTT assay. The apoptotic responses of the infected cells

were quantified by ELISA. The levels of the various p63 isoforms were determined by Western blot analyses. To knock down TAp63 expression gene silencing was used. The HaCaT cell line was highly permissive to VSV replication. VSV infection elicited the apoptotic death of the infected cells. Mock-infected HaCaT cells displayed the endogenous expression of deltaNp63alpha. Interestingly, we observed an impressive reduction of the deltaNp63alpha level of VSV-infected cells. The kinetics of VSV replication, apoptosis and suppression of deltaNp63alpha expression correlated strictly. This suggests that the VSV-induced decrease in deltaNp63alpha level is a key event in the apoptotic response of the HaCaT keratinocytes. Both HSV-1 and HSV-2 replicated efficiently and elicited a strong cytopathic effect in HaCaT cells, and apoptosis played an important role in the demise of the infected cultures. The levels of deltaNp63alpha and deltaNp73beta were decreased, while the expressions of the TAp63gamma and TAp73delta were highly increased in HSV-1-infected cells. The knockdown of TAp63 expression enhanced the viability of HSV-1-infected cells. In contrast, in response to HSV-2 infection the levels of deltaNp63alpha, deltaNp73beta and TAp73delta were decreased, while the expression of TAp63gamma remained unaffected.
These data may bear on the pathogenic mechanisms of disease caused by HSV-1 and HSV-2. Moreover, the different effects of HSVs on the expressions of TAp63gamma and TAp73delta may be novel type-specific features of these viruses.

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## INTRACELLULAR REPLICATION OF *FRANCISELLA NOVICIDA* WITHIN *HARTMANELLA VERMIFORMIS*

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*Francisella tularensis* is a gram negative facultative intracellular bacterium that causes the zoonotic disease tularemia. Free-living amebae, such as *Acanthamoeba* and *Hartmannella*, are environmental hosts of several intracellular pathogens. Epidemiology of *F. tularensis* in various parts of the world is associated with water-borne transmission, which includes mosquitoes and amebae as the potential host reservoirs of the bacteria in water resources. *In vitro* studies showed intracellular replication of *F. tularensis* within *A. castellanii* cells. Whether ameba is a biological reservoir for *Francisella* in the environment is not known. We used *Hartmannella vermiformis* as an amebal model system to study the intracellular life of *F. novicida*. For the first time we show that *F. novicida* survives and replicates within *H. vermiformis*. The *iglC* mutant strain of *F. novicida* is defective for survival and replication not only within *A. castellanii* but also in *H. vermiformis* cells. In contrast to mammalian cells, where bacteria replicate in the cytosol, *F. novicida* resides and replicates within membrane-bound vacuoles within the trophozoites of *H. vermiformis*. In contrast to the transient residence of *F. novicida* within acidic vacuoles prior to escaping to the cytosol of mammalian cells, *F. novicida* does not reside transiently or permanently in an acidic compartment within *H. vermiformis* when examined 30 min after initiation of the infection.
We conclude that *F. tularensis* does not replicate within acidified vacuoles and does not escape into the cytosol of *H. vermiformis*. The *Francisella* pathogenicity island locus *iglC* is essential for intra-vacuolar proliferation of *F. novicida* within *H. vermiformis*. Our data show a distinct intracellular lifestyle for *F. novicida* within *H. vermiformis* compared to mammalian cells.

## SCREENING OF *BACILLUS THURINGIENSIS* TYPE STRAINS FOR *CRY* AND *CYT* TOXIN GENES WITH PCR ASSAY and SCANNING ELECTRON MICROSCOPY

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The Western Corn Rootworm, *Diabrotica virgifera virgifera* is currently probably one of the most threatening pest in Hungarian (and European) agriculture, as its soil-inhabiting larvae can seriously damage roots of maize *(Zea mays*) and lead to high yield losses. The chemical pesticides used to control the spread and damage of the insect are losing efficiency due to emerging resistance, so seeking for new ways of pest controls is necessary. Various strains of *Bacillus thuringiensis* produce different parasporal inclusion (crystal-like) proteins, which exhibit specific activity against larvae of *Lepidoptera*, *Diptera* and *Coleoptera,* providing an alternative and perhaps more environmental friendly pest control on insect herbivores. The entomopathogenic activity of this bacterium is principally due to the presence of proteinaceous inclusions that can be distinguished as distinctively shaped crystals by scanning electron microscopy. These inclusions are comprised of proteins known as insecticidal crystal proteins (Cry proteins). Type strains of *B. thuringiensis* were screened for *cry* and *cyt* genes to reveal their potential entomopathogenic effect and target pest range. The screening was carried out with Polymerase Chain Reaction (PCR) using *cry*- and *cyt*-toxin gene specific primers, aiming at the Cyt2, Cry2, Cry3, Cry4, Cry8, Cry14 Cry22, Cry34 and Cry35 toxin families. The PCR products were sequence analyzed for the identification of genetic content. The screening approach proved to be efficient based on positive PCR reactions of documented *cry* genes. The PCR assays were complemented by scanning electron microscopic detection of parasporal crystals.

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## DEVELOPMENT AND IMPROVEMENT OF DETECTION OF HIGHLY PATHOGENIC BACTERIA DURING EXTERNAL QUALITY ASSURANCES FOR THE DETECTION OF HIGHLY PATHOGENIC BACTERIA OF POTENTIAL BIOTERRORISM RISK

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Establishment of External Quality Assurances for the Detection of Highly Pathogenic Bacteria of Potential Bioterrorism Risk (EQADeBa) is an EU-funded project carried out by the Robert Koch Institute (RKI) in co-operation with 23 partners from 20 European countries. In frame of the EQADeBa Project, samples containing highly pathogenic viable and non-viable target bacteria such as *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Brucella abortus* and *melitensis*, *Coxiella burnetti*, *Burkholderia mallei* and *pseudomallei* with accompanying bacteria were transported from RKI to the participant laboratories. The main goal was to organize a permanent network and to take part in the harmonization process of biosafety and biosecurity issues and improve our diagnostic capacity and knowledge. Between 2009 and 2011 three external quality exercises were prepared. During the first round 15 samples containing inactivated microorganism, during the 2nd and

3rd round 15 samples containing viable bacteria in soft agar (5/15 samples to react in a timely manner) and 15 samples containing inactivated bacteria in different matrices such as PBS, Spree river water and mouse cell culture (refer to isolate, environment and tissue) were sent. All samples were handled under bsl3 conditions. Immunological, cultivation and molecular methods were used to identify the target bacteria. Rapid screening tests resulted in high specificity but low sensitivity compared to other methods. After the 1st round specific media were introduced to increase the specificity of the culturing methods. The automated identification systems (Biolog, API) could be used for the typical strains from target bacteria. Slide agglutination using home made polyclonal sera against Yersinia sp., Burkholderia sp. and *Francisella tularensis* were applied to support the selective cultivation. Conventional and real-time PCR assays (home-made and commercial kit) were used on all samples. PCR reactions were carried out both on isolated DNA from the original samples and primocultures without nucleic acid isolation. Due to appropriate optimization procedures (time, temperature, interpretation of real time PCR results), all target bacteria were identified accurately. During the EQADeBa project, new sample processing, cultivation, rapid screen tests and molecular methods were introduced or old ones were improved to make the detection of potential bioterrorism agents more efficient and reliable. However, our methods need further improvement to give more rapid and accurate results to authorities, clinicians and epidemiologists.

## THE M3 PROTEIN OF MURID HERPESVIRUS 4 (MUHV 4): GENE CLONING, PROTEIN EXPRESSION AND PURIFICATION

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For recent studies on the pathogenesis of human gammaherpesviruses – the Epstein-Barr virus (EBV) and the Human herpesvirus 8 associated with Kaposi's sarcoma (KSHV) – animal models are often used, such as the infection of mice with their natural pathogen called Murid herpesvirus (MuHV-4). Several strains of this virus were isolated from free-living rodents at the territory of Slovakia from 1980. The most frequently studied MHV-68 has a genome structure similar with human gammaherpesviruses. In its pathogenesis in experimentally infected mice, of special interest is the development of tumours, such as lymphomas. The M3 protein encoded by the MuHV-4 genome was found to have high and specific binding affinity to a broad spectrum of chemokines (either of murine or human origin). Its immunomodulatory effects enhance virus replication in the host and promote reactivation from latency. Based on previous results describing some differences in properties of natural M3 protein between strains MHV-72 and MHV-68, we aimed to prepare recombinant proteins of both strains needed for further chemokine blocking studies. Two recombinant plasmids pET26b(+) with the M3 gene sequence inserted and supplemented by the 6xHis sequence were prepared. The M3 protein was expressed in two *E.coli* strains BL21 (DE3) and Rosetta (DE3) under the inductor effect of IPTG. The cell cultivation conditions necessary for purification of pure native M3 protein by affinity chromatography were optimised to achieve protein production of a good functional quality and in amounts suitable for crystallization and subsequent diffraction measurements. The analysis of the M3 protein binding sites to chemokines or their receptors in association with its anti-chemokine activity will help to understand the functions of M3 protein in virus-host interactions.

Recombinant viral vectors exploiting unique properties of M3 protein could provide benefits of prolonged cure effect in use in human medicine e.g. in therapy of cancer or autoimmune diseases.

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## THE EXAMINATION OF ZEARALENONE-INDUCED CYTOTOXIC EFFECTS AND OXIDATIVE STRESS PROCESSES IN *SCHIZOSACCHAROMYCES POMBE*

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The mycoestrogen zearalenone (ZEA) is a secondary fungal metabolite that can contaminate feed and foodstuff. It is produced by different *Fusarium* species and can cause worldwide serious health problems for animals as well as humans. In mammalian cells ZEA binds to oestrogenic receptors and causes well characterized symptoms. Its cytotoxic and oxidative stress inducing effects were studied on *S. pombe* fission yeast cells. Minimal inhibitory concentration was 1000 µM determined by microdilution method (M27A) according to the NCCLS standard. The growth inhibition at 100 µM, 500 µM and 1000 µM by ZEA was determined in shaken cultures in supplemented minimal broth at 30 $^{o}$C. After 20 hours, the 100 µM ZEA treatment did not induce any remarkable inhibitory effect. In the presence of 500 and 1000 µM ZEA, the growth inhibition was 83 % and 84 %, respectively, and it decreased to 40 % and 38 % after 36 hours treatment. 100 µM ZEA did not affect the survival rates but it was approximately 0 % after 30 min treatment in the presence of both 500 µM and 1000 µM ZEA. The retarded growth kinetics indicated adaptation was investigated, where 250 µM ZEA pretreatment was used as defined as subinhibitory concentration from survival rates. The kinetics of ZEA uptake was determined photometrically and showed fast process, where the 80% of ZEA was taken up by the yeast cells in the first 10 minutes.
ZEA treatment notably increased the intracellular concentrations of superoxide anion, peroxides, and hydroxyl radical. However, the altered chromium reduction capacity also supports the imbalance of the oxido-reduction state of cells. Although *S. pombe* does not contain oestrogenic receptor homologue sequences, the ZEA caused cytotoxic effects and induced oxidative stress in fission yeast suggested first time an aspecific mode of action of this eukaryotic cell.

## INVOLVEMENT OF P63 IN THE HERPES SIMPLEX VIRUS (HSV)-INDUCED DEMISE OF CORNEAL CELLS

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The transcription factor p63 plays a pivotal role in the development and maintenance of epithelial tissues, including the ocular surface. In an effort to gain insight into the pathogenesis of keratitis caused by HSV-1 and HSV-2, we determined the expression patterns of the p63 and Bax proteins in the Staatens Seruminstitute Rabbit Cornea cell line (SIRC). SIRC cells were infected with HSV-1 or HSV-2 at various multiplicities and maintained for different periods of time. Virus replication was

measured by indirect immunofluorescence assay and Western blot analysis. Cell viability was determined by MTT assay. The apoptotic response of the infected cells was quantified by ELISA detecting the enrichment of nucleosomes in the cytoplasm. Western blot analysis was used to determine the levels of p63 and Bax proteins. Indirect immunofluorescence assays and Western blot analyses demonstrated the presence of glycoprotein D (gD) in SIRC cells infected with HSV-1 or HSV-2, and the pattern of gD expression was consistent with efficient viral replication. MTT assay, ELISA and annexin V staining revealed that both HSV-1 and HSV-2 replicated efficiently and elicited a strong cytopathic effect in the SIRC cell line, and apoptosis played an important role in the demise of the infected cells. Although the proportions of dead cells were comparable in the HSV-1- and HSV-2-infected cultures, the early apoptotic population was larger in the cultures infected with HSV-1 than in those infected with HSV-2. Thus, the apoptosis-inducing effect of these viruses is different. Western blot analyses demonstrated that mock-infected SIRC cells displayed the constitutive expression of deltaNp63alpha. The expressions of the Bax-beta and TAp63gamma isoforms were considerably increased, whereas the level of deltaNp63alpha was decreased in the HSV-1-infected SIRC cells. Experiments involving the use of acyclovir showed that viral DNA replication was necessary for the accumulation of TAp63gamma. Although the expression of deltaNp63alpha decreased in the HSV-2-infected SIRC cells, TAp63gamma was not detectable and the level of Bax-beta increased slightly. Together, these data indicate that a direct, virus-mediated cytopathic effect plays an important role in the pathogenic mechanism of herpetic keratitis. The cytopathogenicity of these viruses is complex, and involves apoptotic mechanisms. Further results clearly demonstrate that HSV-1 and HSV-2 modulate the patterns of p63 and Bax expression in a type-specific manner. By disturbing the delicate balance between the pro-survival deltaN and the pro-apoptotic TA isoforms of p63, these viruses may cause profound alterations in the viability of the ocular cells and in the tissue homeostasis of the ocular surface.

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## SOME ASPECTS OF REGULATION OF OXIDATIVE STRESS PROCESSES IN YEAST

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Cells, especially single cell organisms like yeasts, have to frequently adapt to changing external environment to maintain their homeostasis such as heavy metals, oxidative stressors, toxic compounds etc. Unbalanced homeostasis can be the consequence of mutations, too. In response to these adverse changes involve a variety of programmed stress responses such as the sensing stress, the signaling pathways and the resulting compensatory changes in gene expression and metabolism. Oxidative stress represents an imbalance between the production and manifestation of reactive oxigen species (ROS) such as the superoxide anion ($O_2^{\cdot-}$), the hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^{\cdot}$) and a biological system ability to readily detoxify the reactive intermediates or to repair the resulting damage. Chemically, oxidative stress is associated with increased production of oxidizing species or a significant decrease in the capability of antioxidant defenses consist of a combination of enzymatic (superoxide dismutases, catalase, glutathione peroxidase etc.) and non enzymatic system including small radical scavengers like glutathione, thioredoxin and glutaredoxin. In the fission yeast *Schizosaccharomyces pombe,* the mitogen-activated protein kinase (MAPK) Sty1p and transcription factors Atf1p and Pap1p are critical for the response to oxidative stress.

Phosphorylation of the trascription factors and their localization to the nucleus result in changes in gene expression associated with the core environmental stress response (CERS) as well as stress-specific gene expression. In addition, transcription factor Pap1p respond to specific stress stimuli to regulate non-CERS gene expression.
These results explain the adaptation and cross-protection processes at molecular level. Knowledge of these processes in yeast may contribute to understand the complex phenomena of oxidative stress toxicity of the chromate anion /Cr(VI)/, the $Cd^{2+}$, the patulin mycotoxin, and the HIV-1 vpr, etc.

## NEW TAXA OF THE FAMILY TRICHOMONASCACEAE FROM HUNGARY

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*Trichomonascus* was considered to be an endomycete-like genus by Malloch and de Hoog (1998), but its phylogenetic placement was unclear. The attempt to isolate DNA from the herbarium specimen of the holotype material of *T. mycophagus*, the type species of the genus was unsuccessful [1]. Based on striking morphological similarities Kurtzman [1] described *T. petasosporus*, a heterothallic yeast species, the first cultivated species of the genus. Prompted by the results of multigene sequence analysis Kurtzman and Robnett [2] transferred two species from the genus *Stephanoascus* to *Trichomonascus* and erected the genus *Sugiyamaella* to accommodate *S. smithiae* and related teleomorphic species. They also proposed the family Trichomonascaceae to include the phylogenetically related genera *Sugiyamaella, Trichomonascus, Wickerhamiella* and *Zygoascus*. During our studies aiming at the investigation of the yeast biodiversity in Hungary, numerous yeast strains were isolated which, based on the comparisons of the D1/D2 domain of the LSU rRNA gene proved to be undescribed species of the family Trichomonascaceae. Four strains, isolated from pollen storing cells of honeycomb of honey bee (*Apis mellifera*) sharing identical D1/D2 sequences were described as *Trichomonascus apis* [3]. The strains were haploid and represented opposite mating types. Their ascospore formation was similar to that of the former genus *Stephanoascus*. An other group of conspecific strains of the undescribed species were recovered from rotten wood samples, collected in forests of Hungary. These strains were also haploid and formed ascospores when paired with strains of the opposite mating types.
The D1/D2 sequences placed them in the Trichomonascaceae family, where they formed a well supported subclade with some additional undescribed species. Multigene phylogenetic analysis revealed that the above-noted strains were phylogenetically well separated from recognised teleomorphic genera in Trichomonascaceae. To accommodate these strains the new genus *Spencermartinsiella* and the new species *S. europaea* were proposed [4].

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## PATHOGEN DETECTION FROM WHOLE BLOOD WITHOUT DNA PREPARATION

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Clinical studies have demonstrated the increased mortality caused by sepsis when inappropriate antimicrobial therapy is given. Rapid diagnosis of infections would be a tool for adequate and appropriate antimicrobial therapy in the treatment of a patient with severe sepsis. The use of conventional laboratory techniques for the isolation and identification of invasive infections are time-consuming and hence molecular techniques are beginning to play an increasing role in their laboratory diagnosis. The PCR methods use different protocols to isolate and purify genomic DNA from the samples in an amount and quality sufficient for further nucleotide analysis. The preparation of DNA, however, prolongs the procedure and might introduce further errors into the analytical process. To reduce the duration and to improve the reliability of detection new PCR techniques omitting the DNA preparation step have been developed. This progress could be made by the use of special additives, buffers or modified polymerases. Recently, new PCR mastermixes containing these or similar components have shown up in the market to enable robust direct DNA amplification of highly inhibitory samples. Here we describe a rapid, simple and accurate procedure for detecting pathogens from blood without DNA preparation steps. Both sera and anticoagulated peripheral blood samples were used for investigation. The samples originated from Intensive Care Units of University of Szeged, and the study was approved in advance by the local ethical committee. Two mastermixes were compared: one of them are recommended for DNA amplification from highly inhibitory samples (SsoFast Supermix, Bio-Rad Laboratories, Hercules) while the other is dedicated for applications using direct DNA amplification from whole blood samples (Phusion Blood Direct PCR Kit, Finnzymes, Espoo, Finnland). All mastermixes were used according to the manufacturer's instruction. We used two specific primer pairs to differentiate the fungal and bacterial amplicons. The amplicons were investigated by real-time PCR and separated by electrophoresis on 1.5% agarose gels in the case of sera and whole blood samples, respectively. We have obtained similar results both with sera and anticoagulated whole blood samples. Under the conditions used in the present experiments detectable amplification was not observed with the other commercially available PCR kits tested. These PCR procedures omitting prior DNA preparation makes this identification method efficient and time sparing. Whereas conventional PCR procedures for pathogen investigation take about a day to complete, the new technique described can be accomplished within two hours. The fact that there is a significant increase in mortality with each hour delay in the administration of appropriate antibiotic therapy from the onset of sepsis underlay the importance of this new technique.

## THE FELINE IMMUNODEFICIENCY VIRUS AS A SMALL ANIMAL MODEL of HIV

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The feline immunodeficiency virus (FIV) is a lentivirus similar to human immunodeficiency virus (HIV) that undergoes persistent infection and causes an AIDS-like disease in domestic cats. FIV is

sensitive to most HIV drugs and evades immune surveillance through mechanisms similar to those exploited by HIV. Thus, FIV and its natural host, the domestic cat, are considered a reliable small animal model to develop new antiretrovirals and design novel approaches to vaccinate against lentiviruses. Development of an effective FIV vaccine has met with difficulties very similar to those encountered with HIV and non-human primate lentivirus vaccines. Suboptimal immunogenicity, inadequate antigen presentation, and inappropriate immune system activation are believed to have contributed to these disappointing results. This lecture will focus on the advantages and disadvantages to use FIV compared to non-human primate models, and on past and current vaccination approaches carried out with FIV. Findings, implications and transferability to human AIDS of a recent and successful FIV vaccine will be discussed.

## ISOLATION AND IDENTIFICATION OF ACETIC ACID BACTERIA FROM WINE AND VINEGAR SAMPLES IN TURKEY

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Acetic acid bacteria are used as starter for vinegar production. In the present study we aimed to isolate acetic acid bacteria from home made vinegars as well as wine from 6 different cities. A total of 7 home made wine samples from 5 locations and 7 vinegar samples from 3 location in Turkey were collected for isolation of bacteria. These samples were incubated statically at pH 6.0 and 30ºC for 7 days in Hestrin-Schramm (HS) broth medium that contains 2.0 % glucose, 0.5 % yeast extract, 0.5 % polypeptone, 0.27 % $Na_2HPO_4.12H_2O$ and 0.115 % citric acid. Then the cultures were inoculated onto HS-agar (1.5% agar) plates after serial dilution. The growth of the colonies were observed during incubation at 30ºC for 3 days. White to cream colonies with mucous structure were purified by repeated streaking onto agar plates. All bacteria were characterized by Gram staining and by their metabolic properties. These bacteria were further characterized by 16S rRNA amplification and sequencing. The clonalities of the isolates were tested by ERIC-PCR analysis. A total of 42 bacteria were isolated and identified as acetic acid bacteria by classical methods. All isolates were further analyzed by sequencing. The results showed that of 42 isolates 11 were *Gluconacetobacter saccharivorans*, 9 were *Gluconacetobacter hansenii*, 8 were *Acetobacter pasteurianus*, 7 were *Acetobacter ghanensis*, 3 were *Acetobacter malorum*, 2 were *Acetobacter fabarum*, 1 was *Acetobacter lovaniensis*, and 1 was *Gluconobacter oxydans*. All of 11 *G. saccharivorans* isolates 2 and 3 were isolated from wine and vinegar samples, respectively. A clone was common between wine and vinegar samples from Isparta by ERIC PCR. *G. hansenii* isolates were from 3 cities and 4 samples (2 wine and 2 vinegar). *A. pasteurianus* were isolated from 4 wine and 1 vinegar samples of 2 cities, Tekirdag and Isparta. A clonal profile was observed among isolates from 3 distinct wine samples of Isparta. *A. ghanensis* isolates were from 2 cities, Tekirdag and Bursa and all were from vinegar sample and had the same ERIC PCR profile. A. malorum strains that were isolated from 2 wine samples of Tekirdag had the same profile. *A. fabarum* strains were from 2 wine samples of Aydın and these isolates had the same profile. *A. lovaniensis* strain was from vinegar sample from Isparta. *G. oxydans* strain was from wine sample of Tekirdag. Our results showed that same acetic acid bacteria may be isolated from samples of distinct cities. Also common our results indicate that same clones may be isolated from wine and vinegar samples.

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## NEW APPROACHES OF SIZE CONTROL IN FISSION YEAST

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Size control is a mechanism ensuring that a characteristic cell size remains constant over generations in a population. There must be 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, initiating DNA replication (S phase) and the onset of mitosis (M phase). Hence two size checkpoints exist in the eukaryotic cell cycle in G1 phase and in G2. The molecular mechanisms of these size checkpoints are still obscure; however, they seem to be evolutionary conserved from unicellular microorganisms up to even human cells. Since the late 1970s fission yeast has been an attractive model organism in size control studies. The cylindrically shaped cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter. 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 is, the shorter its cell cycle will be. 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. The wee1 mitotic inhibitor was found to be mainly responsible for this size checkpoint. In small cells wee1 keeps the cdc2/cdc13 complex (also known as MPF) in an inactive form. After reaching a critical size, wee1 itself becomes inactivated and the cell starts to prepare for mitosis. Former models suggested that MPF accumulated (proportional to the increasing cell size) in the constant size 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. Recent experimental data seems to give us a new clue how size control might even operate. Namely, a spatial gradient of a mitotic inhibitor (pom1, indirectly acting on wee1) is generated along the cell cortex, having a maximal value at the cell tips, and a minimum at the centre. As the cell grows, local pom1 concentration at the cell centre decreases and finally in late G2 it drops below a critical level, which is no more able to delay mitotic onset. As a consequence, initiation of mitosis is connected to reaching a threshold in cell size. The connections between genes, proteins and molecules participating in these biochemical reactions can be described with the help of ordinary differential equations (ODEs). These equations are constructed from different parts: synthesis, degradation, activation, etc. From all these equations we get a whole ODE system, and if we solve it, we can get more information about the described biological system. As all the previous models were created according to the former hypothesis, our aim was to develop a model with the new results built in. Apart from analyzing the cycle of wild type cells we also made simulations of wee1$^-$ cell cycle mutants, because size control acts differently in these cells.

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## EPSTEIN BARR VIRUS (EBV) VACCINE DEVELOPMENT STRATEGIES AND THE DESIGN OF A POLYEPITOPE VACCINE

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EBV (HHV 4) is a gammaherpesvirus which has developed several latency strategies when residing in B lymphocytes (latency I, II/III). At primary infection, the virus replicates in the oropharyngeal mucosa. By high probability, the EBV infection induces infectious mononucleosis (IM), which is a powerful immune response with atypical mononuclear cells in the blood and enlarged lymph nodes. The virus encodes several oncoproteins; and has been associated with various lymphatic tumors such as Burkitt´s lymphoma, Hodgkin´s lymphoma, non-Hodgkin B and/or T cell lymphomas in immunocompromized patients as well as nasopharyngeal carcinoma. The vaccine design so far has been concentrated on the construction of subunit and/or recombinant virion envelope glycoproteins such as gp350/220, aiming to induce relevant neutralizing antibody formation and providing satisfactory protection. The latter was tested in experimental animal (monkey) models and/or in a few human volunteers with not always satisfactory effects, since it did not prevent seroconversion. In addition to these efforts, synthetic peptide and/or polyepitope vaccines have been tested. These carried mainly the antigenic determinants of latency associated EBNA3 protein(s) and/or of the latent membrane proteins LMP1/LMP2. Their efficacy brought similar inconsistent results. We report the design of a synthetic mucosal polyvalent epitope vaccine, in which the protective viral epitopes were bound to pathogen mimicking microparticles (PMM). The careful selection of the EBV epitopes coming from gp350, gp110, gp42, LMP1, EBNA1 and EBNA3c polypeptides was made by means of computer algorythms to ensure the selection of non-overlapping immunogenic peptide motifs. In addition, the PMM contained a T helper cell binding epitope, a B-lymphocyte blocking epitope a nonspecific (toll-like) receptor binding epitope. The EBV specific epitopes carried by the PMMs efficiently target the dendritic cell (DC) uptake as demonstrated *in vitro* in a monocyte derived DC line. In these, PMM elicited a massive interleukin production (IL-1β, IL-2, IL-6, IL-8 and TNF α were tested. Finally attention was devoted to the choice of a potent adjuvant (essentially a combination of poly I/C and LPS) ensuring synergistic effect on receptors (TCR) of helper as well as cytotoxic T cells. We decided to test the protective effect of PMM *in vivo* using a rabbit model, which was based on intravenous inoculation of living B95-8 cells ($1x10^6$ cells/ml containing about $10^9$ copies of EBV DNA were given per rabbit). Experiments are in progress to evaluate the protective effect of the PMM vaccine in different epitope combinations.

## MOLECULAR METHODS FOR THE IDENTIFICATION OF *MYCOBACTERIUM* ISOLATES WITH ANIMAL ORIGIN

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Reference laboratories are challenged to identify *Mycobacterium* isolates to the species level in order to define an isolate as true pathogen or environmental and potential contaminant. The primary task of our laboratory is to confirm or disprove the presence of tuberculosis pathogens in animals. Besides the pathogens of bovine and avian tuberculosis, we regularly isolate non-tuberculous Mycobacteria (NTM). The traditional identification method differentiated *Mycobacterium* isolates on the basis of phenotypic characteristics and additional biochemical tests. These characteristics do not allow precise identification of all the species, thus leading to false identification results. As classical morphological and biochemical methods are time-consuming and error prone, we introduced molecular biological methods for the identification of our *Mycobacterium* isolates. All Ziehl-Neelsen positive

*Mycobacterium* isolates are tested in a multiplex amplification system that can identify the genus *Mycobacterium* and then distinguish between *Mycobacterium tuberculosis* complex (MTC) organisms, *M. avium, M. intracellulare* and M. spp. MTC isolates are further tested with GenoType MTBC kit which permits the genetic differentiation of *M. africanum, M. bovis* BCG, *M. bovis, M. caprae, M. microti* and *M. tuberculosis*/"*M. canettii*" strains on the basis of gyrase B gene polymorphisms. All MTC isolates from sporadic outbreaks of bovine tuberculosis and wildlife cases proved to be *M. bovis* ssp. *caprae* during the last 6 years. *M. avium* isolates are further tested for the presence of insertion elements IS900, IS901 and IS1245. *M. avium* ssp. *paratuberculosis* (MAP) is a slow growing, IS900 positive, mycobactin dependent subspecies of *Mycobacterium avium* complex (MAC) causing Johne's disease in ruminants (paratuberculosis) with a yet unproven role in human Crohn's disease. Since 2006 we isolated more than 350 MAP strains from different animal species (sheep, red fox, wild boars, red deer, cattle) from all parts of Hungary. *Mycobacterium avium* ssp. *hominissuis* (MAH) strains are IS900 and IS901 negative, while IS1245 positive. Since 2006 we isolated almost 60 MAH strains from wild boars, swine, red deer, birds and cattle. *Mycobacterium avium* ssp. *avium* (MAA) strains are IS900 negative and IS901 and IS1245 positive. During the last years we isolated more than 90 MAA strains.
The identification of NTM poses still a problem. Over the past ten years more than 45 novel NTM have been described. However, some clinically significant human pathogen strains can be identified by GenoType *Mycobacterium* CM/AS kit but great majority of the animal isolates could not be identified by this method. For the accurate identification of the NTM isolates we introduced rpoB and tuf gene sequencing. The 48 isolates tested were successfully identified by this method.

## DNA-DAMAGING EFFECTS OF UV-INDUCED PHOTOLYTIC DEGRADATION PRODUCTS OF PHENYLUREA HERBICIDES, ANALYZED BY COMET-ASSAY

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Phenylurea herbicides, like diuron, monuron, linuron, are photosynthesis inhibitors killing the entire plant by this effect. They are highly persistent and remain in the environment long time after use. Moreover, they could be toxic and carcinogenic to animals and humans. In this study, DNA-damaging properties of decomposition products of diuron, monuron and fenuron via UV-induced photolysis and combination this method with ozonation were investigated with comet assay. Comet assay, or Single Cell Gel Electrophoresis (SCGE) assay was developed in late 1980's and was first described by Singh et al. in 1988, as a fast, effective way of measuring DNA damage or repair in individual cells. The name comes from the resulting image, obtained after the process as a „comet" with a distinct head and tail. The head is composed of intact DNA, while the tail consists of damaged (single-strand or double-strand breaks) or broken pieces of DNA. The comet assay is used in the alkaline and neutral version described in the literature. In our study we used the alkaline version to estimate the DNA damage after treatments of E. coli cells with different decomposition products of the three herbicides. The experiments were performed according to the work of de Mattos et al. (2008), with some modifications. We embedded the treated E. coli cells in a low melting point agarose gel. After gelification the blocks were incubated for 16 h, at 55 °C, in the dark, in a lysing

solution (0.1 mM EDTA (pH 8.0), 0.5 M NaOH, 0,05% SDS). Then, the blocks were washed three times in cold Tris/EDTA buffer and placed onto the teeth of the comb of the electrophoresis gel apparatus. Agarose (0.76%) was suspended in diluted alkaline buffer and heated for complete homogenization. After cooling, it was poured in the tray of the horizontal gel electrophoresis chamber and the comb, containing the blocks with the lysed E. coli cells, was fitted. Following gelification, the comb was removed, leaving the sample blocks in the gel. The electrophoresis was carried out in alkaline buffer at 7 V for 20 h. After electrophoresis the gel was neutralized by soaking in a solution containing 30 mM NaCl and 50 mM Tris/HCl (pH 6.0). Following this step, the gel was stained in ethidium bromide solution (in TBE) and visualized in a transilluminator.
The described method was applicable for the detection of DNA-degrading effects of the photolytic products of all the three investigated herbicides.

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## INVESTIGATION OF BACTERIAL FUNCTIONAL COMMUNITY STRUCTURE WITH RISA-AFTER PRECULTURING (RISA-APC) METHOD

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Microorganisms play a key role in soil development and preservation; moreover, they could indicate the soil health and conditions. The relatively fast and dynamic changes in the bacterial communities could be good indicators for the soil quality and also indicate the influence of the different environmental factors. There are different works, investigating the connections between the bacterial community structure changes and soil quality. In this study we provide a simple method for obtaining quick data regarding the functional structure of bacterial communities in the soil. Our approach has been named as RISA-APC method (ribosomal RNA (rRNA) intergenic spacer analysis, after preculturing). The base of this technique is: preculturing the bacteria of the soil samples in distinct media for a short time and after that, performing a molecular diversity analysis of the developed microbial communities. The region of the rRNA gene cluster between the small (16S) and large (23S) subunits in bacteria is called the intergenic spacer region (ITS). The ITS length polymorphism after PCR could be visualized with gel electrophoresis, and the resulted mixture of DNA fragments is characteristic, such as a barcode and indicates the composition of the investigated bacterial community. The RISA analysis of DNA samples, extracted from mini-colonies appearing after preculturing of aliquots of the soil samples on solid media, solidified with agarose, supplemented with different carbon sources, could supply us information about the functional diversity of the bacterial communities. Three soil types were analysed, deriving from wheat field, forest and sandy soils with RISA-APC. The carbon sources were: carboxy-methyl cellulose, xylane, chitin, starch, tributyrine, casein and protocatechuic acid. For the investigation of the heavy metal tolerant bacterial communities, we used YEG media supplemented with $CuSO_4$ or $CdCl_2$. Our RISA-APC method clearly correlated, as regards the complexity of RISA-fingerprints, with the expected basic taxonomical complexity of the soil types and with the carbon search used for preculturing. The RISA-APC method developed in our study proved to be a useful tool for the comparison of different soil types, and for the examination of changes in the soil bacterial community structure, despite the fact

that this approach does not provide bases for a precise species or genus identification. Furthermore, this method could be combined with various statistical methods to analyse these correlations in detail.

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## MALDI-TOF MASS SPECTROMETRY – A HIGH-THROUGHPUT TOOL FOR PROTEIN-BASED IDENTIFICATION OF MICROORGANISMS And ITS TAXONOMIC RESOLUTION

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Methods for rapid identification and differentiation of microorganisms at the levels of genera, species, subspecies and strains are required for medical diagnostics, quality control of biotechnological processes and holdings of culture collections as well as for environmental research. Despite of the enormous potential of sequence information on genes or even complete genomes for the differentiation of microorganisms, DNA-based approaches have not yet satisfied the urgent need for an automated and cost-efficient high-throughput tool for classification and identification of bacteria, yeasts and fungi. Phenotypic markers could not be outcompeted and novel strategies for their efficient analyses have been developed. Early studies revealed the capacity of Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS) for differentiation of intact microbial cells already 15 years ago. The development of standardized protocols for sample preparation, analytical software, automated hardware and comprehensive databases allowed the commercialisation of MALDI-TOF MS identification systems which entered already the market of medical diagnostics and are gaining increasing acceptance in other microbiological applications. MALDI-TOF mass spectra of exponentially growing bacteria are dominated by peaks of ribosomal proteins which are conservative macromolecules with the potential to provide information on the phylogenetic relationship. However, the taxonomic resolution of MALDI-TOF MS and its relevance within the arsenal of diagnostic methods remained to be examined. While the identification of bacterial species turned out to be the strong point of MALDI-TOF MS, only little has been known so far about its discriminatory power for strains, subspecies or taxonomic ranks above the species level. The capabilities and limitations of MALDI-TOF MS for differentiation and identification at different taxonomic levels were evaluated in this study for genera of the suborder *Micrococcineae and* for species, subspecies and strains of the genera *Microbacterium*, *Bifidobacterium*, *Staphylococcus, Campylobacter and Escherichia*. Established methods like sequence analyses of conservative genes, analyses of chemotaxonomic markers, DNA-DNA hybridization and RiboPrinting represent a graduated scale of taxonomic resolution and were used for comparison in order to define the most appropriate areas of application for MALDI-TOF MS.

## LABORATORY DIAGNOSTIC METHODS IN SUSPECTED DISSEMINATED LYME DISEASE: A COMPARISON OF DIFFERENT TECHNIQUES.

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Both direct and non direct laboratory tests for confirmation of *Borrelia burgdorferi* s.l in biological samples obtained from patients with clinical diagnosis of suspected Lyme borreliozis have been in use. In the present study we have compared two direct laboratory tests (PCR/RFLP and cultivation) and two non direct tests ELISA and Western blot) for detection of *Borrelia burgdorferi* s.l in the 26 blood samples of patients with disseminated form of LB. The two methods, PCR and WB clearly showed the highest rate for the confirmation of the disseminated LB. The PCR/RFLP analysis enable us to identify cultures in 10 cases as *B. garinii*, two isolates were *B. afzelii* and one *B. burgdorferi* s.s. In 2 samples a coinfection of *B. garinii* and *B. afzelii* was also detected. In one case we observed coinfection of *B. garinii* and *Anaplasma phagocytophilum*. The electron microscopy of the samples showed an interesting morphology of several cysts and granules around the spirochetal cells. The confirmation of the pathogen in the samples, using cultivation method, electron microscopy and detecting of the pathogen DNA in cultures by PCR-RFLP has often been a reason for the antibiotic treatment. The results of the study presented here identify an additional problem with serology for antibody to *B. burgdorferi* and indicate that a lack of standardization can lead to marked variability of results reported by different methods.

## NEW TARGETS FOR GENETIC STRAIN IMPROVEMENT IN THE CELLULASE PRODUCER *TRICHODERMA REESEI*

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The ascomycete *Trichoderma reesei* is an industrial producer of enzymes for degradation of lignocellulosic polysaccharides and recombinant proteins under the expression signals of the main cellulases. Genes encoding carbohydrate active enzymes (Cazymes) involved in plant cell wall degradation are found in non syntenic regions within the genome of *T. reesei*. Expression of the genes in these so-called Cazyme clusters is regulated by a number of different transcription factors but also controlled by a putative protein methyltransferase LAE1. Deletion of lae1 leads to a loss of expression of the different cellulase and hemicellulase genes under including conditions (cellulose, lactose and sophorose) and results in the inability of the lae1 deletion strain to grow on cellulose as carbon source. Overexpression of lae1 under constitutive expression signals enhanced the expression of these (hemi)cellulase genes on different inducing carbon sources. The data thus provide an explanation for the clustering of genes encoding the major plant cell wall degrading enzymes in the genome of *T. reesei*, and imply that the heterochromatin structure is a major determinant of cellulase gene expression and hence a suitable target for strain improvement.

## INFLAMMATORY CARCINOGESIS: I. GENERATING TUMOR CELLS IN THE INFLAMMASOME

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Chronic inflammatory processes frequently culminate in oncogenesis. Some pathogens possess

"oncogenes" (LMA of EBV; kaposin and G-coupled proteins of HHV-8; E6 & E7 of HPV16; the NFkappaB-driven xenotropic murine leukemia virus-related virus, XMRV, in the prostate; CagA of *H. pylori*; enterotoxigenic *B. fragilis*). Some non-oncogenic pathogens induce cellular oncogenes (the *vav* oncogene by *Mycoplasmataceae*; the c-*ski* oncogene by the liver fluke *Opisthorchis viverrini*). Commonly, the promoters of tumor suppressor genes (p53, RB, PTEN, RUNX3, WEE) [PNAS 108:4908-13 2011] are silenced by epigenetic DNA methylations; histone deacetylations and/or by the neutralization of tumor suppressor gene mRNA transcripts by micro/siRNAs. Exceptionally, the oncogene is activated first and then it induces the inflammatory process (the Ret oncogene in papillary carcinoma of the thyroid) [Nat Rev Cancer 10:2-3 2010]. Most of the inflammatory chemo-, lympho- and cytokines and molecular mediator proteins (the ligand & receptor of the chemokine CXCL12 & CXCR4 stromal-derived growth factor; IL-1beta, IL-6, IL-8; IL-17; intranuclear trasnslocation of NFkappaB for the activations of "inflammatory genes"; TGFbeta, IFNgamma, TNFalfa; high mobility group proteins, etc), as well as the anti-inflammatory agents and mechanisms (ASA, ibuprofen, curcumin, resveratrol, ethyl pyruvate; IL-10, IL-17; cyclooxygenase inhibitors,.etc) have been well recognized. The most commonly activated oncogenes are the c-SRC, RAS, WNT sonic hedgehog (splitting E-cadherin, thus allowing intranuclear transfer of beta-catenin, opposed by the Dickkopf proteins); the insulin-like growth factors; the anti-apoptotic genes, especially Bcl-2/$Bcl_{XL}$, and the genes that sustain stem cells (Myc, Nanog, Sox2 & 4, Moloney virus insertion site, BMI-1, Oct4, etc). The most common "cell survival pathways" solidifying the oncogenic transformation are the STAT and PI3K/AKT. The environments of inflammatory carcinogenesis are referred to as "epigenetic field of cancerization" and "epigenetic switch linking inflammation to cancer." Epigenetically mediated silencing of tumor suppressor genes, or activation of oncogenes may be reversed by demethylations (azacytidine,. etc), or histone deacetylase inhibition (trichostatin, etc). Cells targeted for inflammatory carcinogenesis often linger in the state of autophagy. In these cells the pro-apoptotic beclin and the anti-apoptotic Bcl-2 vie for supremacy. If the autophagic cell dies, the host escapes carcinogenesis. If dsDNA breaks are repaired, mismatched gene fusions occur, and the state of mitotic catastrophe is resolved, the host will have to confront the rise of malignant cells. Cancer cells will overcome immune reactions (immune T cells; cytolytic antibodies) of the host by generating Treg cells, treacherous traitor T cells, and myeloid-derived suppressor cells. The subverted tumor microenvironment will support the tumor cell colony with growth factors including VEGF for neoangiogenesis. The tumor cells will spread through lymph channels and blood vessels and will kill the host. The tumor cells die with their host, unless extracted for transplantation or *in vitro* culturing. Under these conditions, the tumor cells will prove their immortality and independence from their environment by producing their own growth factor ligands and receptors in autocrine circuitries; this includes androgens and AR in prostate cancer cells. As the Sox 2 gene arises from a long RNA at 3q26.3-27, it exhibits its ancient relationship with RNA, as this was exercised in the "RNA-DNA Virus World" over three billion years ago. In oncogenesis, the stem cell DNA replevies its ancient faculties without severing its auxiliary relationship with its predecessor, the RNA.

## INFLAMMATORY CARCINOGENESIS. II. THE CELLULAR DNA IS INDUCED TO RESUME ITS ANCIENT FORMATION EXISTING IN THE ERA of THE "PRIMORDIAL GENE POOL" and IN THE GENOMES of THE FIRST UNICELLULAR EUKARYOTES

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A primordial gene pool of the "Virus World" existed in the precellular era [1, 2]. Can oncogenic DNA be viewed as the result of a regression in time and structure to the wild-type ancestral independent and immortal DNA of the "primordial gene pool of the Virus World"? Is the somatic cell-dependent subservient DNA the result of a series of "taming mutations" for the acceptance of service, senescence and mortality in the multicellular host of DNA mutations, the unigenic events downgrade the molecule to be functionally insufficient; whereas the oncogenic mutations are unique, multigenic, serial and sequential, resulting in an upgraded molecule being in hypercycle without telomere loss, of high fusogenic capacity, resistant to physico-chemical damage, thus redeeming its forfeited faculties of independence and immortality.

The medical oncologist observes the relative ease and frequency, the stem cell- or the somatic cell-dependent DNA exerts for its oncogenic transformation, as if reversal to its immortal state of existence were avidly pursued, especially in distress. Once established, the oncogenically transformed DNA remains close to indestructible. of many events of distress, the inflammasomes offer all the opportunities to be taken advantage of avidly and with ease by the stem cell- (or somatic cell-) DNA for its reversal to its primordial formations. The main inducers are reactive oxygen and nitrogen species, dsDNA breaks with mismatched gene fusions, tissue hypervascularity and an armada of inflammatory chemo-, lympho- and cytokines. Transformation is initiated by microRNA mediators and epigenetic events (gene methylations; histone deacetylations) acting first reversibly on the promoters of tumor suppressor genes (silencing them) and oncogenes (activating them). In time, the initially reversible epigenetic changes solidify into irreversible constitutive mutations. "Cancer" is diagnosed for a physiological event, originally inscribed into the wild-type primordial DNA for its selfish existence in longevity.

This inherent faculty of the ancestral DNA remains preserved but suppressed in the stem cells and germ cells, and probably even in their descendant, the somatic cell-dependent subservient DNA. Upon distress, be it an inflammatory condition, irradiation or chemical exposure, or plane senescence of its host, the cell-serving DNA exerts a violent and selfish act in an attempt at the replevying of its abandoned faculties, its primordial independence and immortality.

The internal interventions to stop this act are those of the small interfering siRNAs neutralizing mRNA transcripts of the oncogenes, or the epigenetic reactivation of tumor suppressor genes by demethylating their suppressed promoter genes and by reacetylating their histones with deacetylase inhibitors. The external interventions are our attempts at destroying the oncogenes and the cells harboring them with chemo-radiotherapy, at the risk of inducing new second cancers. Small molecular targeted therapy works for the inhibition of the oncogene-encoded kinases. Multiple oncogene activations require further gene- or . oncolytic virotherapy. Some cancer cells yield to therapeutic vaccinations.of their hosts.

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## MONITORING THE EFFECT OF AGRICULTURAL SOIL INOCULATIONS ON THE INDIGENOUS MICROBIAL COMMUNITY BY MOLECULAR BIOLOGICAL METHODS

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Rhizobacteria are capable of stimulating plant growth by improvement of plant nutrition, production of phytohormones, and suppression of disease-causing organisms. In the field, the success of inoculation by plant growth promoting rhizobacteria (PGPR) depends on many environmental factors, the most important of which is the survival of these bacteria in the plant rhizosphere. A mixture of seven PGPR bacteria was applied in agricultural fields 1-2 weeks prior to sowing in the spring of 2010. Previously, the identity of the inoculants was determined using classical microbiological techniques and their PGPR characteristics were verified by laboratory investigation. In order to assess changes occurring within the indigenous soil microbial community as a result of inoculation, we used the terminal restriction fragment length polymorphism (T-RFLP) fingerprinting technique investigating the 16S rRNS gene.

We aimed to design a model system in which the terminal fragments (T-RFs) of the seven bacteria could easily be resolved from each other, therefore, the full length of the 16S rRNA gene in the case of each strain was determined. Three regions of the 16S rRNA gene – containing the variable regions V1-V3; V3-V5 and the V6-V8 – were assessed by computer simulation testing various restriction enzymes. The primer-enzyme combination, distinguishing the seven T-RFs best was chosen containing: the HEX-27F-534R primer pair (V1-V3 region) and the *Hin*6I restriction enzyme, which produces T-RFs of the length of 80, 153, 205, 237, 371, 471 and 524, respectively. The *in silico* results were tested empirically with the strains, which proved the theoretical TRF values. The trouble with detecting the relative abundance of the inoculants is that they are generally occurring soil bacteria. The seven bacteria have to be detected against the background of the T-RFLP fingerprint of the indigenous microbiota. The diversity of eight uninoculated soil samples originating from four agricultural fields was investigated by T-RFLP using three enzymes, of which the results were analyzed in combination. The bacterial community of two control samples (I6-0 and SZG-0) were very distinct from the other six samples. We compared the community fingerprints of the control, uninoculated soil samples (0) and the inoculated samples 1, 3 and 5 weeks after inoculation with the bacterial mixture. We investigated whether we were able to trace changes in the relative abundance of genera closely related to the inoculants. We detected a significant increase in the relative abundance of strain-3 in the case of two soil samples. We observed significant changes in the microbiota, and the separation of the control samples, as well as that of the 1-week samples, was apparent from the hierarchical cluster analysis of the fingerprints of all examined samples (three replicates). The separation of the 3-week and 5-week samples was not as clear.

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## BACTERIOCIN GENES AMONG *E. COLI* STRAINS: THE INCIDENCE DEPENDS ON GENOTYPE AND PHENOTYPE OF *E. COLI* STRAINS

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*E. coli* strains (n = 1670) isolated during years 2007-2010 in the Czech Republic were collected from patients attending regional hospitals in Brno. All strains were tested using commercially available biochemical tests (ENTEROtest 16; Erba Lachema, Ltd., Brno, Czech Republic) for their biochemical activities, and with PCR for the presence to one out of four *E. coli* phylogroups including group A, B1, B2, and D. Moreover, the incidence of 29 bacteriocin-encoding determinants (i.e. colicin and microcin encoding genes) was tested in each individual *E. coli* strain. According to their biochemical activity, 1670 *E. coli* strains clustered into 6 groups containing 709, 50, 47, 744, 103, and 17 strains, respectively. The two largest clusters differed in the incidence of both phylogroups B2 and D and also in the incidence of bacteriocin determinants of mH47, mM, E1 and K. In addition, the incidence of colicinogenic strains was higher in the phylogroup A and B1 ($p < 0.001$) compared to combined B2 and D phylogroup strains. Strains of phylogroup B2 most frequently encoded microcin H47 and strains of phylogroup D microcin B17 and colicin Js.
The incidence of individual bacteriocin encoding determinants is therefore predominantly associated with particular *E. coli* phylogroups and also with *E. coli* strains of specific biochemical activity.

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## THE GENETIC MECHANISMS BEHIND THE CEPHALOSPORIN RESISTANCE OF *BACTEROIDES* SPP.

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*Bacteroides* are generally accepted as β-lactamase producers with high prevalence, and therefore it is not exceptional that they are almost 100% resistant to normal β-lactams. Behind this high resistance rate the ubiquitousness of their normal cephalosporinasae gene, *cepA*, is which was found to be characteristic of the Division I of *B. fragilis* strains. The expression of *cepA* might be enhanced by insertion sequence elements, like IS*1224*, as it is the case for other *Bacteroides* antibiotic resistance genes. We recorded the MIC values for ampicillin, amoxicillin/clavulanate, cefoxitin, piperacillin/tazobactam and imipenem, and production of β-lactamase activities for 40 *Bacteroides* strains together with the *cepA, cfxA, cfiA* and IS*1224* genetic background. A characteristic genomic segment of *cepA* was also detected by PCR. The prevalence of the *cepA*, *cfxA* and *cfiA* β-lactamase genes were 82.1, 17.9 and 3.6 %, respectively, and 93 % of the strains exhibited β-lactamase activity measured by nitrocefin assay. The *cfiA*-positive strains did not harboured the *cepA* gene but there were non-fragilis *Bacteroides* strains that still carried a *cepA* gene. The 'common' chromosomal region of *cepA* was found in 63% of all *cepA*-positive strain including some non-fragilis *Bacteroides* too. To account for very high β-lactamase activity produced by some *cepA*-positive strains IS elements could be detected in the corresponding upstream regions.

## ANTIBIOTIC RESISTANCE AND VIRULENCE MECHANISMS OF BACTEROIDES: A GENOMIC APPROACH

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*Bacteroides* are the best known, among the most extensively investigated and the clinically most relevant human anaerobic pathogens. Beside that anaerobic pathogens despite their significant pathogenic potential are often overlooked are usually important members of the human normal flora of the gut, genito-urinary tract, oral cavity and skin. The *Bacteroides* constitute a significant proportion of the microbiota of the intestine and there they exert important symbiotic functions. Their most frequently isolated and most pathogenic species is *B. fragilis* which is a type strain for anaerobic bacteria and for an early-diverged ancient bacterial phylum, the Bacteroidetes/Chlorobi group. The current genomic and metagenomic approaches for *Bacteroides* served with important observations for their physiology, pathogenicity and antibiotic resistance that bear prominent clinical relevance too. At present we know the genomic sequence of 8 *Bacteroides* strains (including 3 *B. fragilis* type strains) and for 71 other there are ongoing projects. Using the aid of genomics we could understand action of the main pathogenicity factors of *B. fragilis*, the abscess causing capsular polysaccharides (CPSs), their molecular regulation and role in normal circumstances. The invertible nature of some chromosomal regions in addition to the CPS operons was observed too in the case of this organism. The recognition of conjugative transposons (CTns), other than the tetracycline resistance elements, in the case of the production of the *B. fragilis* enterotoxin (CTn*86*) was also a straightforward result. By means of inverse PCR of the carbapenemase gene (*cfiA*) of *B. fragilis* the insertion site and the genetic environment were determined in the genome of the *B. fragilis* NCTC 9343 strain. 16 operons homologous to the *Pseudomonas mexA-oprD* resistance system were detected in the genome of *B. fragilis* NCTC9343 of which the *bmeABC* efflux effectors conferred resistance to several antibiotics. Microarray expression experiments helped to fine-map the genes of *B. fragilis* in its oxidative stress response and to examine the effect of the tetracycline resistance CTns on the expression of chromosomal genes of the *Bacteroides* host. Genome sequencing projects revealed large plasmids besides the chromosomes in the case of *B. fragilis*.

Our studies involving nucleotide sequences and protein expression of a 5.6 kb *Bacteroides* plasmid suggested its role in interaction of the carrying *Bacteroides* with the host. The methagenomics on the investigation of the composition of the microbiota of the intestinal tract in normal and in 'disturbed' circumstances also revealed the importance of *Bacteroides* and complex interaction of the participants possibly influencing the overall physiology of the human body.

## EFFECT OF BIOGAS-DIGESTATE ON DEHYDROGENASE ENZYME ACTIVITY IN DIFFERENT TYPE OF SOILS.

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Using different soil fertilizer materials, as manure is becoming less available, and application of the inorganic fertilizers raises many questions. Therefore, it is increasingly important using compost and biogas-digestate as soil improvement material. The microbial communities activity ensure the fertility of the soil. The most important is the activity of the dehydrogenase enzyme. This enzyme group

catalyzed the hydrogen transfer in the metabolism of biological oxidation. The aim of this study was to examine the effect of the biogas-digestate on dehydrogenase enzyme activity (DHA) in different type of soils, when two different methods were used. The german standard (ISO 23753-1:20058E) were tried to replace the harmful methanol using hungarian standard (MSZ-08-1721-2-1986). The applied biogas-digastate were taken from Kaposvár Sugar Factory of Hungarian Sugar Ltd. The dose is equal to 16,7 $m^3$ /ha and 533 kg organic matter/ ha. The treatment was performed in three different soils: brown forest soil, calcareous chernozem and carbonate meadow soil. The DHA were measured after the treatment in the 0, 7, 14 and 28 days in both standard besides controls. The results showed the increase of the DHA in all type of soils. We found the ISO standard results are approximately ten times higher, and the method is much more sensitiv, than the hungarian standard. In Hungary the Soil Information and Monitoring System applied the methanol using standard to determine the DHA, hence we can't replace to the german method, because the results, after preliminary validation, are too different. In the case of the carbonate meadow soil the DHA values were after the treatment whit the hungarian standard 3,37 whit the german standard 29,50 mg formazan/ 1 g soil/ 24 h. After 14 days were 3,69 and 32,62 mg formazan/ 1 g soil/ 24 h. Therefore, due to the sensitivity it's worthy to considere to use the german standard to testing the effects of the soil fertilizers.

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## FRUCTOSYL TRANSFERASE ACTIVITY OF PECTINEX ULTRA FOR PRODUCTION OF NOVEL OLIGOSACCHARIDES

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Oligosaccharides with biological background have attracted high interest both scientifically and industrially over the past years. Bioactive oligosaccharides play an important role in molecular recognition events such as immune recognition, cell-cell communication and initiation of microbial pathogenesis. Although there are some oligosaccharides, which have received particular attention because of their favourable features, being low in calories and noncariogenic, and acting as selective energy sources for beneficial microorganisms in the intestinal flora. These facts consequently has stimulated that the biological interest of oligosaccharides is growing very rapidly, and necessitates the development of efficient synthesis reactions. The enzyme-catalyzed synthesis of oligosaccharides represents an interesting alternative allowing the control of both the regioselectivity and the stereochemistry of bond formation. Furthermore in the last some years comes to front the interest of tailor-made oligosaccharides with specific sizes and containing specific glycopyranosyl-residues. Oligosaccharides now can be produces through glycosyltransferase activity of enzymes. This work was focused on creation of transglycosyl bioconversion applied commercial available Pectinex ultra® (Novozyme) from Aspergillus aculeatus mainly containing polygalacturonase. The enzyme reaction was carried out on 60 $^0$C, pH5.5, with enzyme having 1 U/ml hydrolytic activity. Samples were taken at time intervals and boiled for 10 minutes for stop the reaction before analysis. Bioconversion were analysed by measurement of reducing sugar with BCA method, by determination of glucose concentration with GOD-POD method and by monitoring of saccharides with HPLC. Effects of saccharose as donor and different acceptors (mannose, maltose, lactose) on fructosyltransferase activity were investigated. Basically, media with 10 (w/v) % carbohydrate content (5% sucrose donor and 5% acceptor) were applied for investigation of acceptor specificity. Only in the cases of maltose and lactose as acceptors, oligosaccharides were detected. The highest amount of oligosaccharides was

established on 1st day with maltose and between 2nd and 3rd day with lactose. Effects of ratio of donor to acceptor were checked. Optimal ratio of lactose or maltose to saccharose was 1:3. In case of lactose, effects of ratio of enzyme to substrates (20 %, 30 % and 40 % total carbohydrate content) on glycosyltransferase activity were also investigated. It was determined, that with enhancement of substrate concentration, the synthesis of oligosaccharides can improve. Applying 40 (w/v) % substrate concentration and 1:3 ratio of lactose:saccharose 9,78% oligosaccharide (according to total carbohydrate content) was achieved. More studies are needed to enhance yield of oligosaccharides, but these results are very promising for development technology for production of novel oligosaccharides (sugar analogues) that play an important role in food and pharmaceutical industries.

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## CYTOLETHAL DISTENDING TOXIN (CDT-V) OPERON IS FLANKED BY P2-LIKE PHAGE ELEMENTS IN BOVINE *ESCHERICHIA COLI* O157

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Cytolethal distending toxins (CDT) are the prototype of inhibitory cyclomodulins. CDT is considered a relatively new virulence factor detected in several Gram-negative pathogenic bacteria. So far five types of CDT have been recognized in *Escherichia coli* (CDT-I to V). CDT-V is the most recently described variant which was identified in Shiga-toxigenic (STEC), enterohaemorrhagic (EHEC) and enteropathogenic (EPEC) *E. coli* strains of various serotypes. In the present study we cloned and sequenced the cdt-V operon and its flanking regions from an stx- and eae-negative atypical bovine *E. coli* O157:H43 strain. The cdt-V operon showed only some single nucleotide polymorphism (SNP) to the sequenced cdt-V genes, which however did not change the amino acid sequence. The cdt-V operon was flanked by phage-like sequences which showed strong homologies to P2, WPhi and L-413C phage open reading frames (orf). These phage-like orfs were not detected in *E. coli* strains producing CDT-I, CDT-III and CDT-IV or in other pathogenic *E. coli* and in *E. coli* K-12 strains. At the same time the prophage sequences found downstream of the operon characterized eight CDT-V producing strains tested, while the upstream sequences were found only in the bovine *E. coli* O157:H43 strains (n=3). The fact that we could not induce phage from the CDT-V *E. coli* O157 strains suggests that cdt-V, similarly to the other cdt operons, could also be disseminated by transduction, and during the adaptation to the new bacterial host the phage became temperate.

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## CHARACTERIZATION OF ANAL HPV GENOTYPES AMONG HUNGARIAN HIV POSITIVE AND HIV NEGATIVE MSM GROUPS

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The anal cancer is the most common cause of death among the non-AIDS diseases. It's clear that the infection of HPV cause the anal cancer primarily. The men who have sex with men (MSM) are a risk

group of HPV infections, especially in the HIV infected population. The anal intraepithelial neoplasia (AIN) develops more frequently in HIV-positive MSMs and it progress to the anal cancer rapidly. In our study HPV genotype testing was performed on a subset of 92 MSM patient anus samples (79 HIV-positive, 13 HIV-negative) and 43 women cervix samples. 37 different HPV genotypes (including 14 presumed high-risk (HR) types and 23 presumed low-risk (LR) types) could be detected from a sample at the same time using Roche Linear Array assay. In addition to the medical examination the MSM patients also filled in a detailed questionnaire (sexual behaviour, drug use, STI-s). The female cervical samples were collected from histology diagnosed P3 and P4 stage patients of Gynaecological Department. The inform consent was signed by all of the patients. High level of HPV infections among HIV positive patients was observed. 93,5% of men (97,5% HIV-positive, 61,5% HIV-negative) and 65,1% of women were HPV positive. 57,1 % of women had only one genotype, while among the HIV-positive MSM, only 15,1% had one, 17,4 % had two, 20,9 % had three, 8,1% had eight, and 1,2 % had 12 genotypes. Prevalence of HR HPV types were 88,6% of HIV positive men and 46,5% of women, LR HPV types were detected 75,9% in HIV-positive men and 30.2% in women. HPV genotypes CP6108, 59, 11, 68, 70, 83, 39, 72, 35, 69, 82, 40, 71, IS39 were characteristic observed only in MSMs. The high prevalence of HPV infections is clearly influenced by the sexual behaviour of the individual, and HIV-infection. Prevalence of HPV-infection, and negative correlation with CD4 number was observed. HPV59 genotype is significantly correlated with passive and passive/active sexual role. An evidence based overview of the anal HPV co-infection of Hungarian HIV-infected MSMs, the HPV diversity and frequency were investigated. The wide varieties of HPV genotypes were detected and co-infection with multiple genotypes was common. We can conclude that HIV-positive MSMs compose the primary risk group of the anal HPV infection and clinical lesions, and as a final outcome, of anal cancer.

## ISOLATION AND CHARACTERISATION OF *ORNITHOBACTERIUM RHINOTRACHEALE* FROM WILD AND DOMESTICATED BIRDS IN HUNGARY

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Respiratory tract infections are causing considerable economic loss in the poultry industry around the world. Fungi, viruses, and bacteria - acting alone or in combination - are indicated as causes of respiratory diseases, usually influenced by non-infectious factors as well. Clinical signs caused by these pathogens are rather variable and can show quite a resemblance. Isolation and identification of the pathogen is essential for the definitive diagnosis and for an effective treatment. *Ornithobacterium rhinotracheale* has been isolated from chickens, turkeys, pigeons and from a wide range of other avian species. Recently, 18 serotypes (designated A to R) have been determined in agar gel precipitation (AGP) and ELISA tests. Most of the isolates (97 %) represent four major serotypes (A, B, D and E). The majority of the isolates from chicken (94 %) and most of the isolates from turkey (57 %) belong to serotype A. Our work aimed the investigation of the prevalence of *O. rhinotracheale* in wild and domesticated birds in Hungary, isolation of the pathogen and characterisation of the isolated strains. Altogether 388 samples (173 choanal swabs, 135 tracheal swabs and 80 lung samples) were investigated. Twenty *O. rhinotracheale* strains were isolated from 2 pigeons, 4 chickens, 13 turkeys and 1 goshawk (*Accipiter gentilis*). Isolates were identified by strain-specific PCR and analysed in traditional biochemical tests. All strains were oxidase positive and catalase negative, they showed urease activity and produced acid from glucose. They did not produce

indole and had no nitrate reductase activity and gave variable results in lactose and sucrose probe. Antimicrobial susceptibility of *O. rhinotracheale* strains was determined by Kirby-Bauer disk diffusion method. All strains were resistant to nalidixic acid, sulphamethoxazole trimethoprim and sulphonamides, and were susceptible to ampicillin. Amoxicillin and doxycycline were also effective against the majority of isolates. PCR products (a 784 bp fragment of the 16S rRNA gene) of two strains were sequenced. The obtained sequences showed high similarity (99-100 %) to the *O. rhinotracheale* sequences in GenBank. PCR fingerprinting techniques have been used for further characterisation of *O. rhinotracheale* isolates. Three patterns were obtained by ERIC-PCR based on enterobacterial repetitive intergenic consensus sequences and four patterns were recovered by RAPD-PCR (random amplified polymorphic DNA) using a single, universal M13 primer. The latter method might be eligible to replace AGP in serotyping.

## ALTERED CO-EXPRESSION OF CD46 MEASLES VIRUS RECEPTOR ISOFORMS IN OTOSCLEROSIS

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Otosclerosis is a complex bone remodeling disorder of the human otic capsule associated with persistent measles virus infection. The main cellular receptor of measles virus is a transmembrane glycoprotein (MCP, CD46), which has 14 well-described splicing variants. The aim of this study was to investigate the possible involvement of altered CD46 expression pattern of the otic capsule in the the pathologic background of otosclerotic bone remodeling. Surgically removed ankylotic stapes footplates (N=51) were analyzed by histopathological and molecular biological methods, respectively. Measles virus sequences were detected by RT-PCR and alternatively spliced mRNA sequences of CD46 isoforms were amplified by using a nonconventional nested RT-PCR. The expressed CD46 isoforms were identified by sequencing of the PCR products. Pooled protein isolates from otosclerotic and healthy tissues were used for western blot detection of CD46 proteins. The presence of viral RNA was associated exclusively with the histopathological diagnosis of otosclerosis. Concerning CD46 expression pattern, four novel and disease associated CD46 variants were detected in otosclerotic bone. Our findings suggest that the unique CD46 isoform coexpression pattern of otosclerotic bone might determine the susceptibility for persistent measles virus infection in the otic capsule and be the basis of the pathogenesis of otosclerosis.

## INVESTIGATION OF A TRAVEL ASSOCIATED LEGIONNAIRES' DISEASE CLUSTER

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Two related cases of travel associated Legionnaires' disease were reported to the Department of Epidemiology (NationalCenter for Epidemiology) in October 2010. An elderly married couple stayed at a wellness hotel in Komárom-Esztergom County during the incubation period. In case of a travel-associated Legionnaires' disease cluster an epidemiological investigation involving environmental sampling is necessary. Water samples were collected at the potential sites of exposure. The water distribution system was sampled at multiple points including the water outlets in the room where the patients stayed. In the spa centre of the hotel, samples were collected from the pools, after the sand filters (filtered water), and special water features (massage jets, head showers, water spouts). Samples were processed by standard cultivation methods for *Legionella*. Samples collected from the water distribution system and the pools were negative for *Legionella*. However, *Legionella* was detected from a ceiling water spout (3 900 CFU/L *Legionella pneumophila* 1), and from the jacuzzi's filtered water and buffer tank (1 200, 2 000 CFU/L respectively, *Legionella pneumophila* 1 and 2-14). Clinical *Legionella pneumophila* 1 isolate from the fatal case (cultured from the bronchoalveolar lavage of the male patient) was compared to the water isolates from the above samples using rep-PCR, total genome restriction PFGE and monoclonal antibody typing. The clinical and environmental *Legionella pneumophila* 1 isolates were identical by all typing methods and belong to one of the most virulent Mab types (Knoxville). The similarity of the strains confirms the hotel spa centre as the likely source of infection. The operation of the spa centre was suspended; pools and sand filters were disinfected, and repeatedly sampled. *Legionella pneumophila* was only eliminated after two shock disinfection processes. This case draws the attention to the fact that *Legionella* may be a risk factor in spa pools even when the bacteria is absent from the pool water. Sand filters and internal surfaces of the water features can be the reservoirs and thus infective sources of *Legionella*. It is an immediate priority in Hungary to introduce regulation and mandatory monitoring of *Legionella*, especially in aerated warm pools which pose the highest risk of infectious aerosol formation.

## NOVEL CRYPTIC dsRNA-VIRUSES ARE WIDELY DISTRIBUTED IN CAPSICUM CHINENSE PEPPER CULTIVARS

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Symptom-free plants often harbor viruses, and members of two dsRNA-virus families, the cryptoviruses and the endornaviruses are known not to induce symptoms in infected plants. Plant cryptic viruses belong to the *Alphacryptovirus and Betacryptovirus* genera of *Partitiviridae* family, and have a small, segmented dsRNA genome in the size range 1-3 kbp. Cryptic viruses are widespread in horticultural species and may be persist in almost all members of a species/cultivar without being detected. The presence of cryptoviruses has been reported previously in *Capsicum annuum* and *C. frutescens*, but a systematic survey to uncover the persistence and diversity of cryptic viruses in additional Capsicum species has not been carried out., To collect information about the presence of the cryptic viruses we investigated the dsRNA-pattern in a collection of eight *Capsicum* species (*Capsicum annuum*, *C. baccatum*, *C. chacoense*, *C. chinense*, *C.*

*eximium*, *C. frutescens*, C. *praetermissum* and *C. pubescens*) and their cultivars. In *C. chinense*, *C. frutescens*, *C. chacoense and C. annuum* cultivars one or two pairs of small dsRNA bands in the size range 1-3 kbp were found. These dsRNAs probably represent genomic dsRNA of putative cryptic viruses and on the basis of the length of dsRNA pairs at least three different cryptoviruses may occur in these *Capsicum* species. To prove the cryptoviral origin of the dsRNAs detected in *Capsicum chinense*, dsRNA was isolated from '9006' cultivar and a 540 nt-long partial sequence of the viral genome was determined. DsRNA contained an ORF fragment on the plus strand that encodes a predicted protein of 163 amino acid residues. The deduced amino acid sequence of the partial ORF contains four of the six characteristic sequence motifs (motifs V-VIII) of putative RNA-dependent RNA polymerases (RdRp) of RNA viruses. A comparison of the amino acid sequence of the putative RdRp with sequences in the NCBI database revealed highly significant similarities to RdRp of viruses of the *Partitiviridae* family. The deduced amino acid sequence is not identical to the sequence of the RdRp of *Pepper cryptic virus 1* isolated from *Capsicum annuum* cv. 'Jalapeno M'; we found only 57.2 % similarity. Therefore we conclude that the partial dsRNA encodes the RdRp of a novel cryptic virus: *Capsicum chinense cryptic virus*.
This dsRNA-virus is widely distributed; it is present in 80% of tested *Capsicum chinense* cultivars. It is also found in phylogenetically related *Capsicum frutescens* cultivars.

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## PHENOTYPIC PLASTICITY, SPECIES SORTING AND COMPETITION IN BACTERIAL COMMUNITIES FACING ENVIRONMENTAL CHANGES

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Metacommunity theory predicts that dispersal plays a crucial role during community assembly. However, little is known about how dispersing bacteria cope with new environmental conditions and how they compete with local resident communities. To address these questions we implemented a transplant experiment with dialysis bags where bacterial communities from brackish and freshwater samples were incubated either separately or in a 1:1 mix under both environmental conditions. The non-mixed samples allowed us to estimate the effect of the new environment on the respective source community, whereas the mix allowed us to estimate the effect of competition between the dispersed and local communities. After that we repeated the experiment but used water samples that were pre-adapted to the environment they were transplanted into. In both cases the bags were incubated for 3 days and then bacterial abundance, biovolume and production were measured. Active bacterial community composition (ABCC) was determined using 454 sequencing of 16S rRNA. In both cases measured parameters of the non-mixed communities were only affected by the environment, suggesting strong phenotypic plasticity. This was supported by the observation that ABCC in the 1st experiment was mainly influenced by the source but not by the environment. In the 2nd experiment, however, the environment was also significant. These results suggest that the first response of a community to the challenges of a new environment is through phenotypic plasticity, whereas species sorting requires longer adaptation. When both source communities were mixed, the measured parameters differed from expected values based on the performance of the un-mixed samples. Moreover, ABCC analysis showed that generalist taxa that occurred in both original source communities were more successful in the mixes than in un-mixed samples. These results suggest that

competition has significant effects on both bacterial community composition and function.

## INCIDENCE DENSITIES AND SUSCEPTIBILITY OF GRAM-NEGATIVE RODS IN A ROMANIAN TERTIARY HOSPITAL

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To evaluate the susceptibility patterns of Gram-negative rods isolated during a two year period and to monitor the incidence densities of certain epidemiologically important resistance phenotypes. Non-duplicate, clinically significant isolates recovered during 2009 and 2010 were considered, others than urinary tract isolates. Susceptibility results were interpreted according to CLSI 2010 edition. Although there was an increase in 2010 compared to 2009 in the susceptibility of Pseudomonas aeruginosa strains to carbapenems (imipenem: 42% vs. 55%, meropenem 44% vs 53%), piperacillin-tazobactam (36% vs 47%), cefepime (37% vs 46%), these changes were not statistically significant. Incidence densities of carbapenem resistant *Pseudomonas aeruginosa* strains decreased from 22 in 2009 to 13/100.000 hospital bed-days in 2010 ($p<0,05$, t test). Time series analysis revealed a descending trend over the two year period. In *E. coli* strains (127 strains in 2009, 103 strains in 2010) susceptibility increased significantly ($p<0.05$, chi square test) to amikacin (from 75% to 97%), third and fourth generation cephalosporins (from 65% to 75% and from 69% to 83%, respectively). In case of other Enterobacteriaceae there were no significant changes. Incidence density of ESBL producer *E. coli* and *K. pneumoniae* strains increased from 20 in 2009 to 22/100 000 bed-days in 2010 ($p=0,77$, t test). In the second half of 2010 we identified the first four carbapenem-resistant Enterobacteriaceae strains in our hospital (one *Klebsiella pneumoniae* and three *Enterobacter cloacae* strains). In spite of the decreasing tendency of the incidence of some phenotypes, resistance in Gram-negatives is still a major problem in our hospital.
The emergence of strains with new resistance mechanisms is of great concern.

## HIGH RESOLUTION METHYLATION ANALYSIS OF THE HUMAN CD40 PROMOTER IN EPSTEIN-BARR VIRUS (EBV) POSITIVE AND NEGATIVE CELL LINES

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Epstein-Barr virus, a human Gammaherpesvirus, is associated with a series of human neoplasms. Latent membran protein 1 (LMP1), a potent oncoprotein of the virus, activates cellular genes via the NF-kB pathway. Because there are four NF-kB binding sites in the regulatory region of the cellular CD40 promoter, the expression of the CD40 transcripts is potentionally regulated by LMP1. The CD40 gene encodes a transmembran protein, which has an important role in the regulation of various immunological processes. In the current study we analysed the expression and methylation patterns of the CD40 gene in lymphoid and epithelial cell lines. We found a variable CD40 expression level in EBV negative and positive Burkitt's lymphoma (BL) and lymphoblastoid cells. There was a relatively high CD40 mRNA level in EBV latency type III cells (BL41-E95B, CBM1-Ral-STO), characterised

by the presence of EBV nuclear antigenes EBNA1-6 and membrane proteins LMP1, -2A, -2B. CD40 expression was low in the EBV negative BL line BL41 as well as Rael, a latency type I EBV positive cell line expressing only EBNA1. The highest CD40 promoter activity was observed in the EBV positive nasopharyngeal carcinoma cell line C666, that expresses EBNA1 only. There was no CD40 expression, however, in the EBV negative cervix carcinoma cell line HeLa. Analysis of CpG methylation showed that a promoter distal region was highly methylated in all of the lymphoid cell lines studied. In contrast, the same region was hypomethylated in the epithelial cell lines C666 and HeLa. A promoter proximal region containing a series of transcription factor binding sites proved to be hypomethylated in all cell lines except Rael, where two highly methylated CpG dinucleotides were observed. We found five highly methylated CpGs in the first exon of HeLa, the only cell line not expressing CD40. Our data suggest that highly methylated sequences in the first exon of CD40 may contribute to the silencing of the human CD40 promoter.

## SPECIES ASSIGNMENT AND ANTIFUNGAL SUSCEPTIBILITIES OF BLACK ASPERGILLI RECOVERED FROM OTOMYCOSIS CASES IN IRAN AND HUNGARY

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Otomycosis, also known as fungal otitis externa, has been used to describe a fungal infection of the external auditory canal and its associated complications, sometimes involving the middle ear. Many fungal species have been identified as infectious agents in otomycosis, with *Aspergillus* and *Candida* species being the most common. In tropical and subtropical regions, *Aspergillus* is considered to be the predominant causative organism, with *Aspergillus niger* as the most frequently described species. In this study, the species assignment of black aspergilli isolated from otomycosis cases in Iran and Hungary was carried out using sequence analysis of part of the calmodulin gene. The results indicate that *A. niger* is not the only black *Aspergillus* species involved in otomycosis cases. *A. awamori* and *A. tubingensis* are also able to cause ear infections. The species distribution of black Aspergilli responsible for otomycosis was found to be different in the two countries: while *A. niger* dominated in Iran, *A. awamori* was the predominant species in Hungary. Antifungal susceptibility tests have been carried out against 5 antifungal drugs including amphotericin B, fluconazole, itraconazole, ketoconazole and terbinafine.

All isolates were highly susceptible to terbinafine, while exhibited moderate susceptibilities against amphotericin B and ketoconazole. *A. niger* and *A. awamori* were found to have higher MICs for ketoconazole than *A. tubingensis*, in accordance with previous findings.

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## SOIL RESPIRATION AND MICROBIAL ACTIVITY OF UNDISTURBED SOIL COLUMNS UNDER DIFFERENT NITROGEN MANAGEMENT

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In recent years considerable attention has been given to the continuous increase in greenhouse gases being emitted into the environment. As soils are known as a significant sink and source of these gases agricultural practice especially the ploughing and nitrogen management have critical role to control the rate of greenhouse gas emission. While gas emission from the soil surface has been widely investigated, relatively few studies have been made on the transport and concentration of greenhouse gases within the soil profile and the microbial background mechanisms. The aim of the present work was to investigate the surface $CO_2$ fluxes during a vegetation season together with the microbial biomass and microbial activity in undisturbed soil columns. Six undisturbed soil columns were prepared from the set-aside plot in a long-term experiment set up by the Georgikon Faculty of Agriculture of the Pannon University in Keszthely. The columns were 90 cm high and 40 cm in diameter. The soil columns had different treatments: 1) control with no plants; 2) mineral fertilized with no plants; 3) no fertilizer and maize plants; 4) mineral fertilized and maize plants; 5) manure and maize plants; 6) mineral fertilized plus manure and maize plants. Soil surface $CO_2$ fluxes were measured by gas sampling from a closed-chamber inserted into the top of each column at zero and at 30 minute after closure. Gas samples were taken by a gas-tight syringe and injected into evacuated vials. The concentration of $CO_2$ was measured by gas chromatograph. Soil samples were also taken to measure active microbial biomass by substrate-induced respiration (SIR) and microbial activity was measured as fluorescein-diacetate hydrolising activity (FDA). Treatments had significant effect on SIR and FDA although the effects of individual treatments could not be distinguished. Manure treatments caused significantly higher microbial biomass and activity during summer. On the other hand the presence of maize did not appeared in the SIR and FDA values. We established significant correlation between SIR and FDA ($r = 0.596$; $p=0.0001$). Surface $CO_2$ flux was highly influenced by soil temperature. Three peaks of $CO_2$ fluxes were observed during the 141 days period, the first was 9 to 11 days after seeding of maize, the second at day 86 (21 of July) in all treatments while at day 37 (2 of June) only at manure treatments. The mean values of $CO_2$ fluxes varied between 21 and 2052 mg $CO_2 \cdot m^2 \cdot hour^{-1}$. The correlation between surface $CO_2$ flux and SIR was marginally significant ($r = 0.302$; $p=0.073$) while between $CO_2$ flux and FDA was significant ($r = 0.47$; $p=0.004$).

## ISOLATION OF UNCOMMON SPECIES OF CORYNEBACTERIA FROM CLINICAL SAMPLES

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Uncommon starins of corynebacterium isolated at various department of Semmelweis University, Budapest, were identified by the sequencing of the 16S rDNA gene, and by the VITEK automated system. From the 26 isolates 15 proved *C. striatum*, 3 *C. jeikeium*, 2 *C. accolens*, and 1-1 *C. amycolatum, C. minutissimum, C. diphteriae, C. pseudodiphtericum*, respectively. The strains were cultured from the following specimens; wound infection: 8 *C. striatum*, 2 *C. amycolatum*, 1 *C. accolens*, 1 *C. minutissimum*, 1 *C. diphteriae* (non-toxin producer); bronchus lavage: 3 *C. striatum*, blood culture: 3 *C. jeikeium*, 1 *C. minutissimum*; conjunctiva: 1 *C. pseudodiphtericum*; sputum: 1 *C.*

*striatum*; trachea: 1 *C. striatum*; central venous catheter: 1 *C. striatum*; punctatum: 1 *C. striatum*. The genetic relationship between the *C. striatum* and the *C. amycolatum* strains has been investigated by pulsed-field gel electrophoresis (PFGE). To our understanding this is the first report of clusters of *C. striatum* in Hungary and the first isolation of *C. diphteriae* from skin infection.

## FIRST REPORT ON INCN PLASMID-MEDIATED QUINOLONE RESISTANCE DETERMINANT QNRS1 IN PORCINE *ESCHERICHIA COLI* IN EUROPE

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Plasmid-mediated quinolone resistance (PMQR) of enterobacteria encoded by qnr genes is an emerging concern in human and veterinary medicine. Here we aimed to study PMQR of porcine *E. coli* in two large piggeries in Romania and Hungary. The studies identified PMQR E. coli strains in 34% of piglets in the Romanian farm. Clonality of six qnrS1 E. coli strains representing the Romanian pig farm was established by MLST, and the qnrS1 plasmids were characterized by plasmid transfer and by PCR-based replicon typing. The six tested strains were assigned to three different MLST types. All proved to carry IncN plasmids representing the first IncN-borne *qnrS1* gene to be identified in *E. coli* from food producing animals. DNA sequences flanking the qnrS1 gene showed ≥99% homology with the corresponding resistance region of the pINF5 plasmid from *Salmonella Infantis* isolated from chicken carcass and of IncN plasmids from human clinical *E. coli* strains. Thus our data suggests that transfer of qnrS1 plasmids occurs between *Salmonella* and *E. coli* of animal and human origin with pigs representing one of the potential reservoirs. Furthermore we report on identification and characterization of the *qnrS1* gene in porcine *E. coli* for the first time in Europe.

## SCREENING FOR EXOPOLYSACCHARIDES (EPS) SECRETED BY SOIL BACTERIA

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Secretion of bacterial exopolysaccharides (EPS) in the soil is beneficial. Several genera of soil bacteria produce remarkable amount of EPS. The secretion of bacterial EPS provides protective niches for the microbes. Furthermore it is important for colonization and surviving under hostile environmental circumstances. EPS delay the drying of the soil by improving its structure, decrease the boundness of the soil as well as adsorb useful nutrients. EPS provide excellent surface of intensive microbial life. Besides the beneficial effects on the soil based on their broad diversity of chemical composition, they are widely used in the pharmaceutical, food, laundry chemical, biosurfactant and in petroleum industry as oil-drilling mud. They are also functioning as emulsion paints, glue, foil and moreover in Japan it is known that biodegradable cutlery is made of EPS. Some of them such as xanthan, dextran, levan, cyclodextrines, alginate are produced on industrial scale. Due to growing interest in EPS we are keen on investigating them, as they carry great potential of developing various

useful biodegradable products. Efforts were made to produce various EPS in shake flask fermentation applying bacterial strains of Hungarian soil fertilizer products called BactoFil A10, B10 and Cell. EPS were isolated from the fermentation broth and dried. The compositions of the secreted EPS were investigated by high perfomance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC). Based on our experience EPS producing capability of our bacterial strain collection was investigated *in vitro* applying various circumstances and culture media. In the case of EPS producer isolates several fermentation media were tested in order to reach as high yield as possible. One of the best EPS producers has achieved a theoretical yield of 23,1% cultivated in a high saccharose containing medium.

## ANTIBACTERIAL EFFECTS OF SILVER-DOPED PHOTOCATALYST USING VISIBLE LIGHT

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Recently, photocatalysis has received enormous attention for pollution control and indoor air purification. In current systems inexpensive photocatalyst, typically $TiO_2$ is illuminated with an UV source. In order to expand the applications of photocatalysis there is a need to develop materials showing photocatalytic activity above wave-length 380 nm. The deposition of different noble metal nanoparticles on $TiO_2$ photocatalysts has been widely used to extend the light absorption to the visible region. Visible light irradiation combined with Ag-doped photocatalyst is expected to make a significant contribution to the development of an effective surface and air purifier. The Ag-doping yields catalysts that, beside being photochemically active, also possess antibacterial effect. In this work the standard $TiO_2$ (Evonik P25) plasmonic photocatalyst was used as a control, and its antibacterial activities was compared with the Ag-doped version. Our objective was to study also the effect of doping on the optical properties of the standard $TiO_2$ catalyst. Mechanically stable polymer-based composite layers were prepared using the spray coating technique. The antibacterial activities of the nanocomposite layers against different nosocomial pathogens were investigated under irradiation with visible light ($l \geq 380$ nm). Standard culture method was used to measure semi-quantitatively the antibacterial activity against methicillin resistant *Staphylococcus aureus* (ATCC 43300) and *Pseudomonas aeruginosa* (ATCC 27853). The effect of Ag-doped photocatalyst was also investigated in solution on the isolated cell wall components of *Escherichia coli* (DH5α) (isolated in our laboratory) and *Staphylococcus aureus* (obtained from Sigma) by electron microscopic examination. The Ag-doped $TiO_2$ photocatalyst shows much higher photo-oxidation rate than the Evonik P25 $TiO_2$ due to the lower band gap energies. This second generation photocatalyst absorb also the photons in visible wavelength range, because of the plasmon band of silver in also in the visible range. The nanocomposite films were shown antibacterial effect on both test bacteria, however the Ag-doped nanocomposite was more active. The colony forming unit values were decreased by the increasing catalyst surface concentration and light exposure time. However, the antibacterial activities of the photocatalysts differed also according to the supporting polymer-based layers used in the different experiments. The electron microscopic examinations clearly showed the destructive effect of the Ag-doped $TiO_2$ on the sacculus of *Escherichia coli* and *Staphylococcus aureus* in parallel with the exposure time of the visible light.

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## DE NOVO GENOME PROJECT OF THE MYCOTOXIN-DEGRADING *RHODOCOCCUS PYRIDINIVORANS* AK37

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*Rhodococcus* pyridinivorans AK37 is an effective aromatic hydrocarbon- and mycotoxin-degrading soil actinomycete and represents a genus of considerable industrial interest. Up to date only four *Rhodococcus* genome projects were published (*R. jostii*: McLeod et al. 2006, *R. erythropolis* PR4-Sekine et al. 2006, *R. opacus* B4, Yamashita et al. 2007 and R. equi 103S: Letek et al., 2010) and all the investigated species belong to distinct clusters of the rhodococci. *R. pyridinivorans* is loosely associated with the *R. rhodochrous* clade, which group has a significant environmental relevance and known to degrade a wide variety of aromatic ring containing xenobiotic compounds (e.g. mono- and polyaromatic hydrocarbons, endocrine disruptors and mycotoxins). Even so species of this clade have not been subjected to complete genome sequencing yet. The complete genome sequencing was performed with a combined strategy of ABI SOLiD 4 sequencing and Roche 454 sequencing technologies. The usage of this hybrid method enabled to generate few, long contigs, preventing the build up of repetitive low complexity regions. Genomic DNA of *R. pyridinivorans* AK37 was sheared with a Hydroshear instrument to gain approximately 2 kb large fragments for ABI SOLiD and 0,6 kb fragments for the Roche system, respectively. The combined sequencing strategy generated 65 contigs and preliminary investigation of the contigs revealed that *R. pyridinivorans* AK37 has a moderate sized genome, comprising 5,242,905 bp (68% G+C) arranged in a single chromosome without the occurrence of any plasmid. The chromosome contains 5,151 predicted protein-encoding genes which are exceptionally rich in aromatic ring processing enzymes (117) and especially in oxygenases (63). The genome of strain AK37 is most similar- beside the other rhodococci- to those of nocardial and mycobacterial strains. In order to gain a physical map of the entire genome, gaps between the contigs have to be filled. Based on the results of the *de novo* genome project 2D proteome/MALDI MS and transcriptome analyses were performed to investigate the aflatoxin B1 metabolism of strain AK37 in order to reveal the key enzymes of the degradation. Unfortunately identification of any potential enzyme of the degradation pathway was failed and it can be hypothesized that cometabolic pathways or constitutively expressed enzymes are responsible for the aflatoxin B1 degradation in *R. pyridinivorans* AK37. However, the up-regulation of genes required for DNA repair was observed in the presence of aflatoxin B1 and the enzymes were successfully identified.

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## A NOVEL METHOD TO MEASURE LOW YIELD BIOGAS PRODUCTION

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A novel, cheap and easy to use method has been developed to measure low gas yields in laboratory bioreactors, based on the principle of bubble counting via digital imaging techniques and pattern recognition. No external hardware control is required for the measurements, and the device can be turned into a multichannel tool without further detector accessories. The device consists of L-shaped glass pipes, a white tray (as carrier and background behind the pipes) with colour marks making the sight interpretable for a pattern recognition apparatus, dyed liquid, a digital web camera and a computer with a pattern recognition software. The biogas leaves the reactors as bubbles in the glass tubes and the software recognizes bubbles in the camera frame. The method proved to perform outstandingly according to the testing and calibrating measurements against standard gas flow and revealed a short periodicity in the gas yields of two parallel laboratory scale mesophilic biogas reactors providing well analyzable data about them. For exact characterization of the oscillation, the data were converted into Morell wavelet spectra, which showed that every feeding period had a characteristic and similarly shaped wavelet profile, thereby this rhythm must have had an immanent source in the community metabolism.

## DISTRIBUTION OF DIFFERENT IRON UPTAKE SYSTEMS AMONG *KLEBSIELLA PNEUMONIAE* STRAINS ISOLATED FROM WOUND, URINARY AND BLOOD STREAM INFECTIONS

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It is a well known fact that iron uptake systems are parts of the bacterial virulence factor repertoire. Last year at this forum we already presented data on the distribution of different iron uptake systems and other virulence factors of *K. pneumoniae* strains isolated from blood stream infection (BSI) and urinary tract infection (UTI). In the present study we gathered data on iron acquisition systems for *K. pneumoniae* isolated from wound infections (WI), and compared them to our data available for UTI and BSI strains. Enterobactin and aerobactin phenotypes were tested by means of the "cross feeding" method using indicator strains specific for the respective siderophore types. The kfuB and irp1-ip2 genes were looked for by the help of polymerase chain reaction to show the presence of the Klebsiella ferric iron uptake (KFU) and yersiniabactin systems in the isolates. 88 BSI and the same number of UTI isolates, and 110 strains of WI were included into the study. The yersiniabactin system presented with a significantly higher frequency ( $p<0,05$) in WI isolates compared to the UTI isolates (UTI 23,9%, WI 12,7%). Incidence of the remaining three iron uptake systems did not display statistically significant differences among the three clinical isolate types. Enterobactin occurred with the highest frequency (UTI 84,1%, BSI 83%, WI 85,5%) followed by the KFU system (UTI 46,6%, BSI 46,6%, WI 35,5%) and the yersiniabactin system (UTI 23,9%, BSI 15,9%, WI 12,7%) while the aerobactin regime showed the lowest prevalence (UTI 5,7%, BSI 9,1 %, WI 5,5%). Data were consolidated to reveal combinations in iron uptake systems prevalence. Enterobactin as a sole iron uptake system showed significantly higher prevalence in the WI (52,7%) isolates compared two BSI (37,5) and UTI (29,5) isolates, respectively. Similarly, yersiniabactin as sole iron uptake system was significantly more frequent in WI isolates (6,8%) compared to BSI (0,9%) isolates. The prevalence of any of the

siderophore types as a single iron uptake system was analysed also in the context of the three isolate types. The prevalence as a sole iron uptake system was significantly higher in WI isolates (60%) isolates or in BSI isolates (52,5%) than in UTI (37,5%) isolates. The prevalence of isolates possessing more than one iron uptake system was also calculated. This category was significantly more frequent in UTI (56,8%) isolates compared to WI (36,4% ) isolates. According to the revealed tendency UTI isolates had the highest prevalence in carrying multiple iron uptake systems followed by BSI isolates, and eventually the lowest frequency was found in WI isolates. The inverse pattern was found when prevalence of a single iron uptake system was analysed, i.e. WI isolates presented with the highest and UTI isolates with the lowest frequency. These data suggest that having multiple iron acquisition system of *K. pneumoniae* strains may yield an advantage during the formation of urinary tract infections. Further studies are needed to validate this hypothesis.

## EFFECTS OF 2-ARACHIDONYL GLYCEROL ON HUMAN MACROPHAGES DURING *CANDIDA PARAPSILOSIS* INFECTION

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*Candida parapsilosis* (Cp) is an emerging pathogen with an increasing clinical importance. The fungus has several virulence factors that are known to play important role in pathogenesis. One of these factors is the secretion of hydrolytic enzymes such as aspartyl proteases and lipases. Our team has previously shown that lipase mutants have significantly decreased virulence in various infection models, and are killed more efficiently by macrophages. Additionally, primary human PBMC-derived macrophages, when infected by lipase mutant Cp, showed at least two-fold higher cox-2 (cyclooxigenase 2) overexpression compared to those infected with lipase-producing (WT) Cp. COX-2 is responsible for the synthesis of prostaglandins, the key mediators of inflammation. One of the substrates of COX-2 is the 2-arachidonyl glycerol (2-AG), which is produced mainly by monocytes, and is a very important endocannabinoid signaling molecule with immunomodulatory properties. 3D structure analysis of Cp lipase confirmed that 2-AG and the degradation product of 2-AG, AA (arachidonic acid) could be a possible substrate for this particular enzyme. Considering these facts, we investigated the potential role of the endocannabinoid system in fungal infections. We used exogenous 2-AG during infection of primary PBMC-DMs by wild type and lipase-negative mutant Cp cells. After 12 hours of incubation we examined the expression of COX-2 using RT-PCR. The results showed that exogenous 2-AG could increase the COX-2 expression in macrophages infected by WT cells almost by the same level as that was observed in the case of lipase mutant infection. Further experiments including CB2R (cannabinoid receptor 2) antagonist SR144528 indicated that free 2-AG level has a very important role in immune reactions. It is known that 2-AG can bind to CB2R, which is an important part of the endocannabinoid system and is involved in the immune response as well. Emerging evidence also suggest that endocannabinoids can behave as both pro- and anti-inflamatory mediators, but further experiments are necessary to clarify the exact role of the endocannabinoid system in fungal infections.

## COMPLETE GENOME SEQUENCE OF THE LIGNOCELLULOSE-DEGRADING COMPOST INHABITING BACTERIUM, STRAIN K07

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Plant cell wall is the most abundant and renewable source of organic carbon in the biosphere. Microbes adapted to lignocellulose as sole source of energy and carbon express a series of different hydrolases (cellulases, xylanases, mannanases) to degrade this recalcitrant substrate. The most complex hydrolytic enzyme producers -*Saccharophagus degradans, Cellvibrio japonicus, Terendinibacter turnerae*- belong to gamma proteobacteria. Earlier we isolated an outstanding cellulose degrader, compost inhabiting, mesophilic, new species new genus candidate bacterium (strain K07) whose taxonomic position is near to the above mentioned group. Here we report the complete genome sequence of strain K07. The 4,243 -megabase genome (G+C contents 51,26%) is well equipped with genes for plant cell wall degradation. The sequence analysis of the annotated genome reveals 62 predicted glycoside hydrolases (GH) belonging to 28 different GH-family. Many of these enzymes exhibit unusual domain architecture, including the presence of long polyserine linker adaptors (PSL; 25-61aa) between the polysaccharide binding- and hydrolytic-domains. Like *C. japonicus* and *S. degradans* strain K07 also encodes a large set of GH43 putative xylanase enzymes. One part of predicted carbohydrate binding modules of K07 also resemble to *Saccharophagus* while the use of malectin for polysaccharide binding in several GH enzymes is seem to be peculiar feature of strain K07. To sum up the genome of strain K07 is remarkably similar to that of the *S. degradans* and *C. japonicus* consistent with the utilization of lignocellulose as main substrate for these microbes.

## OCCURRENCE AND POPULATION STRUCTURE OF *ASPERGILLUS FLAVUS* ISOLATES INFECTING MAIZE IN SOUTHERN HUNGARY

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*Aspergillus* ear rot of maize is caused by various Climate change affects the occurrence of fungi and their mycotoxins in our foods and feeds. A shift has recently been observed in the occurrence of aflatoxin producers in Europe, with consequent aflatoxin contamination in agricultural products including maize in several European countries not facing this problem before.

Although aflatoxin contamination of agricultural products including maize is not treated as a serious threat to Hungarian agriculture at present, these observations led us to examine the mycobiota of maize seeds collected from Southern Hungarian maize fields in 2007-2008. Based on calmodulin sequence data, the most prevalent species was

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## TESTING ANTIFUNGAL EFFECT OF RAGWEED EXTRACT

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The wormwood-leaved ragweed (*Ambrosia artemisiifolia*) millions of lives made difficult by the effects of allergens from year to year. Pollens themselves not only locally, but from the origin reaching several kilometers can cause problems. In Canada ragweed is known as a herb for hundreds of years. It is suitable for haemostasis and antispasmodics. The letter made brew is used for scalp wash, skin diseases, infected wounds and inflammation of the eye. The biggest pollen productor is the ragweed in the pollen season. Today in Hungary about 5 million hectares of agricultural land contaminated with ragweed. The problem of ragweed at least 60 billion HUF extra expense in the budget. These means direct agricultural damages and public health problems. In agriculture, the main damage caused by water and nutrient absorption. In addition wormwood-leaved ragweed seeds may become mixed with crop seeds, which is negative factor for export. In Europe the ragweed was studied mainly caused by pollen allergy in terms. However, the plant contains biologically active constituents. We want to demonstrate that the ragweed, which is a weed, contains biological active - for example antifungal - compounds. We tested the microbiological activity of ragweed extracts in laboratory experiments. Our extract doesn't contain allergenic Amb a I and Amb a II proteins (harvesting before flowering), so it is not dangerous for humans. The biological activity of ragweed-extracts were tested against *Monilinia laxa* isolated from *Prunus domestica*. In studies we dose the substrates of the plant extract in different quantities and by that "poisoned agar" method examined the inhibitory effect of the active ingredient for fungi development. We did our experiments in classical Petri dishes with medium and we used plant shoots (*Prunus cerasus)* in vitro too. In latter case we sprayed the extract to the leaves, then we infected them with the plant pathogen *Monilinia laxa*. Our results suggest that ragweed contains biologically active substances, which inhibit the growth of fungi, depending on the concentration of active ingredients of the plant, and the time varies depending on the inhibitory effect. The minimum effective concentration was 150 mg agent per Petri dishes. This amount is for 1 week inhibited the *Monilinia laxa*. We detected full fungicidal effect at 450 mg agent per Petri dish above do not have developed the fungus in the month under observation. The inhibitory effect of ragweed has been clearly demonstrated in our experiments. Organic growers probably will use our extracts in plant protection. Part of the defense and treatment costs a wormwood-leaved ragweed processing plant can be built. Harvesting and processing a lot of people would work. To avoid confusion, we do not intend to cultivate wormwood-leaved weed fallow, because, unfortunately, there is plenty of it.

## INDUCTION OF CHITIN SYNTHESIS DURING ECHINOCANDIN B PRODUCTION IN *ASPERGILLUS NIDULANS* VAR. *ROSEUS* ATCC 58397

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Echinocandins are potent antifungal drugs showing fungicidal effect against several *Candida* strains including the azole resistant *Candida albicans* isolates and also showing fungistatic effect against some filamentous fungi including *Aspergillus fumigatus*. As inhibitor of β-1,3-glucan synthase,

echinocandins inhibit the synthesis of the cell wall biopolimer β-1,3-glucans. Interestingly the echinocandin B (ECB) producer *Aspergillus nidulans* var. *roseus* ATCC 58397 was more sensitive to ECB than the non producer *A. nidulans* FGSC A4. In a standard microdilution method the MEC (minimal effective concentration) values for *A. nidulans* var. *roseus* and for *A. nidulans* were 0.5 μg/ml and 2.5 μg/ml, respectively. The high sensitivity of *Aspergillus nidulans* var. *roseus* can be explained well with the low activity of β-1,3-glucan synthase in this strain. The presence of ECB increased significantly the resistance of *Aspergillus nidulans* var. *roseus*. According to RT-PCR experiments ECB induced several genes involved in chitin biosynthesis and altered the activity of some other cell wall biogenesis genes as well.
Changes in the composition of cell wall (increased chitin content under ECB producing conditions) also supported the view that the increased chitin biosynthesis can be responsible – at least partly – for the inducible ECB resistance of *Aspergillus nidulans* var. *roseus*.

## CHARACTERIZATION OF "*ASPERGILLUS NIDULANS* VAR. *ROSEUS*" ATCC 58397

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The isolate *Aspergillus* (*Emericella*) *nidulans* var. *roseus* ATCC 58397 is industrially important due to its production of the antifungal compound echinocandin B (ECB) Beside of ECB, *A. nidulans* var. *roseus* also produces the carcinogenic sterigmatocystin, which is chemically related to the well-known aflatoxins. This strain was characterized as an ECB-producer *A. nidulans*-like fungus, having smooth-walled ascospores like *A. nidulans* but showing slow growth at 25 °C similar to *Emericella rugulosa*. The taxonomical position of *A. nidulans* var. *roseus* - similarly to the other subspecies from the *Aspergillus* genera – still remains questionable, and therefore even the name "*A. nidulans* var. *roseus*" is not a validly published name. Polyphasic characterization of the echinocandin B producer *Aspergillus nidulans* var. *roseus* ATCC 58397 strain was carried out to elucidate its taxonomical status. According to its carbon source utilization and secondary metabolite spectrum as well as the partial β-tubulin, calmodulin and γ-actin gene sequences *A. nidulans* var. *roseus* belongs to the *Emericella rugulosa* species.
Interestingly, auxotroph mutants of *Aspergillus nidulans* var. *roseus* ATCC 58397, *E. rugulosa* CBS 171.71 and CBS 133.60 formed stable heterokaryons on minimal medium with several *A. nidulans* strains and in the case of *A. nidulans* var. *roseus* even cleistothecia were developed.

## PRODUCTION OF GLUTAMINASE A BY *ASPERGILLUS NIDULANS*

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Glutaminases (EC 3.5.1.2) catalyze the hydrolytic deamidation of L-glutamine resulting in the release of ammonium and production of L-glutamate. Glutaminase is crucial for fermentation of soy sauce and several other traditional oriental foods including fish sauce, soybean, and pork sausage. High glutaminase activity increases the amount of glutamate one of the most important flavor components.

Like asparaginases, glutaminases have also received attention with respect to their therapeutic application for treatment of leukaemia. Although the genome of *Aspergillus nidulans* contains a glutaminase gene the production of this enzyme has not been observed so far. In our experiments, *A. nidulans* FGSC A26 possessed high extracellular glutaminase activity in submerged cultures. The enzyme was encoded by the ANID_04809 gene and beside of glutaminase activity showed significant γ-glutamyl transpeptidase activity as well. The ANID_04809 gene was induced only in carbon starving cultures. The produced protein could bound to the cell wall and the formation of ChiB autolytic chitinase (but not of the EngA autolytic β-1,3-endoglucanase) was needed for extracellular appearance of glutaminase activities. Systematic monitoring of carbon starving cultures can be a useful technique to identify further glutaminase producer strains and new fungal glutaminases of potential industrial importance. Glutaminase A formation of *A. nidulans* is a good example that autolysis (production of cell wall hydrolyzing enzymes) can enhance the release of cell wall bound products to the fermentation broth. Developing hyperautolytic mutants or mutants overexpressing cell wall hydrolyzing enzymes can be a promising way of strain improvement.

## PRESENCE OF PILI IN *STREPTOCOCCUS PNEUMONIAE*: CLINICAL SPECIMENS AND ISOLATES FROM HEALTHY CARRIERS

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We have tested 100 clinical strains, isolated at different routine laboratories in Hungary between 2002-2008, as well as 156 strains that derived from healthy children attending day-care centres (2009-2010), all well characterised earlier. Boiled bacterial colonies were used as template. We have used previously described primers for the PCR detection of pili. Among the clinical strains, the pilus was present in 24 cases (24.0%). Seven of these derived from invasive infections, and the rest from respiratory specimens. Half of them came from small children (<4,5 y), and half from adults (41-84 y). The majority of the strains was resistant (R) to macrolides, and had elevated MICs to penicillin (0.25-1.5 mg/L). Their serotypes were: 6 (n=11), 14 (n=7), 19F (n=3), 11A (n=2) and 23F (n=1). Among the carried isolates, only 15.4% (n=24) were pilus positive. These were of the following serotypes: 6 (n=10), 19F (n=6), 14 (n=3), 19A (n=2), 15B (n=1), 3 (n=1) and 18C (n=1). Although the majority of the carried strains was sensitive to antibiotics, nearly half of the pilus + strains was also R to macrolides and non-susceptible to penicillin. Out of the 24 children, only 2 were previously vaccinated with Prevenar. The rate of pilus positive clinical strains correlates well with international data, but it was significantly lower in the carried strains. This suggests that pili are required more for invasion rather than merely for colonisation. Very probably there is no direct correlation between pili and resistance, but rather we found pili only in certain resistant serotypes. These were almost all (45/48) vaccine-types (with the dominance of serotype 6), except for 3 strains (11A or 15B). The conjugate vaccines seem to be quite effective in preventing colonisation with pilus positive strains, hence preventing subsequent invasion in the body.

## DEGRADATION OF ETHYLENETHIOUREA BY SOIL BACTERIA

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Ethylenethiourea (ETU) is a major degradation product of the widely used ethylenebisdithiocarbamate (EBDC) fungicides. These compounds are very instable and in aqueous solution spontaneously decompose within two weeks. Their spontaneous degradation product, the mutagenic and carcinogenic ethylenethiourea (ETU) is more stable and its accelerated degradation by soil microbes would be highly desirable. For this purpose we tried to isolate ETU degrading bacteria from distinct soil and water samples by enrichment technique. Up-growing bacterial communities could only detect if ETU was used as nitrogen source. The isolated strains from the enriched communities were not able to use ETU as sole source of carbon but were able to use ETU as sole source of nitrogen. To follow the ETU consumption from the culture media of the strains we developed and optimized a new colorimetric measurement. This method is based on the fact that 2,6-dichloroquinone-chloroimide gives with ETU a pinkish-yellow product. The amount of ETU with this method could be measured at 405 nm wavelength either in microtiter-plate photometer. The best strains proved to be *Bacillus subtilis* and *Pseudomonas fluorescens* based on molecular level identification using sequences of their 16S ribosomal RNA genes. We made a survey regarding the ETU transforming ability in the genus *Bacillus* and *Pseudomonas* and investigated more than 40 *Bacillus* and more than 60 *Pseudomonas* strains deposited in our bacterium collection. About 10% of the investigated strains were able to degrade 100 mg/l ETU within 10 days of culturing, but 1000 mg/l ETU concentration proved to be full inhibitory for all strains.

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## ORIGIN OF HUMAN VIRUSES

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The presentation will include: i. three general theories on origin of viruses; ii. mechanisms of virus evolution; iii. three types of human viruses: inherited viruses, new acquisitions from (domesticated) animals, zoonotic viruses; iv. emerging and reemerging human virus infections; v. prospects to eradicate viruses other than smallpox; vi. evolution of flaviviruses; vii. how viruses and their vectors are spreading to new areas; viii. effect of climate change on viral diversity, evolution and spread; ix. future prospects of human virus infections.

## ANTIMICROBIAL ACTIVITY OF THYMI AETHEROLEUMS EXTRACTED FROM DIFFERENT *THYMUS* SPECIES

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Antimicrobial activity of essential oils obtained from different *Thymus* species (*T. vulgaris, T. serpyllum, T. pulegioides* and *T. glabrescens*) by distillation using the Neo-Clevenger method was studied. *Thymus* species are rich in volatile oils; phenolic compounds thymol and carvacrol are

considered as dominant volatile compounds. The antimicrobial activity of Thymi aetheroleums was tested against two Gram-negative and two Gram-positive human pathogenic bacteria *Pseudomonas aeruginosa, Cronobacter sakazakii, Listeria monocytogenes* and *Streptococcus pyogenes*, respectively. The opportunistic human pathogenic yeast *Candida albicans* and the saprophytic yeast *Saccharomyces cerevisiae* were also involved in the investigations. Antimicrobial activity of aetheroleums was tested by agar diffusion method and dispersing them in melted agar medium. Formation of inhibition zones and suppression of surface growth were compared considering the different aetheroleums and microorganisms. Volatile compounds of the four different aetheroleums were separated by thin layer chromatography. The following volatile compounds were identified in the chromatograms: borneol (Rf. 0.22), geranyol (Rf. 0.31), linalool (Rf. 0.44), thymol (Rf. 0.67), linalyil acetate (Rf. 0.8) and bornyl acetate (Rf. 0.91). *Pseudomonas aeruginosa, Listeria monocytogenes* and *Streptococcus pyogenes* were highly and equally sensitive to the inhibitory activity of the aetheroleums, while *Cronobacter sakazakii* had limited sensitivity. Sensitivity of the two yeast strains was similar to that of *Chronobacter sakazakii* but *Saccharomyces cerevisiae* proved to be a little more sensitive than *Candida albicans*. Inhibitory activity of the aetheroleums was found directly correlating with the thymol content of the aetheroleums. From this respect *T. vulgaris* and *T. serpyllum* were the best species.

Based on the experimental results it can be concluded that volatile compounds of the essential oil extracts of *Thymus* species are very efficient disinfectants and can be applied in vaporizers against bacteria and yeasts present in the air, body surface, mucous membranes and environment. However, antimicrobial activity of different *Thymus* species could be significantly different, what was found to be in relation with the thymol content of the investigated *Thymus* species.

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## MYCOBIOTA AND FUMONISIN CONTENT OF FIGS AND DATES PURCHASED IN HUNGARY

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Fumonisins are carcinogenic mycotoxins which were originally identified in *Fusarium verticillioides*. According to recent findings, fumonisins are also produced by some black *Aspergillus* species including and Aspergilli are able to produce fumonisins in high quantities on agar media with low water activities. Data on the occurrence and role of this species in fumonisin contamination of agricultural products with high sugar content are needed to clarify the importance of *A. niger* and *A. awamori* in human health. In this study, we examined the mycobiota and fumonisin contamination of dried fig and date samples collected in Hungarian outlets.

The samples came from Iran and Tunesia. Black Aspergilli could be isolated from all except one of the fig samples. Apart from black Aspergilli, the aflatoxigenic *A. flavus* was also identified in most of the samples. Regarding dates, black Aspergilli could be recovered from all samples. Some of the samples were also contaminated by *A. flavus*. Species assignment of the isolates was carried out using sequence analysis of part of their calmodulin gene. *A. tubingensis* dominated on both figs and dates, however, A. niger could also be isolated from fig samples. Fumonisin content of the figs was examined using reversed-phase high-performance liquid chromatography/electrospray ionization –

ion trap mass spectrometry. Only one of the fig samples was found to be contaminated by fumonisins at a rate of 150 g/kg. The fumonisin isomers detected include fumonisins $B_{1-4}$, and some small isoforms. Regarding dates, all samples were found to be contaminated at rates ranges between 700-7000 g/kg. The fumonisin isomers detected were the same as those observed in figs, although fumonisin B1 was the predominant isoform detected in dates.

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## *ASPERGILLUS AWAMORI* CAUSES BLACK MOLD ROT AND FUMONISIN CONTAMINATION OF ONIONS IN HUNGARY

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Black mold rot caused by black Aspergilli is an important post-harvest disease of onion worldwide. Usually *Aspergillus niger* is cited as the causative agent based on morphological criteria. Isolates of this species and its close relative, *A. awamori* have been found to be able to produce both ochratoxins and/or fumonisins. In this study, the mycobiota and fumonisin contamination of mouldy onion bulbs purchased in Hungary were examined. All except one of the examined mouldy samples were found to be contaminated with black Aspergilli. Black Aspergilli could be isolated both from the outer dry and the inner fleshy scales of onion bulbs. Species assignment of the isolates was carried out using sequence analysis of part of the calmodulin gene. Sequence data revealed that all black Aspergilli isolated from onions belong to the *Aspergillus awamori* species, which species has recently been revalidated as a taxon closely related to *A. niger* based on multilocus sequence data and AFLP analysis. The range of fumonisin isomers present in the onion samples was also examined using reversed-phase high-performance liquid chromatography/electrospray ionization – ion trap mass spectrometry. Two of the examined onion samples were found to be contaminated with fumonisins at a relatively low rate (about 0.3 mg $kg^{-1}$). This is the first report on fumonisin contamination of onion bulbs. The fumonisin isomers observed include fumonisins $B_{2-4}$, 3-epi-$FB_4$, iso-$FB_1$ and an iso-$FB_{2,3}$ form. The range of fumonisin isomers detected in the onion bulbs indicates that probably *A. awamori* is responsible both for black mold rot and fumonisin contamination of onions in Hungary. Examination of the level of ochratoxin contamination of the onion samples is in progress.

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## STRUCTURE-EFFICACY RELATIONSHIP IN *RHIZOPUS MICROSPORUS* VAR. *OLIGOSPORUS* ANTIBIOTIC PEPTIDE

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Today, there is a substantial demand for new, safely applicable antimicrobial agents, because of the increasing number of the microbial infections. The antibiotic peptide secreted by *Rhizopus microsporus* var. *oligosporus* (ABP) is interesting in this respect, because it has an antimicrobial

effect against some Gram-positive bacteria, and can be safely used in food fermentation, or in the agriculture. In its mature form, it is a small molecular mass (~5.1 kDa), basic (pI=8.6) protein consisting of 49 amino acids, which contains two domains and is stabilized by five disulfide bridges. The tertiary structure of the protein is very similar to the defensin-like molecules: 2 filaments connected by one β-sheet. It has been produced by heterologous expression system in Pichia pastoris. For understanding the connection between the structure of ABP and efficacy, mutant ABPs with different structure were created via in vitro, random, PCR-based mutagenesis. In the next step, the ABP, and two mutant forms of the peptide, which showed difference in their predicted structures compared to the original ABP, were produced in a heterologous expression system by *P. pastoris* (EasySelect Pichia Expression Kit, Invitrogen). The antimicrobial activity of the partially purified proteins was investigated under different environmental conditions (temperature, pH, and proteinase treatment). Based on our investigations, it is supposed that the increasing number of the β-sheets and/or filament regions in the protein structure implicates the stability under different pH and temperature conditions and improves the antimicrobial activity. After the understanding the antimicrobial effect and further animal and plant model experiments (pharmacokinetic properties, toxicity) ABP and its structural mutant variants will be promising compounds as biopesticides, food preservatives, and pharmaceutical agents in the future.

## NEW GENERATION SEQUENCING OF THE *CHLAMYDIA TRACHOMATIS* INFECTED AND INTERFERON-GAMMA TREATED HUMAN NEUTROPHIL GRANULOCYTE TRANSCRIPTOME

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The neutrophil granulocyte is a key cell type involved in the *Chlamydia trachomatis (C. trachomatis)* mediated inflammation. Interferon-γ (IFN-γ) is the major cytokine in the elimination of *chlamydia infection*. Even though the importance of neutrophil granulocytes, the impact of *C. trachomatis* infection and IFN-γ on the neutrophil gene expression has not been studied. New generation sequencing was used to discover the impact of the *Chlamydia trachomatis* D infection and IFN-γ on the transcriptome of human neutrophil granulocytes. Our data showed that the *C. trachomatis* infection altered the expression of host genes involved in inflammation and innate immunity. These genes included matrix metalloproteinases, complement cascade members and different proinflammatory cytokines. Interestingly, we found that several adaptive immunity related genes were also induced including members of the antigen processing and presentation machinery and T-cell chemokines MIG, I-TAC, IP-10 and RANTES. Protein level measurement of MHC-I, CXCL9 and CXCL10 expression was supported the new-generation sequencing data. Our experiments support the role of neutrophil granulocytes in the *C. trachomatis* mediated inflammation, and also highlight their involvement in antigen presentation, T-cell recruitment and activation.

## THE EFFECTS OF SUBSTRATE CHANGE ON THE OPERATION OF A WASTEWATER SLUDGE DIGESTER

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Investigating the microbial communities of biogas reactors is a highly relevant area of microbiological studies, since biogas is one of the primary alternative energy sources of today and digesters form an integral part of modern waste management techniques. Three laboratory scale sludge digesters were fed with different substrates during the course of our experiment. Community structure was studied with PLFA and DGGE analysis in order to determine whether a change in substrate elicits a shift in the composition of the microbial community. The gas output was measured by a newly developed bubble counting method. One reactor was set up as control, while the other two were treated with different substrates as parallel experiments. The treated digesters were first fed with protein, then starch and finally sunflower oil. 75 percent of the dry mass of the originally used concentrated wastewater sludge was replaced with the alternate substrate. Characteristics specific to the substrate applied were observed on the gas output graphs, suggesting that, at least on a physiological level, the community did react. Changes were also observed in the distribution of chemotaxonomic and genetic markers, meaning that the change in substrate affected the abundance of the community constituents. Based on the results of the cluster analysis of fatty acid biomarkers all the samples from the control reactor proved to be similar, as was to be expected. Samples taken from the treated reactors during the feeding with oil proved to be similar to the controls, suggesting sunflower oil is not utilized by the microbes. This was underlined by the comparative analysis of the molecular markers and the reduced gas output. The samples taken during the treatment with starch and protein form a common group on the dendrogram. While the treatment with protein instantly raised the gas output, according to the gas output graphs the utilisation of starch commences only after a longer period of adaptation. There was, however, considerable difference in the length of this incubation period between the two treated digesters. The results of the DGGE analysis also highlight a difference in community composition before and after treatment with starch.

## ROLE OF MORPHOLOGICAL CHANGES IN THE VIRULENCE OF *CRYPTOCOCCUS NEOFORMANS* AND *CRYPTOCOCCUS GATTII*

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*Cryptococcus* genus comprises different species which are causative agents of fatal diseases, especially meningitis. Among them, *Cryptococcus neoformans* and *Cryptococcus gattii* are the species more frequently isolated from patients. *Cryptococcus neoformans* is largely associated with HIV infections, although in the last years new predisposing factors have been described. Their incidence is particularly devastating in developing countries, and it is estimated that they cause more than 650.000 deaths per year. In contrast, *Cryptococcus gattii* is more prevalent among immunocompentent individuals. *Cryptococcus* possesses a polysaccharide capsule around the cell body which is considered the main virulence factor of the yeast, since it exerts a large number of effects on the host immune response. In addition, during infection, *Cryptococcus* undergoes two important morphological changes, which are observed at different times of the infection. During the first hours of interaction with the host, the capsule suffers an increase in its size. We have observed that this increase confers resistance to stress condition. The second morphological changes occurs after a few weeks of infection, and it involves a significant increase in the total size of the cell, which yields the appearance of cells with a diameter larger than 30 microns, which we have denominated as

"giant cells". The formation of these forms is associated with significant changes in the cell, which affect both the cell body and the capsule. The presence of these cells poses a problem for the immune system, since they cannot be phagocytosed. In our group we are investigating the role of these morphological changes in the virulence of *Cryptococcus neoformans* and *C. gattii*. We are trying to identify genes involved in the regulation of capsule growth and on the formation of giant cells, using genomic approaches. We have identified several candidate genes that could be involved in capsule regulation, and we are testing their phenotypes in vitro and in vivo. In addition, we are using the non-conventional host *Galleria mellonella* (greater wax moth) to investigate their role in virulence. We have found that after infection *C. neoformans*, the yeast cells similar morphological changes as those observed in mammals. Using this model, we are also testing the importance of capsule growth and giant cell formation in the virulence of *C. neoformans*. Finally, we are investigating the role of morphogenesis in *Cryptococcus gattii*, and our preliminary results indicate this species induces more efficiently the formation of giant cells, compared to *C. neoformans*. This particular feature could have important consequences, because it might provide a mechanism by which *C. gattii* is able to infect immunocompromised individuals.