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WHICH MEMBER OF THE ANTIBIOTIC CLASS SHOULD WE USE FIRST?

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The choice of which antibiotic to use is complicated by the rapid rise in the proportion of resistant strains. Traditional views about the emergence and spread of resistance often are still focussed on the mutation rates and the introduction of mobile DNA elements. Resistance has two components; the development of resistance in real time and the spread of closely-related resistant strains (often clonal) from one patient to another, subsequently leading to the dissemination from one healthcare facility to another. The containment of this latter spread is often an infection control problem rather than a formulary issue. The control of resistance emergence is, however, often directly dependent on the choice antibiotic given. It has been known for some time, that higher doses of antibiotics would prevent the emergence of resistance and that sub-inhibitory concentrations facilitate its appearance. This has been quantified to identify the mutant prevention concentration, which identifies that certain members, often the most active, of a class of antibiotic are more capable at preventing resistance emergence than the rest. This has been shown with quinolones, where less active members of this drug class have been able to select widespread resistance. Examples of this have been the use of nalidixic acid to treat *Shigella* infection in the developing world, resulting in widespread ciprofloxacin resistance and the emergence of fluoroquinolone resistance in *Acinetobacter* spp. Similarly, the emergence of vancomycin resistance in Enterococcus spp has been attributed the use of the growth promoter avoparcin, also a glycopeptide. Less obvious examples where less active members of a drug class have been instrumental in resistance emergence, for the drug class as a whole, are seen with the extended-spectrum beta-lactamases (ESBLs) and, more latterly, the carbapenemases. The early emergence of the ESBLs in *Klebsiella* spp has been correlated with the use of slow-penetrating, and thus less active, cephalosporins, such as ceftazidime. Similarly, there is evidence that the less active carbapenems could select resistance not only to themselves but also to the more active members of this drug class; for example, the introduction of drugs such as ertapenem may select carbapenem-resistant strains, particularly in non-fermenting bacteria, although this drug would not be targeted against them.

Often we do not have a choice as to which antibiotic within a drug class we should use. However, when we do have a choice, there may well be a flaw in the adage that, we should start with the less active members first so that the more active ones may be used later. Resistance in a drug class often applies to all members of it but, even if it does not, the success of other members of the drug class may be compromised as resistance to the initial drugs used may be precursors to the rest. Resistance is extremely difficult to eradicate and mostly we are unsuccessful. So attempts should be made to prevent its emergence in the first place. With this in mind, in many cases it would be preferable initially to use the more active members of a drug class in order to suppress the development of resistance, using less active members of the class when the risk of resistance is known to be low.

TESTING THE PHENOL DEGRADING ABILITY OF STRAINS ISOLATED FROM ACTIVATED SLUDGE OF INDUSTRIAL WASTEWATER

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Industrial wastewater of a coke-oven plant contains several organic and inorganic pollutants - including aromatic cyclic compounds, thiocyanate, nitrate, ammonium - in concentrations that are toxic to the environment. A laboratory model system was constructed for the biological purification of chemically pre-treated wastewater. Previous studies based on total DNA community analysis showed high diversity of bacteria that may account for the degradation of phenols and thiocyanate. The aim of this study was the experimental testing of the phenol degradation using isolated strains.
More than hundred strains were isolated from activated sludge with spread plate technique applying inorganic phenol and thiocyanate containing media. Strains were grouped according to their ARDRA (Amplified Ribosomal DNA Restriction Analysis) patterns and with one or two strains from each group sequence analysis was carried out. The phenol degradation ability of the selected strains was tested in liquid cultures measuring the optical density and the phenol concentration (colorimetric method using 4-amino-antipyrine). Several strains (*Pseudomonas*, *Alcaligenes*, *Comamonas* etc.) proved to be able to remove phenol from the inorganic liquid medium containing phenol as the sole carbon source, some of them even in elevated concentration.

DETECTION OF HANTAVIRUSES IN HUNGARIAN SHREW (MAMMALIA: SORICIDAE) SAMPLES

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Hantaviruses are rodent-borne viruses of the Bunyaviridae family. Some of them are important zoonotic agents causing haemorrhagic fevers and renal failures. In central Europe Belgrade-Dobrava and Puumula viruses are the most important human pathogen hanatviruses. Certain wild mouse and vole species are the main reservoirs of them. Shrews (Soricidae) are widespread small insectivorous mammals, which are frequent in the tropical and temperate zones of the Earth. Hantavirus-related antigens were detected several years ago in European shrew species in the USSR and in Yugoslavia; and hantaviruses were isolated from far-eastern shrew samples. Virus isolation attempts from European shrew samples were, however, so far unsuccessful. Recently a novel hantavirus, the Seewis (SWSV) virus was detected by molecular methods in European common shrew (Sorex araneus) samples collected in Switzerland. Phylogenetically, SWSV grouped with other recently identified shrew-borne hantaviruses. In this study shrew samples collected in central European countries were tested for the presence of hantaviruses, using universal hantavirus RT-PCR assays. Samples were obtained from Hungary, between 1997 and 2005. Hantavirus nucleic acid was detected in six Sorex araneus specimen. Phylogenetic analysis of the sequences revealed clustering of the strains corresponding with their geographic origins. The investigations revealed that the hantavirus infection rate of central European shrews is relatively high. Clinical symptoms and pathological lesions were not recorded; therefore shrews are probable reservoir hosts of these viruses. Because shrew-

hantavirus related human infections were not detected so far, the zoonotic potential and possible public health impact of these viruses shall further be investigated.

MOLECULAR DIVERSITY OF THE ARBUSCULAR MYCORRHIZAL FUNGI OF *JUNIPERUS COMMUNIS* IN SEMIARID SANDY GRASSLANDS OF THE GREAT HUNGARIAN PLAIN

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Arbuscular mycorrhiza (AM) is a mutualistic symbiosis formed by the majority of the terrestrial plants and the members of the fungal phylum Glomeromycota. Although some data about the AM fungi (AMF) of the Carpathian Basin have been published, a comprehensive molecular diversity study of the AMF of natural habitats in the area is missing. The main aim of the work presented here was to study the in planta molecular diversity of AMF colonizing *Juniperus communis* in semiarid sandy grasslands on the Great Hungarian Plain.
Our sampling sites were the grasslands close to Bugac, Fülöpháza and Tatárszentgyörgy. The roots of ten trees were sampled at each sampling site. The samples have been collected three times a year in spring, summer and autumn since the autumn 2006. After total DNA extraction from the roots, an approximately 550 bp long part of the SSU region of the nrDNA was amplified using the AMF specific NS31-AM1 primer pair. The amplicons were cloned into pGEM-T Easy Vector and transformed into JM109 E. coli competent cells. 30 insert containing clones were selected for each sample for subsequent RFLP analysis using AluI, HinfI and TaqI enzymes. Several clones of each RFLP type were sequenced on both strands. After checking the electrophoregrams, the sequences were analysed using appropriate programs (Staden Program Package, ClustalX, MultAlin, Mega4.0, BLAST). More than five hundred clones gained from AMF colonizing *Juniperus communis* have been screened and more than three hundred have been sequenced up to now. No nrDNA sequences of non-Glomeromycota fungi was detected. Our results show that the AMF colonizing *Juniperus communis* on the Great Hungarian Plain belong to at least 10 distinct phylotypes. Majority of these phylotypes clustered into the *Glomus* GroupAb and GroupAc, but some lineages grouped into the families Gigasporaceae and Diversisporaceae. Some of the sequences formed distinct lineages when sequences of known glomeromycotan taxa had been also included into the phylogenetic analyses. We may assume that those phylotypes represent undiscribed arbuscular mycorrhizal fungi.

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DEVELOPMENT OF A REAL TIME QUANTITATIVE PCR ASSAY BASED ON PRIMER-PROBE ENERGY TRANSFER FOR THE DETECTION OF PRRSV IN CLINICAL SAMPLES

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most rapidly evolving

RNA viruses. For the detection of the virus, several molecular diagnostic methods have already been reported, however, one of the most commonly used primer pair is proved to amplify non-PRRSV sequences under routine diagnostic laboratory conditions. The other risk factor is the continuously changing genetic material of the virus, therefore, based on the sequence analysis of the emerging new strains, constant update on the molecular techniques is needed. The real time quantitative RT-PCR methods using hydrolising (TaqMan®) probes are very sensitive even to a single mismatch on the probe-target region. The system, we developed is based on FRET: the 3' end labelled probe will attach next to the 5' end labelled reverse primer, and energy will be transferred from the donor electrophore (FAM) to the acceptor (Texas Red) resulting in detectable and measurable light emission in positive cases. The intensity of the light signal depends on the quantity of the template in the mixture. The advantage of this method is that it uses polymerase enzyme that do not have exonuclease activity, thus perfect match is not needed between the probe and the target region. The sensitivity of the assay was measured using the Lelystad European reference strain, the Ingelvac MLV™ North American vaccine strain, and a Belarus strain belonging to the European Subtype 3. Known amounts of target RNA, prepared from all three strains were ten fold diluted from 10^{12} to one copy/µl. The assay was able to detect even the final dilution (one copy) in all three cases. The specificity was controlled using 35 different Hungarian Type 1, and Type 2 strains. To exclude the cross reactivity of the system porcine circovirus type 2, swine influenza virus (H3N2, and H1N1), classical swine fever virus, porcine respiratory corona virus, Aujeszky disease virus, porcine parvovirus, porcine cytomegalovirus positive samples were also tested and resulted negative.
The melting point analysis revealed, that increasing number of mismatches will lead to decrease in the melting point of the product, and 5' end mutations have less effect in the melting point changes.

TICK-BORNE ENCEPHALITIS OUTBREAKS THROUGH RAW MILK CONSUMPTION IN HUNGARY

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The average yearly number of tick-borne encephalitis (TBE) cases in Hungary was around 280 until 1997 when the number of cases dropped and remained on a significantly lower level (average ~80/year). The most likely reason for this dramatic decrease seems to be an abiotic factor (since that time hospitals have to pay for the serological tests), but other factors may also play a role. The number of TBE patients in Hungary in 2007 was also low: altogether 62 persons were diagnosed by virological laboratory methods. However, this was the first year when nearly half of the TBE patients acquired alimentary viral infection. Thirty TBE patients were detected having consumed raw goat-milk, without a history of tick-bite in the critical time.
The first milk-borne outbreak started in May, with 5 confirmed patients. Serological data of 8 examined goats of the suspect source farm showed that 2 goats underwent the virus infection, but only one was identified with a recent infection. The second outbreak started in August and resulted in 25 TBE patients. From the suspected farm 75 goats were examined for the presence of TBEV antibodies. One animal out of the 4 seropositives suffered recent infection. The largest milk-borne TBE outbreak occurred in Hungary in 1992, with 26 cases, but it did not constitute such high proportion of clinical TBE cases. This suggests that the real number of patients infected through tick-bite was in fact higher in 2007, but remained unknown. The milk-borne outbreaks with high number

of patients in a short time arose the interest of clinicians, epidemiologists, veterinarians and the media as well, and consequently were investigated extensively. Our experiences show that more attention should be paid to this alimentary route of infection, especially because of the increasing popularity of „natural foods" including raw goat milk in Europe.

EBV LATENCY SPECIFIC EXPRESSION OF LAMIN A/C IN HUMAN LYMPHOID CELL LINES

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Lamin A/C proteins are members of the nuclear lamina forming a supporting meshwork in the nuclear periphery. Lamin A and its truncated form, lamin C contribute to gene regulation, RNA-splicing and delivery of complexes to their place of function, i.e. they serve as a platform for molecular processes. Lamin A/C proteins are usually not expressed in cells of hematopoietic origin. In our experiment we used the method of Western Blotting to examine the protein level of lamin A and C in B-cell lines harbouring Epstein-Barr virus (EBV) genomes of different latency types. We found that in cell lines with the most restricted EBV latency (latency I) lamin A/C were hardly detectable. In contrary, in lymphoblastoid cell lines (latency III) and in the NPC cell line C666-1 (latency II), lamin A/C were highly expressed. Further studies are in progress to elucidate the potential role of latent EBV products in differential lamin A/C expression.

PSEUDOMONAS AND OTHER OPPORTUNISTIC PATHOGENS IN HOSPITALS' WATER DISTRIBUTION SYSTEMS

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Nosocomial infection is one of the major risk factors for hospitalized patients. A potential – and usually underrated – source of infection is the water distribution system. A previous study using molecular techniques has shown the presence of various opportunistic pathogens in drinking water samples from a hospital. Aim of the presence study was (1) to compare the presence of facultative pathogens in 3 hospitals using both culture dependent and molecular techniques, (2) to characterize the isolates of the most frequently detected species, *Pseudomonas aeruginosa* in detail.
Five 10 L samples were collected at each hospital. Samples were concentrated by filtration, and processed by (1) cultivation on selective-differentiating media (2) DNA extraction followed by taxon-specific PCR and denaturing gradient gel electrophoresis. *Pseudomonas aeruginosa* was detected according to MSZ EN 12780:2003. For characterization of *Pseudomonas aeruginosa*, a total of 30 strains collected from 10 hospitals were used; strains were characterized by BOX-PCR.
Presence of most relevant, potentially water-borne nosocomial pathogens was investigated, namely *Acinetobacter, Burkholderia, Sphingomonas, Legionella pneumophila, Stenotrophomonas maltophilia, Pseudomonas aeruginosa*. Colonies with characteristic morphology of the target species were isolated and confirmed by taxon specific PCR. Results showed high frequency of false positive. Taxon specific PCR directly from total community DNA was successful for *Legionella, Pseudomonas* and *Stenotrophomonas*. Microbial diversity was highly variable between and within

hospitals by both culture dependent and independent techniques. DGGE was successful for 2 of the 3 hospitals. *Pseudomonas aeruginosa* was detected one of the most frequently detected species, counts were exceeding 1000 CFU/100 mL in some samples. Isolates were highly diverse according to their BOX-PCR fingerprint. However, identictal patterns were observed for strains of different origin as well, while often more types were isolated from a distribution system. Results suggest that Ps. aeruginosa colonizes tap faucets in high numbers, while in the pipelines the counts remain low.

EMERGENCE OF "-OMICS" AND OTHER TECHNOLOGIES FOR THE CURRENT DATA DELUGE REQUIRE A NEW WAY OF THINKING FROM MICROBIOLOGISTS

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Technological advances, the "-omics" technologies and the exponential increase of computing power have generated a dramatic accumulation of microbiology data. Hand in hand with new techniques of data mining, new disciplines such as Bioinformatics, Computational Biology, Systems Biology and Predictive Microbiology have sprung out of these developments. The sheer data deluge made traditional microbiologists change their attitude towards numerical and computational sciences and spectacular results have been achieved by applying complexity-centred techniques to microbiology problems. The increase in the amount of data has negative effects, too. It is easier to get contradictory results all supported by observations. The interpetation of data sets and induced conclusions has become more important than getting to them. Predictive microbiology serves as an example, how important it is to have rigorous foundations to mathematical / statistical techniques applied to food microbiology problems. This process itself is not unexpected and examples of similar developments can be found in the history of science. What is new, however, is the tangible emerging of complexity, in its mathematical sense. Complexity is not the same as complicated-ness. It is at the transition stage between predictability and unpredictability. It is the phenomenon when not only there are many constituents in a system, but the number of links (interactions) between them is also big. We point out in this talk, that the data on interactions in a system is becoming more and more important, which has profound effect on the way we describe and model complex microbiological systems.

SCREENING OF HIV AND HEPATITIS MARKERS IN HUNGARIAN PRISONS

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Nowadays the highest risk groups for hepatitis C (HCV) infection has become the intravenous drug users (IVDU) and prisoners. In Hungary the most imperiled group for HIV infection are homosexuals. IVDU, homosexuality and other risk factors - like tattoo, piercing, promiscuity, alcoholism - as well are present in Hungarian prisons. Prison staff like guards, healthcare worker may also be at greater risk to become infected. We got a chance to screen for HIV and hepatitis markers in jailhouses. The survey was carried out in12 institutes, the participation was voluntary.

We tested more than 3500 blood samples for hepatitis B (HBsAg) and C (antiHCV) markers and

determined HIV Ag/Ab (>3000 prisoners and nerly 500 staff). We used ELISA tests for serological screening and those samples which proved positive for antiHCV, we retested by PCR technique. We found tenfold higher antiHCV prevalence (4,5%) in prisoners than in the staff (0.4%) which is equal to that of the normal population. The HBsAg prevalence was 1,5% in prisoners. We found an HIV positive case as well. The prevalence of infected people was much lower than it was expected by data in literatures. Maybe the epidemiological situation is better in Hungary or those who thought they were positive, did not participate in this program. The ratio of PCR positivity among the antiHCV positive cases was lower than expected, probably because some participants had recieved interferon therapy before. Those who proved to be HCV RNA positive were offered interferon therapy.

FINGERPRINTING TECHNIQUES IN THE EPIDEMIOLOGICAL INVESTIGATION OF LEGIONELLOSIS

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Legionellosis is a mandatory notifiable disease in Hungary. However, there is no legislation concerning environmental monitoring or disease control. In 2007, the Nat. Institute for Environmental Health and the Nat. Centre of Epidemiology have published a Guideline on Legionnaire's Disease and its Prevention which regulates the procedure of epidemiological investigation for travel associated and nosocomial cases of legionellosis. Identification of the infective source through comparative fingerprinting of environmental and clinical is indispensable for the prevention of further infections. Since the publication of the Guideline, 2 confirmed nosocomial and one confirmed travel associated case was reported. Epidemiological investigation including environmental survey and sampling was carried out in accordance with the Guideline. Hot and cold drinking water samples were collected at each site. Legionellae were isolated by membrane filtration on GVPN (ISO 11734:2004). Legionella isolates were confirmed and serotyped by latex- and micro-agglutination. Rep-PCR, whole genome PFGE and intact cell MALDI-TOF MS) was used for identification of the potentially epidemiologically relevant environmental strains. Case #1 was a fatal confirmed nosocomial case in North-Hungary in Jan. 2008. Legionella was present in 88% of the hot water and 80% of the cold water samples collected in the hospital (range was 10-6750 and 10–520CFU/L, respectively). A total of 34 *Legionella* isolates were collected, all of which were L. pn. sg 1. Isolates were identical by all of the applied fingerprinting methods. Results suggest that the entire water distribution system was colonized by a single strain. As clinical *Legionella* isolate was not available in this case, comparison with the environmental strains was not possible. However, the hospital's water system is the most possible source of infection based on high levels of *L. pneumophila* in the majority of water samples and the fact that the patient was hospitalized during the entire incubation period. Case #2 was reported as a fatal presumptive travel associated case of legionellosis in April, 2008. Potential site of infection was a pension in Nógrád County. *Legionella* counts in the hot water samples collected at the site were extremely high (40000-81000 CFU/L), cold water system was not colonized. The 15 environmental isolates were L. pn. sg 1 (11 strains) and 3 (4 strains). Three fingerprint types were differentiated by rep-PCR and MALDI MS. The clinical isolate (L. pn. sg 1) has shown 96% similarity to one of the environmental strains based on its PFGE pattern, which indicates the pension as the probable site of infection. Case #3 was a confirmed nosocomial case in April 2008, involving two hospitals in Pest County. Epidemiological investigation of this case is still in progress. Rep-PCR and MALDI-TOF MS both differentiated isolates at sub-serotype level.

As both methods are considerably faster and less expensive than the "golden standard" techniques PFGE or MLST, they provide efficient pre-screening during epidemiological investigations.

HAS HIV EVOLVED TO INDUCE IMMUNE PATHOGENESIS?

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We present mathematical and simulation models on important aspects of the evolution and pathogenesis of the human immunodeficiency virus (HIV). HIV induces a chronic generalized activation of the immune system, which plays an important role in the pathogenesis of AIDS. We investigated whether this ability of the virus is an evolved (adaptive) trait, likely to be retained during future evolution, or a coincidental side-effect of the recent jump to the new human host species, which might be lost eventually. We argue that selection favours the ability of HIV to induce immune activation at the local sites of infection (e.g. lymph follicles) but not at the systemic level. HIV may benefit from increased immune activation, because it replicates primarily in activated $CD4^+$ T lymphocytes; by raising the level of immune activation, the virus can thus increase its supply of susceptible target cells. We developed mathematical and simulation models to investigate under what conditions the ability to induce immune activation (and increased target cell supply) provides a selective advantage to the virus. We distinguished between systemic immune activation that affects the whole target cell population and local activation that is confined to the neighbourhood of the inducing virus. The former was implemented in a spatially homogeneous mathematical model, while the latter was investigated in a simulation model involving local bursts of infection. In both settings, we assumed that activated target cells are more susceptible to infection. In our models, selection favoured the ability of HIV to induce local but not systemic immune activation. Increased systemic activation increases the total virus level; however, it benefits all virus variants equally and is therefore selectively neutral. In contrast, local sites of infection are colonized by just a few viruses and therefore the benefit of locally increased target cell supply can be reaped largely by the inducer viruses. We thus conclude that the generalized immune activation that is likely responsible for pathogenesis is probably not directly under selection in the evolution of HIV. However, it may arise as a side-effect of local immune activation, which is likely to be under selection. According to our models, the future evolution of HIV may depend on the predominant range of the immune activation effect, and on whether short- and long-range activation effects can be decoupled.

DESCRIPTION OF NEW TYPE FUMONISINS BELONGING TO THE FBX SERIES

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The fumonisins, a group of polyketide-derived, structurally related mycotoxins produced mainly by *Fusarium verticillioides* and *F. proliferatum*, can cause severe diseases, including

leucoencephalomalacia in horses, pulmonary edema in pigs, cancer in rats, moreover, the consumption of fumonisin-contaminated maize and maize-based products has been associated with a high incidence of human esophageal cancer. The fumonisin analogs can be classified into four main groups, identified as the fumonisin series FA, FB, FC, FP and the novel developed group of FD. Fumonisins are characterized by a aminopolyhydroxyalkyl carbon chain containing 19-20 carbon atoms which is mono or diesterified mainly with propane-1, 2 ,3-tricarboxylic acid. By means of liquid chromatography/electrospray ionization ion-trap multistage mass spectrometry (MS) Bartók et al. (2006) detected 37 new fumonisins; among them the backbone of 12 compounds (FBX series) were esterified by other carboxylic acids such as cis-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid. Here we report the detection and partial structural determination of further four new, minor fumonisin mycotoxins belonging in the recently described FBX series by applying a new, highly sensitive ion-trap MS equipment. The masses of the protonated molecules and of the characteristic product ions as well as the characteristic neutral mass losses from the protonated molecules suggested their structure. The relative quantities of the new minor compounds were expressed as percentages of FB1 toxin.

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ROLE OF EPIDEMIOLOGICAL INVESTIGATIONS IN DAILY DUTY OF A CLINICAL MICROBIOLOGY LABORATORY

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Aim of epidemiological investigation of bacterial isolates is to demonstrate any similarity or difference comparing them. These examinations are directed towards clarifying relations between the single isolates or supporting a supposed outbreak. Usually, this process is time-consuming, but in case of community-acquired infections, quick results have less consequence than that of hospital-acquired infections (HAI) are needed. Neither manifestation of a HAI could not be established without a detailed analyse of the strains isolated in one ward or within a short interval. Greater discriminating power is attributed to molecular biology tools but performance of them has been localized to special reference laboratories. Repetitive PCR based DNA chip method Diversilab (bioMérieux) was used at the Microbiology Department of Semmelweis University during a 4-months period to adapt for clinical microbiology purpose. A numbers of 44 *Pseudomonas aeruginosa*, 44 of different *Enterococcus* species, and 86 methicillin resistant *Staphylococcus aureus* (MRSA) strains were examined, as well. Strains were originated from clinical samples. Dendograms created by the homology of sequences of *P. aeruginosa* strains tested showed incidence of one clone between of newborns of one perinatal intensive centrum but majority of the isolates proved to be sporadical. MRSA strains sourced from the Clinic of Cardiovascular Surgery belonged several well-distingushed clones; isolates from invasive infections outlined incidence of South-German and New York clones predominating in Hungary. Enterococcal isolates from animal samples differed significantly from that of human origin but results of earlier performed pulsed field electrophoresis were verified. The 4-hour method is fully automated; no staff of special experiences is needed.

NANOTECHNOLOGY AND FOOD SAFETY

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The ability of microbes to attach different surfaces provides them the possibility to survive under harsh conditions and also defence against different disinfectants, biocides. Surface attached microbes are a threat to the food industry, since they are a constant source of contamination. The investigation of attachment is difficult – generally a combination of traditional methods (staining, epifluorescent microscopy, traditional plating, scanning electron-microscopy) should be applied and different molecular methods (i.e. FISH) can also be useful. Using nanotechnology in the investigation, like atomic force microscopy, quartz crystal nanobalance provides further information on biofilm formation. Biosensor OWL is a useful method to investigate biofilm formation. Attachment and biofilm formation can be inhibited by applying Langmuir-Blodgett nanolayer coatings on surfaces. Application of silver or other metal nanoparticles might also be a useful method for killing microbes, however their mode of action and the effect on the environmental microbiota is far from being clear. Nanotechnological methods have continuously improving possibilities to learn more about the microbes, and might also provide effective tools to control the growth of the harmful microbiota, however its impact on the environment should be carefully analysed.

PHYSIOLOGICAL CHARACTERISATION AND MOLECULAR TYPING OF POULTRY MEAT SPOILING BACTERIA

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In these days for production of safety food and protection of consumers' health one of our most important tasks is the reduction and elimination of microbiological risk. Detection, characterisation and identification of pathogenic and food spoilage microbes in raw and mildly treated foods are significant by reason of increased demand for good quality and safety foods. In case of poultry meat Pseudomonas spp. tend to be the most important spoilage bacteria under aerobic storage conditions, but other groups of bacteria are also important in spoilage. Microbiological changes during refrigeration storage of chicken upper legs at 4°C were analysed in two series of examination. Examination of microscopical cell morphology, Gram-staining and different biochemical tests were performed to identify the bacteria. Further additional analyses like colony morphology on WL nutrient agar, determination of the optimal growing temperature (10, 20, 25, 37 and 42°C) were carried out. Typing of dominant spoiling bacteria was performed with different PCR-based molecular methods, such as RAPD and ARDRA. Forty-seven bacteria were isolated from chilled poultry meat samples. On basis of Gram staining two isolates proved to be Gram-positive, while the remaining strains were Gram-negative. It was established that isolates could grow relatively well at 10°C, they showed excellent growing at 20 and 25°C, but at higher temperature (at 37 and 42°C) multiplication was hardly noticeable, so most of the isolates proved to be psychrotrophic microorganisms. According to the miniaturised identification tests majority of the isolates (thirty of them) were distinguished as the members of *Pseudomonas* genus. The other dominant Gram-negative species were *Aeromonas* spp and *Hafnia alvei*. Gram-positive species occurred only in very small ratio (mainly *Staphylococcus capitis* and *Corynebacterium* sp).
Molecular typing of *Pseudomonas* isolates was performed with RAPD and amplified rDNA restriction analysis. ARDRA was done with the application of AluI, HaeIII and RsaI restriction enzymes. Clusters were created according to the digestion motifs of AluI because the other two

enzymes did not have enough discriminatory power. In the first experiment some isolates had the same patterns as *P. lundensis* type strain, some were similar to *P. fluorescens*. In case of the second experiment four clusters were created and only one of them showed similarity with *P. lundensis* authentic strain. Two different oligonucleotide primers were used in case of RAPD analysis. All of the isolates showed different patterns as the consequence of combination of the two primers, so on the basis of these results isolates seemed to be different strains of *Pseudomonas* species. Molecular analyses were done with the other non- *Pseudomona*s bacteria as well.

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PRODUCTION OF OTM1, A BIOLOGICAL ACTIVE EXTRACELLULAR METABOLITE OF FILAMENTOUS FUNGI

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In our continuing search of bioactive metabolites, mycotoxins which show bactericide or fungicide effects producing by filamentous fungi, we found a potent antifungal compound produced in the culture broth of some fungal strains collected from different culture collections. For the selection of the proper secondary metabolites presenting significant antifungal effect 17 isolates representing 6 different genera (*Micromucor, Mortierella, Mucor, Rhizomucor, Rhizopus* and *Gilbertella*) of the class Zygomycetes were selected. The members of this class have significant agricultural importance as postharvest pathogens of agricultural products and as the most frequently isolated causative agents of fungal rots. Other representatives of this group are known to be opportunistic pathogens of humans and animals. These fungi have a substantial intrinsic resistance to most of the widely used antifungal drugs, thus it is very important to found effective natural metabolites against them. The in vitro antifungal activity of ferment broth of numerous fungal strains was determined with 96-well microtiter plate bioassay by measuring the absorbance of fungal cultures at 620 nm. After the initial testing the active metabolite (OtM1) was identified using the fractionation of ferment broth and standard etalon compounds. The identified molecule is produced by the members of a specific fungal genus, which can cause some significant disease on plants. In our study the kinetics of the OtM1 production were investigated in the case of 20 selected isolates. For the qualification and quantification of the OtM1 an isocratic HPLC method was developed using water and methanol as eluents on a Phenomenex Prodigy C18 column. The detection wavelengths were on 230 nm and 254 nm as well as the column thermostat temperature was 35°C. The repeatability, linearity of the analytical technique was tested and the detection limit as well as the quantitation limit was determined. The peak purities in all samples were checked with the ratio of the two detector channel. Appearance of the OtM1 in time was examined after 1, 2, 3, 4, 5 and 6 days of culturing at four different temperature. For all examined strains the maximum levels of OtM1 were detected on days 5-6 at all temperature, but showed different kinetics and amounts. In general the secretion of the OtM1 had a characteristic temperature dependence. Four strains were found to be interesting because of the high amount of the produced the OtM1. The results of this work can provide excellent producer microbes and optimized fermentation conditions for further investigation of the effect of OtM1.

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IMMUNOHISTOCHEMICAL STUDIES ON TRADITIONAL AND RECENTLY EMERGED QX-LIKE INFECTIOUS BRONCHITIS VIRUS STRAINS IN CHICKEN

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Infectious bronchitis virus (IBV), or chicken coronavirus (*Gallus gallus*), is one of the main causes of economic losses within the poultry industry, affecting the performance of both meat-type and egg-laying birds. Besides replicating in respiratory tissues, IBV is able to multiply in various epithelial cells in the kidneys, oviduct, testes and the alimentary tract. IBV has more than 65 serotypes which vary in their ability to cause lesions in the non-respiratory organs. QX-like IBV, a recently emerged serotype, shows high affinity to the kidneys and oviduct causing “false layer syndrome” in case of early infection. The current study is part of a comprehensive experiment to investigate the pathomechanism and the pathogenicity of QX-like strains, and compare them to well-known serotypes. One day old SPF chickens were inoculated with five different QX-like strains (isolated from different pathological conditions) and with the most widespread M41 and 793/B strains. Samples were taken eight times from the 4th till the 42nd days post infection. Immunohistochemical (IH) analysis was performed on samples previously investigated for histopathological lesions, taken between the 4th and the 14th days post infection. The processed samples were taken from the trachea, lung, glandular stomach, small intestines, kidney, ovarium, oviduct and testes. After IH staining, samples were checked and presence of viral antigen was scored under light microscope.
The presence of viral antigen was detected in most of the organs examined, although marked virus replication could be detected only in the upper respiratory tract and renal tissues. The Chinese, French and Hungarian strains showed the highest affinity for these tissues in comparison to the other QX-like and to the conventional IBV strains. Although disappointingly, a majority of the examined samples were negative for viral antigen, which might be attributed either to the rapid clearance of the virus, or to the lack of viral invasion in these organs.

MONITORING AND POTENTIAL MANIPULATION OF SOIL-BIOLOGICAL PROPERTIES IN THE AGRI-FOOD CHAIN

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The soil is a living entity: its main function, the soil-fertility and also the plant-health are highly dependent on its biological characteristics. To improve the soil-biological status, alternative organics, such as the agricultural and municipal wastes are frequently used. Beside the possible heavy-metals accumulation, care should be given for the abundance and functioning of the beneficial “biofertilizers” providing the most-important macro-, micro-elements for plants. Microbes of food-safety importance should also be considered.
The heavy-metals-accumulation, the abundance of beneficial microsymbionts and potential pathogens were studied in four Hungarian representative soils, amended with industrial and municipial sludge- or sheep-yard-manure compost-types at different doses. Host-plants green-pea

(*Pisum sativum* L) and lettuce (*Lactuca sativa*) were used in the short- (3 months) and long-term (4-years) pot-experiments. The colonization (M%) and functioning (A%) of arbuscular mycorrhizal fungi (AMF) and/or the root-nodulation of N_2-fixing *Rhizobium* bacteria were assessed in the rhizosphere of the hosts. The occurrence of microbes having food-safety importance (i.e. *Salmonella* spp, *E. coli*, *Clostridium* spp…etc.) was monitored. NIR spectroscopy, as a fast and non-destructive method was used for the characterisation and discrimination of biosolid-amended soils. On a long-term basis the abundance of the microsymbionts are reduced both by accumulating heavy metals and also as a consequence of the improved soil-nutrient-availability. The microbes of food safety importance slightly increased as a function of the increasing sludge doses probably due to the nutrient availability within a year, however no accumulation was noticed as a function of time. The NIR spectroscopy proved to be an available tool in the detection of the sewage-sludge application in the arable fields. The importance of monitoring the beneficial microsymbionts and the microbes of food-safety importance in the agricultural area is underlined.

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BACTERIOLOGICAL CHARACTERISATION OF THREE ULTRA PURE COOLING WATER PURIFICATION TANKS BY MEANS OF CULTIVATION AND CULTIVATION INDEPENDENT METHODS

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Many industrial institutions suffer from the microbial contamination of cooling water though they use ultra pure water. Microbes exist and form biofilms on different surfaces of the system. Due to the biofilm formation metals are subjected to microbially-influenced corrosion (MIC). In our study, cooling waters were examined in front of and behind different tanks both in the primer (boyler) and the secunder (turbines) cycle of a Hungarian power plant. Three different water cleaning tanks (VT) were examined: I.VT treats the water from the primary cycle with non-regenerated resin; IV.VT cleans waters from both cycles, containing a long time ago regenerated (2-year-old) resin; V.VT treats the water from the secondary cycle and has been regenerated for 1 year ago. Cultivation was carried out on R2A medium. Total cell number was determined by using epifluorescent microscopy after DAPI dying. For molecular studies, 5-9 litres of water samples were filtered simultaneously, then total DNA isolation and community fingerprint was carried out with T-RFLP. Shannon-Waever diversity indices were calculated based on T-RFs. To identify the dominant bacteria in the samples, previously constructed clone library was used for peak identification. The total cell number results ranged between 10^3-10^4 orders of magnitude. The calculated Shannon-Waever diversity indices ranged between 1.97 to 3.15. In case of I.VT the relatively low total cell counts were in touched with high diversity. The opposite results could be detected in case of the two other samples. Cultivation results revealed similar community structure by means of main descent lineages but showed many differences at species level that could be due to the characteristic features of the different water cleaning tanks. The waters could be characterised by the dominance and the high diversity of actinobacteria (*Microbacterium, Rhodococcus, Micrococcus*). Besides, genera *Ralstonia, Bacillus, Staphylococcus*, and still not cultivated bacteria could be found in all samples. T-RFLP analyses revealed characteristic differences in the profiles and showed shifts in the community structure

compositions in case of all water samples. The samples were dominated by β-proteobacteria (*Sterolibacterium, Methylibium, Polaromonas*). Members of α- proteobacteria (*Sphingomonas, Novosphingobium*), CFB (Bacteroidetes) and Firmicutes also could be observed. In case of I.VT, dominant CFB and peak sized by 284 T-RF as well as taxa belonging to β- and γ-proteobacteria which have been found in inlet water disappeared, except *Methylosinus* sp. of that amount increased in the outlet water. In addition, new phylotypes appeared such as Firmicutes, Chloroflexi, division Verrumicrobia, *Rhodoferax* sp. Concerning the tank IV., inlet water was dominated by peak sized by 284 T-RF which shifted to peak sized by 290 TRF in the outlet water. In case of V.VT., genus *Acidovorax* dominated. The dominance shifting was coupled with decrease of diversity in these two samples.

AUJESZKY'S DISEASE VIRUS AS A TOOL IN VARIOUS BIOLOGICAL DISCIPLINES

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Aujeszky's disease virus (AyV) is not only an animal pathogen but also a model organism for the study of molecular mechanisms of neurotropic herpesviruses. Furthermore, AyV is utilized as a tool in several biological disciplines such as neurobiology, genetics, tumor biology, etc. Specifically, using specific mutations, AyV can be rendered to an oncolytic agent, a gene delivery vector and a transsynaptic tracing tool. This talk presents our newest results in the above fields.

THE INVESTIGATION OF NORMAL AND GAMMA IRRADIATED TSE AGENTS IN DIFFERENT CELL-LINES EXPRESSING CELLULAR PRION

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It is known that in TSEs the disease-associated form of prion proteins (PrP^{Sc}) propagate itself in infected hosts by inducing the conversion of the physiological, cellular form of prion protein (PrP^{c}) into the pathological, disease-associated form. Vilette et al. used rabbit kidney epithelial cell line – RK13 – to create a stable transfected form (Rov9) of a cell line which contains the complete coding sequence of the ovine (sheep) cellular prion protein. The expression of PrP^{c} is inducible by doxycyclin (1 μg/ml). They demonstrate that after inoculation of induced Rov9 cells with ovine PrP^{Sc} cells could be propagated accumulating PrP^{Sc} at increasing levels upto the 14-18. passage. To examine the effect of gamma irradiation we used the same Rov9 cell line as well as transiently, continuously (at every second passage) transfected RK13 and CHO cells expressing ovine and bovine PrP^{c}. We propagated all of the different cell lines to the 6th passage and carried out proteinase K digestion using cell lysates for PAGE and Western blot analysis to detect and distinguish the PrP^{C} and PrP^{Sc}. Inoculation of induced Rov9 cells and the other above mentioned cell lines using irradiated infectious brain homogenates (nominal doses of 50 and 200 kGy) we got almost the same Western blot patterns as in case of non-irradiated samples with the only exception that in every case proteins with lower molecular weights appeared. In all cases our results were very similar to those of Rov9's both in irradiated and non-irradiated samples, but transfection of CHO cells appeared to be more efficient than RK13. We concluded that – similarly to our cell free experiments – gamma irradiation is not capable to influence significantly the conversion capability of PrP^{Sc}.

PHAGETERAPEUTIC PRODUCT AGAINST *CAMPYLOBACTER JEJUNI*

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Campylobacter jejuni is Gram-negative, motile humanpathogen causative agent with bipolar flagella, and twisted cell morphology, belonging to the Campylobacteriaceae family. The organism shows oxidase- and catalase-positive activity, and grows under microaerophil circumstances. It can be characterized and selectively grown on Cefoperasone-Charcoal-Desoxicolat-Citrate (CCDA) agar. The majority of human diseases occur in summer and in autumn. Sources of contamination can be food, surface water, wastewater, domestic animlas, rodentia, birds and stools of both ill or asymptomic people. 95% of poultry are asymptomatic carriers. Currently it is one of the most common causative agent of coccidiosis both in developed and developing countries. Symptomes of the disease are fever, stitch, diarrhea, gall bladder inflammation, urethral infection and in severe cases meningitis. Taking the above mentioned into consideration, our aim is to develop a product containing phages tested to host-specificity, analyzed both morphologically and genetically, which is able to infect *Campylobacter jejuni* and can be used to treat meat products by lowering the cases of infections, thus increasing food safety. Samples were taken from wastewater-treatment plants, poultry- and poultry abattoirs and from wild birds. Treatment does not influence neither food-taste, nor the „bio"-qualification, since phages are isolated from nature, no genetic modification will occur and the product is going to be chemical-free.

BIOLOGICAL DEGRADATION OF AFLATOXIN B1 BY SOIL BACTERIA

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Aflatoxin B1 (AFB1) is one of the most potent natural human carcinogens. Beside its mutagenic nature it also exerts toxic and EDC-disrupting effects on mammals, so elimination of AFB1 from food and feedstock would be of great importance to human and animal health.

In this study biological degradation of AFB1 by different oil decomposer soil inhabiting bacteria originating from the strain collection of Agruniver Holding Kft. was examined in liquid cultures and in cell-free extracts. The investigated 24 strains belong to the *Chryseobacterium, Brevibacterium, Gordonia* and *Rhodococcus* genus. For AFB1 monitoring we used two different approaches: immunoaffinity column cleanup with liquid chromatography (LC) and microtitre plate Enzyme Linked Immunosorbent Assay (ELISA) method. The liquid culture experiment resulted in high reduction of AFB1. R. erythropolis AK35 and strain K4 were able to degrade more than 94% of the AFB1 (2ppm) even within one day of incubation. By the use of 16S rDNA sequence analysis we identified strain K4 as R. erythropolis. Interestingly all the five best AFB1 decomposer strains in this study belong to the same species. The ability of crude cell free extracts to degrade AFB1 was studied under different incubation conditions. Aflatoxin B1 was effectively degraded by cell free extracts of strain AK35 and K4. When cell extracts were incubated with proteinase K or heat shock was applied dramatic activity loss was observed. The binding capacity of the surface of the two rhodococcus strains was also measured. According to our results the physical binding of AFB1 was as low as

1,3%. These results confirm that AFB1 decomposition by R. erythropolis is an enzymatic process. The high degradation rate of AFB1 by R. erythropolis AK35 and K4 indicate potential for bio-detoxification application in food and feed processing.

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BIOGAS PRODUCTION ON VARIOUS ORGANIC WASTES IN A TEST LABORATORY SCALE FERMENTER SERIAL

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Biogas is one of the most promising alternative energy sources, which can be produced from many types of organic waste materials. In this project various aspects of biogas production are studied, including the effects of the substrate composition and operational conditions (pH, temperature, microbial consortia…etc.) and the impact of biofilm formation. The main purpose of this work is to find the optimal parameters for biogas fermentation in a test laboratory scale fermentor serial, which is composed of twelve fermenter units. Each unit has a volume of 1000 cm^3 and was designed in a way that all the important parameters of the biological culture can be investigated and controlled. The amount of the produced biogas can be measured continuously by a special volumetric gas measuring device, which is made of glass and contains water. The biogas changes the water level in the calibrated device, which induces an electric sign. Since this sign belongs to a certain amount of gas the accurate volume of the produced biogas can be easily determined. Experiments were carried out under nitrogen atmosphere at different temperatures on three different types of organic waste (waste produced in alcohol production, grass and compost), on glucose and on saccharose. The composition of the gas is analyzed by gas chromatography. It was found that reliable and successful biogas production can be achieved in the test fermenter.

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PRESENCE OF LYMPHOTROPIC HUMAN HERPESVIRUSES, PAPILLOMAVIRUSES AND TT VIRUS IN AMNIOTIC FLUID TAKEN FROM HEALTHY PREGNANTS BEFORE PARTURITION IN HUNGARY

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Pregnant women were examined following healthy pregnancies at term. Lymphotropic herpesviruses have been found by many authors in the urine and blood of healthy neonates. The published

methodologies of sampling suggested transplacental transmission in addition to the perinatal infection during delivery. The work presented is the first systematic screening of amniotic fluids taken from healthy pregnants at term and tested for the presence of human herpesvirus (HHV) types 4, 5, 6, 7 and 8. The presence of all anogenital papillomavirus genotypes was also examined. Amniotic fluids were sampled before arteficial rupture of membranes using a closed vacutainer system. With the permission of the Committee for Ethics 106 amniotic fluid samples and maternal blood samples were examined.
Both amniotic fluids and blood samples were tested for the presence of DNA of lymphotropic herpesviruses. The DNA of human papillomaviruses and TT virus were tested only in the amniotic fluid samples. The DNA of at least one herpesvirus could be deteced in every fourth amniotic fluid sample and in every eighth blood sample. The prevalence of papillomaviruses was 9 of 106 samples. HHV-4, HHV-5 and HHV-7 were found more frequently in the amniotic fluids than in blood samples (7 to 1). The prevalence of HHV-6 and 8 was higher in the blood samples than in the amniotic fluids.
It is well known, that fetal cells can be detected in the maternal circulation. Recently it has been shown, that maternal cells can be transported into fetal tissues, too. Our hypothesis is that the reactivation of latently harboured viruses occurs following materno-fetal transfer of the lymphocytes. The materno-fetal trasport of reactivated viruses cannot be excluded either.
Further systematic follow up will be required to assess post partum pathological, immunological and possible oncological consequences of the late transplacental viral infections.

PRESENCE OF TT VIRUS IN DIFFERENT ORGANS OF WEANED PIGLETS

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Torque teno virus (TTV) is considered an emerging foodborne virus of pigs threatening human health. This pilot study aimed to reveal the presence of TT virus in different organs of piglets (liver, intestine and blood). Samples of 14 weaned piglets from one large herd were tested for the presence of TTV-DNA. Primers specific for swine TTV were used to test the prevalence. PCR products were cloned and sequenced. The number of positive samples was 5/14, 3/14 and 2/14 in liver, intestine and blood, respectively. All the samples (liver, intestine and blood) were positive only from one piglet. Both the liver and the intestine were positive in case of 2 piglets. Two piglets' livers were found to be positive without the positivity of blood or intestine and a piglet had viremia without positivity in other organs tested. No specific clinical signs were assigned to these pigs. The sequences of the PCR products were determined and the sequences of viruses derived from different organs were compared. TT virus is present in some organs of weaned piglets without clinical signs. The sequence of viruses derived from different organs may be not identical.

EFFECT OF HHV-6A ON MONOCYTES AND HIV-1 R5 VARIANT

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Our aim is to examine the effect of HHV-6A on monocyte differentiation, expression of CCR5, and to study the interaction between HHV-6 and HIV-1. Since in vivo monocytes are targets for HHV-6

latency, we establish latently infected cells by HHV-6A. Monocytes are infected with HHV-6A then cell differentiation, CCR5 expression and susceptibility to R5 variant HIV-1 are studied. Cells infected latently with HHV-6 and mock-infected are superinfected with HIV-1, and viral replications are investigated. Negative isolation of monocytes by antibodies, immunofluorescence assay for HHV-6 antigenes, flow cytometry, real-time PCR and reverse transcriptase assay were used. Expression level of CCR5 increased with cell differentiation on mock and HHV-6 infected monocytes, but amount was less on HHV-6 infected cells. Phenotype (size, morphology) of infected cells was different. HHV-6 antigens were not detected, replication was not productive, genome-equivalent in HHV-6A positive cell culture supernatants did not increase with time.
Superinfection of latently infected cells by HIV-1 did not result in HHV-6A reactivation, but suppressed HIV-1 replication was observed. Our preliminary data suggest that HHV-6A alters monocyte differentiation, CCR5 expression, hence the susceptibility to R5 HIV-1. Suppressed HIV-1 replication in co-infected cells might result from these or other factors which require further studies. HIV-1 superinfection did not result in HHV-6 reactivation, hence other methods (autogenic, allogenic stimulation by uninfected and HIV-1 infected lymphocytes) should be investigated.

TAXONOMIC RECLASSIFICATION OF *CANDIDA STELLATA* STRAINS

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Yeasts identified as *Candida stellata* are frequently associated with overripe and botrytized grapes and can survive in the fermenting must until the completion of vinification.
In earlier years, we isolated high amount of *C. stellata* strains from botrytized wines and grapes. The molecular taxonomic examination of these strains revealed that they belong to *C. zemplinina*. The two species could not be distinguished by conventional assimilation and growth tests. To obtain more data about the taxonomic position of these yeasts, we involved additional 33 isolates from our collection and 43 strains deposited in other collections as strains of *C. stellata*. For their reclassification we used molecular methods such as the PCR-RFLP of the ITS1-5.8S rRNA gene-ITS2 and the sequence analysis of the chromosomal region coding for the D1/D2 domain of the 26S rRNA.
Our results demonstrate that the species name *C. stellata* has been used for yeasts, many of which, in view of recent developments of yeast taxonomy, are not conspecific with the type strain of *C. stellata*. Most strains originally identified as *C. stellata* and examined in this study turned out to belong to species that were not known yet at the time of their isolation, such as *C. zemplinina, C. lactis-condensi, C. davenportii* or *Starmerella bombicola*. In some cases we identified *Saccharomyces* (11-80/CCY 26-10-7, 11-61/DBVPG 4171), *Debaryomyces* (11-78/CCY 26-13-1), *Pichia* (11-65/DBVPG 3826) and *Torulaspora* (11-3/RIVE 3-16-1) among strains deposited in the culture collections. Considerable amount of information published about *C. stellata* in wine-making came from the investigation of DBVPG strains, which were found to be strains of *St. bombicola*. The CBS, RIVE, CECT, FAW, Rbst, Rst strains originated from grape and wine and our isolates turned out to be *C. zemplinina. C. stellata* was not found among yeasts newly isolated from noble rotted grapes and botrytized wines either. The findings indicate that *C. stellata* is far less widespread in grapes and natural wine fermentation than hitherto thought.

MOLECULAR AND PHYSIOLOGICAL IDENTIFICATION OF THE YEASTS ISOLATED FROM FERMENTED RIESLING IN BADACSONY

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In Badacsony wine region the Italian Riesling ithe most significant grape variety. Under specific climatic conditions Riesling grapes left on the vine beyond normal ripeness can develop Noble Rot. The result of the activity of non-toxic mold, *Botrytis cinerea*, is the shriveling of the grapes, the evaporation of much of the juice, and the concentration of the sugar. The wines made from these late-picked or selectively-picked berries have not only incredibly intense and concentrated flavors, but also remarkable life span. The objective of this work was to study the evolution of yeast populations and to describe the indegenous yeast microbiota during spontaneous fermentation of Riesling musts from Badacsony. To investigate the yeast microbiota, we isolated yeast strains from late-harvested fresh must in 2006 and 2007. We sampled the musts several times during the fermentation and determined the colony forming units (cfu) to follow the changes of the yeasts populations. We isolated 557 yeast strains in the two vintages. The taxonomic identification of the isolates was done by conventional yeast identification methods based on morphology, sporulation, utilisation of carbon and nitrogen sources, tolerance to 1 % acetic acid, growth at various temperatures. The molecular analysis was done with representative strains of the species found. The PCR-RFLP of the ITS1-5.8S-ITS2 and NTS2 regions of the rDNA and the MET2 gene and the sequence of the 26S region of the rDNA were analysed. We examined the number and the size of the yeast chromosomes by the method of pulsed field gel electrophoresis (CHEF). The results confirmed that the isolates belonged to *Aureobasidium pullulans, Hanseniaspora uvarum, Metschnikovia pulcherrima, Candida zemplinina* and *Saccharomyces cerevisiae* yeast species. The low-fermentative *Hanseniaspora uvarum* and *Candida zemplinina* were the predominating yeasts at the first stage of the fermentation among the yeasts found. In the middle of the fermentation *S. cerevisiae* yeasts became more abundant, increasing to population of 10^7-10^8 cfu/ml. The other species could not be detected after this period to the end of the fermentation. This tendency was observed in both vintages.

DETERMINATION OF HUMAN PAPILLOMAVIRUSES AND TYPES IN CERVICAL SAMPLES BEFORE THE VACCINATION ERA

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Cervical carcinoma is the tenth leading cause of mortality among women in Hungary. The annual numbers of deaths from 1999 to 2003 varied between 465 and 539. Cervical carcinoma is caused by human papillomavirus (HPV) infections. A multicentre study was performed in 1997 by means of cytology and a nucleic acid hybridization probe (Digene). The average HPV prevalence in women who participated in positive or negative family planning programmes was 17.6%. In another survey, the prevalence of HPV among a selected group of women who took part in a carcinoma screening programme was 43.4%. In four age groups, (I) 12-17, (II) 18-24, (III) 25-34 and (IV) 35-45-year-old women were examined by bimanual, colposcopic, cytological and molecular genetic methods. A

short questionnaire was completed by these subjects. In order to achieve a better comparison of the Hungarian data with those from other countries, a new method, HPV PCR (Roche), was introduced for the detection of HPV in 2006. After hybridization to specific oligonucleotide probes, hybrids were detected by colorimetric determination. In the positive cases, the HPV types were determined by linear array (Roche). Parallel cytological examinations were performed with HPV diagnostic methods. 220 (28.0%) of the 788 samples were positive with the PCR method, and 568 (72.0%) were negative. A higher prevalence of HPV was detected by PCR in comparison with the results of the earlier survey where nucleic acid hybridization was used. The highest HPV prevalence by age 35%, was detected in the 18-24-year-old group. The highest HPV prevalence by education 50%, was detected in the lowest educated patient group (<8 years in elementary school). An increasing number of life time partners increased the prevalence of HPV. In the present study, types 58, 35 and 33 were most common in age groups I and II, while types 16, 58 and 35 were most frequent in age groups III and IV. The higher prevalence may be explained by the enhanced sensitivity of the amplified method. The PCR and the linear array procedure can detect 37 types, but the hybridization method only 18 HPV types. Many carcinoma-prevention screening programmes in Hungary are free of charge. However, statistical surveys have demonstrated that screening programmes among women are not effective. A compulsory vaccination program against HPV infection could possibly decrease the mortality rate from cervical carcinoma among Hungarian women.

GENETIC CHARACTERIZATION OF TYPE 2 CANINE PARVOVIRUS STRAINS FROM HUNGARY

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Type 2 canine parvovirus (CPV2) infection is one of the most frequent causes of death in the young, susceptible canine populations worldwide. The clinical manifestation of the disease is characterized by lethargy, vomiting, leucopenia and diarrhoea. Since its emergence in the late 1970’s, several genotypes have been described. The genotyping of CPV2 strains is based on several key changes in the amino acid sequence of the capsid protein (VP2). Soon after the emergence of the firstly isolated genotype (named CPV2), it was replaced by a different antigenic variant named 2a that could be distinguished by the means of monoclonal antibodies. In the mid 1980’s the virus suffered another single mutation and the new variant (CPV2b) quickly spread around the world. Soon after the new antigenic variants (CPV2a and 2b) have completely replaced the original type 2 and are variously distributed and co-exist in canine populations worldwide. Since then a number of further mutations have been described, some of them associated with antigenic differences. Recent investigations have demonstrated that the recently described CPV2c is progressively replacing other CPV types in the Italian canine population. The CPV2c variants have also been reported in other European countries, as well as in South America and Asia. Although the clinical signs and pathological changes induced by the different genotypes of CPV2 are very similar, several studies demonstrated that sometimes there are significant differences in the severity of these changes. The aim of the study was to determine which CPV2 genotypes are currently present in Hungary. Surprisingly, the genetic and phylogenetic investigations of all these strains revealed that all of them were type 2a CPVs. The study also describes a seemingly stable point mutation that occurred in the VP2 region of some of the Hungarian CPV2 strains, which decisively interferes with the outcome of the previously described MboII-based rapid identification test of type 2c CPV strains, leading to diagnostically false genotyping results. Since nowadays the typing of the CPV2 strains is based mostly on investigation

of the viral genome (RFLP, specific PCRs, and sequencing) instead of investigations of the antigenic properties of the virus, the misleading results of such rapid genotyping tests are of extreme interest.

PREVALENCE AND GENOTYPES OF SEN VIRUS IN HUNGARIAN HEALTHCARE WORKERS

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SEN virus was discovered in Italy in 2000 and was shown to be related to the growing family of TTV-like viruses. Along with TTV (Torque Teno virus), SENV has been classified into the Anellovirus genus within the Circoviridae family. TTV isolates are classified into 5 genogroups and SENV genotypes belong to group 3 along with several TTV isolates. Like TTV, SEN virus can also be transmitted both enterally and parenterally. Two of the eight SENV strains (D and H) have been described as possible candidate viruses for inducing posttransfusion hepatitis, because they have been found to be more common in patients with transfusion-associated non-A to E hepatitis, than in healthy blood donors. The clinical importance of SENV is still uncertain, but the great variability observed in this group of viruses makes it important to study their prevalence and nature in the healthy population. In this study serum samples of 185 healthcare workers of a hospital in Budapest were examined for the presence of SENV DNA using polymerase chain reaction. SENV DNA was found in 132 of the healthcare workers (71,4%). SENV-D and H was detected in 42 (22,7%) and 48 (25,9%) samples, respectively. The detected viruses were genotyped using primers specific for SENV-D and H, and by cloning and sequencing of the PCR products.
Several clones from four samples formed a distinct group in the phylogenetic analysis. They may represent a previously undescribed genotype. Also, the SEN virus DNA sequences carried by a symptomless laboratory healthcare worker were followed up for fifteen years. Two strains persisted and could be detected from 1992 to 2000 and from 1996 to 2005. Other strains caused transient infections and were found in only one of the samples.

THE INFLUENCE OF GAMMA IRRADIATION ON CONVERSION OF SCRAPIE PRION IN CELL FREE SYSTEM USING PMCA METHOD

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In TSE the pathological, protease resistant form of prion protein, termed PrP-Sc appears to propagate itself in infected host by inducing the conversion of its host encoded precursor, PrP-sen, into PrP-Sc. Mechanistic details of the conversion are not understood, but involve direct interaction between PrP-Sc and PrP-sen resulting a growing PrP-Sc multimer. Kosicko and coworkers were the first who found that this conversion is carried out in cell free system as well with strain and species specificities. More recent studies have shown that PrP-Sc formation can be amplified indefinitely in crude brain homogenates using protein misfolding cyclic amplification (PMCA) method. The elimination of PrP-Sc from different biological specimens is of great importance. We investigated the possibility of the application of gamma irradiation for this purpose (50 kGy and 200 kGy) supposing that it decreases the amount of PrP-Sc significantly. In that case it is possible to use PMCA method for the calculation of the effect of irradiation. PMCA method was set up for ovine and bovine TSE

samples using Western blot analysis and the amplification rate was evaluated for normal as well as irradiated brain suspensions. We found that the conversion capability did not decrease in irradiated samples not even if we applied an extreme high dose (200kGy). Our results suggest that gamma irradiation itself is not capable to eliminate the pathological prion protein from biological samples.
On the other hand a special conversion method was elaborated for human non irradiated TSE specimens only for diagnostic purposes. This method used platelet for the source of cellular prion protein and applied a special programmable shaker instead of ultrasonic equipment. It was found that a 24 hour treatment resulted in three times amplification in the amount of human PrP-Sc.

TAGUCHI OPTIMISATION OF A MULTIPLEX PCR

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Recently, more and more laboratories have tried to devise a PCR-derived serotyping method for *Streptococcus pneumoniae* instead of the conventional one, based on antisera. The method described by Brito et al. (JCM, 2003), that we followed, uses a multiplex PCR reaction first, dividing the isolates into 6 different groups based on the detected PCR gel pattern. In order to optimise this crucial step, we decided to use the Taguchi method.

	A cpsB-f : cpsC-r1 : cpsC-r2	B cpsO f/r	C Polymerase (1.25U)	D PCR buffer	E $MgCl_2$	F Nucleotide mix
1	10 : 5 : 5 μM	0.5 μM	Taq	Taq	2 mM	228 μM
2	15 : 7.5 : 7.5 μM	1 μM	Tth	Tth	2.4 mM	400 μM
3	20 : 10 : 5 μM	2 μM	AmpliTaq Gold	AmpliTaq Gold	4 mM	571 μM
4	10 : 5 : 2.5 μM					
5	10 : 5 : 0.5 μM					
6	20 : 10 : 1 μM					

This method can evaluate the individual effect of six parameters (A-F), by performing only 18 experiments, varying the parameter levels in an orthogonal arrangement which suppresses the interactions between them. Each parameter can have 3 different values (levels), except the first one (A), which can have six. From preliminary experiments, we have confirmed that the template DNA should be obtained by brief immersion of bacteria directly into the PCR mix, so we have now preset this parameter, as well as presetting the annealing temperature at 60°C. Hence we have set the following parameters in the optimisation: primer concentrations and their relative ratio (cpsB-f, cpsC-r1, cpsC-r2, cpsO-f and cpsO-r primers), concentration of $MgCl_2$ and nucleotide mix, and the quality of polymerase (Taq, Tth or AmpliTaq Gold) and PCR buffer (see Table). As a result, clear and sharp bands were observed in 5 experiments out of the 18, while the reaction did not work reliably in the other cases or did not work at all. Additionally, we also managed to decrease the amount of two primers 10-fold, with respect to the high concentrations described in the original paper. The best results were achieved if AmpliTaq Gold polymerase was used with its own buffer, Taq polymerase with its own buffer, or Tth polymerase with Taq buffer. Since the optimisation was performed, the multiplex PCR was successfully applied for more than 100 strains.

THE EFFECT OF THE 7-VALENT CONJUGATE VACCINE (PCV7) ON PNEUMOCOCCAL INFECTIONS WORLD-WIDE

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Streptococcus pneumoniae (pneumococcus) is responsible for the death of about 1 million children world-wide every year, causing severe community-acquired pneumonia or invasive diseases. To prevent these infections, several vaccines have been developed. The older 23-valent polysaccharide vaccine (Pneumovax, Merck) is ineffective in children <2 years old, who have the highest burden of disease. however, a new conjugate vaccine (Prevenar, PCV7, Wyeth) was developed, where 7 capsular polysaccharides are conjugated to a carrier protein.
The PCV7 was first introduced in the USA in 2000 and later in several European countries. Many papers have summarised the expected or unpredicted consequences of the vaccination with PCV7.
The PCV7 vaccine coverage of the resident bacteria is around 60-85% in the different countries, depending on the local serotype distribution. The major benefit of the vaccination is the radical decrease in both invasive and non-invasive infections (especially otitis media) caused by the vaccine types (VT), and, to a smaller extent, by all pneumococcal serotypes, and even by other pathogens, e.g. *H. influenzae*. On the other hand, an increase in infections due to non-VT (NVT) has been detected everywhere, a phenomenon called the "replacement disease", largely as a result of the significant expansion of already established clones. Additionally, although the vaccine was able to positively influence pneumococcal resistance due to the suppression of resistant VT clones, increasing antimicrobial resistance among the NVTs remains a concern, as also the 2007 CDC report concluded. A special problem arose with the notoriously-resistant serotype 19A, because serotype 19F, which is part of the vaccine, does not provide sufficient cross protection against it. More worryingly, on several occasions, a vaccine-induced serotype switch has been observed, i.e. VT strains changed their capsular type to a NVT, while retaining their genetic background, resistance or virulence ("vaccine escape"). Nasopharyngeal colonisation plays a very important role in the transmission of pneumococci. A great advantage of PCV7 is, that in contrast to the polysaccharide vaccine, it can significantly reduce the carriage rate. PCV7 has another very important indirect effect: it can provide a very good protection not only in the immunised children, but also, because of herd immunity, in unvaccinated children or adults. The groups that benefit most are the <2 months old infants, who are too young to be vaccinated, and the elderly (>65 years).
Finally it can be concluded that the unpredicted negative effects seem to occur to a much less extent compared to the overall beneficial effects of the conjugate vaccine. Even the cost-effectiveness calculations, used for decisions about vaccine usage in several countries, generally showed reasonable benefits. The introduction of the new conjugate vaccines (PCV10 and PCV13) in the very near future will hopefully overcome the problem associated with the very limited number of serotypes present in PCV7, and help to further decrease the burden of pneumococcal diseases world-wide.

OCCURRENCE OF VAN-C POSITIVE ENTEROCOCCI IN DEBRECEN

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Vancomycin resistant enterococci (VRE) are common nosocomial pathogens however, until now they have been rarely encountered in Hungary. In the present study we demonstrate that the prevalence of patients infected by enterococci carrying the vanC resistance gene is rising in the hospitals of the Medical School at the University of Debrecen. Since January 2004 the Bacteriological Diagnostic Laboratory has been screening VRE on 6 mg/l vancomycin containing BHI agar plate according to NCCLS. Clinical samples collected between January 2004 and July 2008 were found to contain various enterococcus strains. Screen-positive isolates were tested for vancomycin and teicoplanin resistance by E-test. Resistant colonies were also investigated by the Vitek2® system. Multiplex polymerase chain reaction was preformed for the detection of the vancomycin resistance genes: vanA, vanB, vanC1/C2, vanD, vanE and vanG. Restriction digestions by HindIII and SalI enzymes were used to differentiate the vanC1 and vanC2 genes from each other. For species identification PCR was used to detect the Enterococcus faecalis and *Enterococcus faecium* D-alanin-D-alanin ligase (ddl) genes or the *Enterococcus casseliflavus, E. faecalis* and *Enterococcus gallinarum* superoxid dismutase (sodA) genes. Overall we identified the vanC1 resistance gene in 9 clinical samples, and the vanC2 resistance gene in 3 clinical samples. In 2004 we found this gene in one E. faecalis isolate from wound secretion and one *E. casseliflavus* isolate from urine. In 2005 another urine sample was shown to contain both E. faecalis carrying the vanC1, and *E. casseliflavus* carrying the vanC2 resistance genes. In 2007 we found 2 urine samples containing vanC2 positive *E. faecalis* strains. In the same year 1 *E. faecalis* and 1 *E. gallinarum* strains were identified, both from urine, and they carried the vanC1 gene. Finally, in 2008 we found 4 samples containing vanC1 positive *E. gallinarum* strains, which indicates, that the prevalence of *E. gallinarum* is alarmingly increasing. These isolates were cultured from blood, ascites and wound secretion. The relationships between the strains will be investigated by pulsed-field gel electrophoresis.

GENOME STUDY OF A HERPESVIRUS ISOLATED FROM A CHONDROSTEAN FISH (*ACIPENSER TRANSMONTANUS*)

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A new family (Alloherpesviridae) has just been approved for the classification of herpesviruses (HVs) of fish and amphibia lacking gene blocks homologous with those of mammalian, avian or reptilian HVs. To date, only six full genome sequences are available from fish or amphibian HVs: Ictalurid HV-1 (IcHV-1), three isolates of Koi HV-1, and Ranid HV-1 and -2 in addition to some partial sequences from other fish HVs. We study the genome of a virus strain obtained from white sturgeon (*Acipenser transmontanus*) from the Snake River, Idaho State, USA (SRWSHV). The virus was isolated and propagated on an epithelial cell line (WSS-2). Viral genome fragments were randomly cloned into bacterial plasmid and sequenced. Missing regions between cloned fragments were amplified by PCR. Design of PCR primers relied on the fact that every gene identified from SRWSHV was similar, in size, position and orientation, to its counterpart in IcHV-1.
Hitherto, 38 genes were fully sequenced, while partial sequence was determined from 7 additional

genes. The cloned or PCR-amplified parts of the SRWSHV genome were assembled into a more than 60 kb contig that spans from the homologue of ORF 24 of IcHV-1 to ORF 68. There were only a few examples for significant discrepancy compared to the corresponding region of IcHV-1. The homologous proteins, found by the BLASTx program with highest scores in the GenBank, were almost invariably from IcHV-1 or in certain cases, from other fish and frog HVs, but never from mammalian, avian or reptilian HVs. These findings and results of the phylogenetic calculations suggest a common evolutionary origin of HVs of Anamnia, and confirm the decision of the International Committee on Taxonomy of Viruses to create a novel virus family for the classification of these HVs. The establishment of a common order (Herpesvirales) for the three main lineages of HVs (infecting molluscs, anamniote and amniote vertebrates) was also supported by our data.

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ADJUVANT MODULATION OF IMMUNE RESPONSE IN MICE AGAINST LCRE PROTEIN OF *CHLAMYDOPHILA PNEUMONIAE*

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Our aim was to evaluate in mice the immunogenicity and protective effect of *C. pneumoniae* (Cp) LcrE protein combined either with Freund's or Alum adjuvant. LcrE protein is a TTSS component of Cp. LcrE was amplified by PCR and cloned into pET vector carrying His tag. The protein was over-expressed in E. coli HB101 and purified by using HIS-select cartridge. BALB/c mice were immunized s.c. with the purified LcrE protein mixed with Alum or Freund's adjuvants 3 times at 3-week intervals. Two weeks after the last immunization the immunized and naive mice were challenged with $4x10^5$ IFU Cp intranasally. Mice were sacrificed 7 days after infection. Cp was cultured from the lungs. Sera were tested for LcrE-specific IgG, IgG1, IgG2a, IgA by ELISA. IgA and cytokines (IL-6, IFN-γKC and MIP-2) were measured in the lungs. Cellular immune response was assessed after in vitro stimulation of spleen cells with LcrE protein or Cp. The number of IFN- γ producing spleen cells upon LcrE or Cp stimulation was determined by ELISPOT assay and the phenotype of IFN- γ producing spleen cells was investigated after depletion of CD4+ and CD8+ spleen cells. The immunization with both protocols resulted in a significant reduction of the number of viable Cp in the lungs after Cp challenge. Equally high titre LcrE-specific IgG production was detected after immunization with the different adjuvants, although higher IgG2a titre was induced by using Freund's adjuvant and higher level of IgG1 was present after Alum-immunization. LcrE-specific IgA level was higher in both the sera and the lungs after using Freund's adjuvant. KC -2 levels in the lungs proved lower in immunized mice afterand MIP Cp challenge, IL-6, IFN- γ. levels tend to be lower in Freund's adjuvant-immunized mice. LcrE-specific proliferation of spleen cells was detectable after both immunization methods. The number of IFN- γ producing cells was higher in the spleens of LcrE+Alum immunized mice upon LcrE stimulation but was lower when stimulated with live Cp compared to non-immunized or LcrE+Freund's immunized mice. Phenotype of LcrE-specific IFN- γ producing cells was CD4+ in Alum-immunized mice, and CD8+ cells were also detected in Freund's immunized mice. When LcrE protein was given in combination with Alum the most widely used adjuvant in humans, or with Freund's adjuvant which is potent but too reactogenic to use in humans, equal level of protection was detected against Cp infection. Lower IgG2a/IgG1

ratio in Alum-immunized mice suggested a shift towards Th2 type immune response. However the presence of LcrE-specific IFN- γ producing cells in the spleen of LcrE+Alum immunized mice indicates generation of Th1 type response also.
Presence of CD4+ LcrE-specific IFN- γ producing cells is concordant with the described protective role of CD4+ cells in chlamydia infection. Decreased production of inflammatory cytokines following immunization might play a role in protection against tissue damage.

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EFFECT OF CLIMATE CHANGE AND GLOBAL WARMING ON THE MYCOLOGICAL SAFETY OF FOODS

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According to the conclusions/predictions of the Intergovernmental Panel on Climate Change (IPCC), the World Meteorological Organization (WMO), the Hungarian VAHAVA (Change – Impacts – Responses) Project, and the National Strategy on Climate Change accepted by the Hungarian Parliament, the climate change may manifest itself rather disadvantageously in the Carpathian Basin. It could have drawbacks on the agriculture, human- and animal health, and diminish food security and –safety. Considering these problems, the lecture will review as part of the latter aspects the decreasing mycological safety of certain crops and stored products due to increasing opportunity for growth of moulds, particularly toxinogenic species. The main ecophysiological factors influencing growth and toxin formation of moulds will be outlined and effects of warming climate and increased occurrence of meteorological extremes will be discussed. In order to strengthening the scientific basis for prevention or mitigation of such effects, international literature on prospects and some results of predictive modelling of mould growth and mycotoxin production will be surveyed.
Finally, the main research and risk management tasks needed to maintain/improve the mycological safety of foods will be summarized.

MOLECULAR INVESTIGATION OF BIOLOGICAL NITROGEN REMOVAL FROM A COAL-COKING WASTEWATER IN A LABORATORY MODEL SYSTEM

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A controlled laboratory model system was set for the biological treatment of coke owen wastewater. The first aerobic stage served for the removal of phenols and thiocyanate and the second stage for the removal of nitrogen compounds (with the conventional two step process of nitrification and denitrification). Applying molecular biological techniques (T-RFLP: terminal restriction fragment length polymorphism, molecular cloning and sequence analysis) microbial background of nitrogen removal processes in the activated sludge were investigated. The T-RFLP screening of the gene amoA (encoding the subunit of the ammonia monooxygenase enzyme) showed that, the community

of ammonia-oxidizing bacteria was not diverse and the members of the genus *Nitrosospira* were the dominant ammonia-oxidizers. Cloning and sequencing a segment of gene nxrB (encoding the subunite of the nitrite oxidoreductase enzyme) revealed that the preponderant nitrite-oxidizing bacterium was Nitrobacter winogradsky in the nitrification reactor.
Analysis of the complete microbial community of the activated sludge from the denitrifying reactor by cloning a partial sequence of 16S rDNA showed high level of bacterial diversity: members of α-, β-, γ-, ε-Proteobacteria, Fibrobacteres and Bacteroidetes were identified. The effect of switch from acetate to methanol as organic carbon source for denitrification was also tested. Results of T-RFLP combined with molecular cloning and sequencing showed that due to this change proportion of methilotrophic bacteria increased in the microbial community. Two structurally different but functionally equivalent nitrite reductases (encoded by the genes nirS and nirK) were used to investigate diversity of denitrifying bacteria by cloning. Sequence analysis of clones revealed high level of phylogenetic diversity of denitrifyers and showed that the β-Proteobacterial genera *Thauera* and *Alcaligenes* were the dominant denitrifying bacteria.

PATHOTYPING OF NEWCASTLE DISEASE VIRUS (NDV) BY TAQMAN-MGB REAL-TIME PCR

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A real-time reverse-transcription polymerase chain reaction (RRT-PCR) has been developed to detect and pathotype Newcastle disease viruses (NDV) from clinical samples. The assay utilizes degenerate oligonucleotide primers and minor groove binder (MGB) TaqMan probes that target a highly variable region of the fusion protein (F) gene of NDV that corresponds to the cleavage site of the F0 precursor protein, the key determinant of pathogenecity.
The specificity of the assay was demonstrated by testing NDV strains collected from various host species, time periods and geographic regions. The pathotypes of 27 lentogenic and velogenic NDV strains representing all the known genotypes (I-VIII), 5 mesogenic/lentogenic vaccine strains and several clinical isolates were determined successfully with the method.
In the experiments no false negative test result was observed. On the other hand, no cross-reaction or false positive result was obtained with the 24 heterologous avian pathogens involved in the specificity assay or with NDV isolates belonging to other pathotype. It is worth noticing that NDV pathotyping RRT-PCR was also able to detect non-standard F0 cleavage sites (e.g. 112R-R-R-K-R-F117 in vaccine strain Mukteswar). The RRT-PCR specific for both lentogenic and velogenic (mesogenic) strains had high analytical sensitivity, detecting approximately 1-10 and 2-24 copies of the target molecule per reaction, respectively. The detection limit was also determined in terms of 50% egg infective dose (EID50) by using dilution series of virus stock solutions generating values of 10+1.0 and 10-2.3 EID50/ml for lentogens and velogens (mesogens), respectively.
The practical applicability of our assay has been proved by: 1, Using stool samples as a source for NDV purification, which is considered to be an important requirement if live birds are examined, especially for intestinal infections; 2, carrying out the RRT-PCR in different thermocyclers and 3, comparing the specificity test results with those of a gel-based pathotyping PCR assay.
The sensitivity of the assay is comparable to the values of universal PCR detecting assays or, in the case of the titrated lentogenic virus stock solution, it was below the previously published results. The

results of this study suggest that the described TaqMan-MGB assay has the potential to be used for the rapid detection and pathotyping of NDV isolates, including exotic (emerging) strains, irrespective of the country or host of origin and the time of virus emergence.

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REAL-TIME AMPLIFICATION BASED QUANTIFICATION OF THE METHYLATION LEVEL OF CPG -110 IN THE HUMAN INTERLEUKIN-10 PROMOTER

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Previously, we observed that the methylation state of CpG sites in the proximal 400 kb IL-10 promoter sequence correlated well with the lack of IL-10 mRNA expression. The widely used DNAse sensitivity, bisulphite sequencing and restriction fragment assays allow semiquantitative evaluation of epigenetic silencing. The methylation pattern of the -110 CpG dinucleotide of the IL-10 promoter was examined as C/T polymorphism after sodium bisulphite modification.
Two different, real-time detection methods were tested: using SybrGreen detection and methylation specific primers targeting the polymorphic nucleotide with their 3' end or using TaqMan MGB probes labelled with 6-FAM and VIC dyes for the polymorphic C and T, respectively, in a multiplex real-time PCR assay. Methylation levels were calculated from the absolute copy numbers of C (methylated) and the T (unmethylated) sequences. The CpG -110 methylation levels of IL-10 producing i.e. proximal promoter unmethylated cell lines (Jurkat, JJhan) and primary lymphocytes ranged between 0,47% and 12,74% according to the Taqman method. The same parameter for cervical cell lines and keratocytic cell lines ranged between 71,09% and 100,76%. The SybrGreen methylation specific PCR tended to overestimate the methylation level by 6% in the former group and underestimate it by 6% in the latter group. Also the intraassay variation was lower in the Taqman measurements than in the SybrGreen ones: the mean intrarun variation was 0,72% vs. 7,23% while the mean interrun variation was 2,71% vs. 10,10%. According to the experiments, the methylation level of the critical CpG residue of the IL-10 promoter is readily measured by real-time amplification using methylation specific Taqman probes.

THE SUCCESS OF THE ANTI-MICROBE EFFECT OF THE SPICES ARTIFICIALLY SOURED IN DRY FERMENTED SAUSAGE

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The originality and the taste of dry-fermented sausages depend mainly on spices and ripening process applied. Due to the increasing consumers' expectation for food safety, the producers intensively enhanced the clarity along the meat processing. Thus spices became a marked source of microbial contamination to date. Contrary, there is lot of scientific evidences about the occurrence of in vitro antimicrobial substances in spice plants that is not considered in the shelf-life calculations. Because

those antimicrobials can be susceptible for processing conditions, thoroughly study was conducted to explore the relationship among the spices and ripening agents that are used also for microbial growth inhibitors. *Escherichia coli* (35033), *Enterococcus faecalis* (ATTC51299), *Staphylococcus aureus* (112002) and a *Bacillus cereus* of sausage origin were applied in various liquid, agar and meat based media for detection of inhibitory effect. Natural form and extracts with different solutions of 6 spices (paprika, white and black pepper, garlic, caraway, rosemary) were tested both with filter paper disc method and mixed in grinded meat. SRE ripening agent containing glucono-delta-lactone as acidifying compound was also applied in some combinations. As sole factor, natural form of spice plant rosemary and garlic proved to be effective against Gram-positive bacteria. The acidification due lactate producing hydrolysis of SRE strongly (with 5 order of magnitude) decreased the count of *B. cereus*, however, the Gram-negative intestine bacteria survived it.
The antimicrobial effect of spices previously grinded and salted or dried for sausage processing dropped to 1/10 of natural form. Minute combination effect was observed when two or more types of additives were applied in grinded meat. The widely variable antimicrobial content of spices might be responsible for the lack of significant relationships.

The study was carried out in the framework of RET 09/2005 NKTH project.

GENETICS AND BIOCHEMICAL CHARACTERIZATION OF LACTOSE TRANSPORT IN THE FILAMENTOUS FUNGUS *ASPERGILLUS NINDULANS*

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The heterodisaccharide lactose (1,4-O-β-D-galactopyranosyl-D-glucose) occurs mainly in mammalian milk where it makes up 2-8 % of the dry weight. Although fungi usually do not encounter lactose in their native habitats, some yeast and most multicellular fungi can utilize lactose as a carbon source, although often at only low rates. For this reason, lactose has been used as a preferred carbon source for penicillin biosynthesis by Penicillium chrysogenum in the 60's and 70's to bypass carbon catabolite repression. It is currently also the only soluble carbon source for cellulase and recombinant protein production under cellulase expression signals in the fungus *Trichoderma reesei*.
In the yeast *Kluyveromyces lactis*, the LAC12 locus encoding a lactose permease is located immediately upstream of a GH2 family ß-galactosidase-encoding gene (LAC4), with which its shares a bidirectional promoter. In *Aspergillus nidulans*, genetic analysis of lactose utilization has detected eight loci (lacA-lacH) to be involved, including two putatively encoding intracellular ß-galactosidases (lacC, lacG). In agreement with this, we only had detected intracellular ß-galactosidases in *A. nidulans* during growth on lactose. However, a lactose permease – the essential prerequisite for this pathway – has not yet been identified from *A. nidulans*, and also not from any other filamentous fungus. In this study, we will describe a lactose permease of *A. nidulans* named lacA. By disruption of this locus we demonstrate that it is heavily involved but not essential for growth on lactose. We will also demonstrate that this lactose permease is the orthologue of the *K. lactis* LAC12, and that this locus has differently evolved in different filamentous fungi. We will also provide evidence that the clustering of LacA and LacG (the latter encoding an intracellular ß-galactosidase) has been maintained during evolution. Consequently, the expression of lacA and lacG is coregulated with one example being the CreA-dependent carbon catabolite repression.

MOLECULAR CHARACTERIZATION OF PHOTOAUTOTROPHIC PICOPLANKTON ASSEMBLAGES IN HUNGARIAN SHALLOW LAKES

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Members of the photoautotrophic picoplankton (APP) are the major primary producers in marine and freshwater ecosystems (up to 50% in Lake Balaton). Furthermore, intermittent, shallow soda lakes of the Carpathian basin, that frequently dry out entirely, are extremely rich in APP. They can dominate up to 100% the phytoplankton and these ponds have the highest APP abundance ever found in aquatic environments. Identification of APP on the basis of classical methods is not possible in most cases because of the small size (< 2 μm) and the lack of distinct morphological characters. In the last few years molecular techniques have played a central role in picoplankton studies and it seems that limited morphological diversity hides significant genetic diversity. Here we summarize the results of a five-year study that was referred to identify the members of APP and to reveal seasonal and temporal dynamics in Lake Balaton, in Lake Fertő and in the soda lakes of the Danube-Tisza Interfluve. In our analysis we applied several PCR-based molecular tools, such as the direct sequencing of isolated picoalgal strains, denaturing gradient gel electrophoresis, cloning and sequence analysis of environmental samples. We used marker molecules with universal (SSU rDNA) and with restricted occurence only in phototrophic organisms (phycocyanin operon) for our molecular investigations. The results showed that both the picocyanobacterial and picoeukaryotic APP have significant genetic variety in the investigated Hungarian shallow lakes and the abundance of certain APP genotypes is correlated with environmental factors (temperature, trophic state). The picocyanobacterial isolates were identified as the nonmarine members of the picophytoplankton clade (*Synechococcus/Cyanobium*) and picoeukaryotes were identified as *Pseudodictyosphaerium / Mychonastes* sp., *Chorycystis* sp., and a putative new Chlorophyta genus was also present.

ANALYSIS OF THE COMPLETE GENOME SEQUENCE OF A HUNGARIAN HEPATITIS E VIRUS STRAIN

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Hepatitis E virus (HEV) causes asymptomatic or clinically observable, hepatitis A-like acute fulminant, self-limiting hepatitis in human. The virus is considered to be a zoonotic agent: apart from successful cross-species infections and serological studies which confirmed the susceptibility of several animal species, human infection after consumption of raw or undercooked meat of infected animals have been reported. Genetic analyses of HEV strains also detected close relationship between viruses of human- and animal origin. The viral genome is about 7,2 kb long, +ssRNA, and codes 3 overlapping open reading frames (ORF). Up to now 4 genotypes are differentiated, among two (3rd and 4th), which contains both human and animal infecting strains. Several studies tried to determine the most effective method of genotyping, but up to now, the full-length sequence-analysis seems to

give the utmost information about the genome of the virus.
The investigated strain was detected in the faeces and liver samples of a domesticated pig, which was kept in a commercial pig farm in Hungary. By pathological investigation gastric ulcer, anaemia, pleuropericarditis and bronchopneumonia was found and determined as the cause of death.
The complete genome sequence of the strain was determined by direct sequencing of overlapping RT-PCR amplification products. All together 17 primerpairs were designed upon a Japanese genome sequence previously published in the GeneBank, and were used for the amplification of the Hungarian strain's genome. In total 7189 nts were determined. The virus was identified as the member of the 3rd genotype. The ORF1 and ORF2 regions the investigated Hungarian strain shows similarity to viruses detected in the UK, Greece, the Netherlands, and also, to a Hungarian strain detected in a human case. The complete sequence shows close genetic relationship to human and animal HEV strains detected in Japan (89 %), Kyrgyzstan (82%), Canada (81%), and in the USA (80%). The polyproline-hinge region of the ORF1 seems to be the most variable part of the genome. Analysis of the amino acid sequence of the coding regions may provide additional information for the investigations on the epidemiological characteristics of hepatitis E virus.

SEQUENCE DIFFERENCES IN THE LONG CONTROL REGIONS OF HPV6 SUBTYPES WITH DIFFERENT PATHOGENIC POTENTIAL AND THEIR IMPACT ON TRANSCRIPTIONAL ACTIVITY

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Three subtypes of human papillomavirus type 6 (HPV6) have been identified. Originally, HPV6a and HPV6b has been described from benign oral, laryngeal and genital papillomas, and are infrequently associated with premalignant or malignant lesions. In contrast, HPV6vc has originally been found in vaginal cancer. The main difference between the subtypes is a cca. 200 bp long similar, but not exactly the same deletion in the long control region (LCR) of HPV6b and HPV6vc as compared to HPV6a. Our aim was to investigate the subtype distribution in different bening and malignant head and neck lesions, and to compare the data to genital subtype distribution.
We examined HPV6 positive samples taken from eight benign oral and laryngeal lesions and two carcinomas of the larynx as well as twelve samples from atypias of the uterine cervix. The definition of the different subtypes was based on the restriction fragment analysis of the viral E6 region. We developed a PCR-RFLP for identification of the three subtypes and used sequencing of the whole E6 gene for confirmation. To study the capacity of oncogene expression, we sequenced the LCRs of the isolates and we tested the transcriptional activities of the LCRs of different subtypes (HPV6a, -6b and -6vc) by means of the luciferase assay after transient transfection into C-33a cells using the standard calcium-phosphate method. Luciferase activities were calculated as the average of three independent experiments. Subtype HPV6b was identified in both laryngeal carcinomas, and subtype HPV6vc from all eight tested benign oral and laryngeal lesions. Interestingly, subtype HPV6a was not found in the tested samples from the head and neck region. All three subtypes were represented in cervical specimens. We did not find E6 and LCR sequence differences between isolates of different subtypes, all sequences were identical to the corresponding sequences deposited in the GeneBank (HPV-6a Accession No. L41216, HPV-6b Accession No. NC_001355, HPV-6vc Accession No. AF092932).
LCR of subtype HPV6a increased luciferase activity thirty-fold, HPV6b showed a tenfold increase, while in case of HPV6vc the increase was ninefold, i.e. luciferase activity of HPV6b and HPV6vc proved to be comparable, while of subtype HPV-6a was consistently five times higher than that of the

other two subtypes in all three repetitions. These results show that the investigated HPV-6 subtypes had different transcriptional activity. As it was shown in case of HPV16 intratypic variants, differences in LCR activity are associated with different transforming potential. Conceivably, the differences found may account for the distribution differences found between the three subtypes.

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GENETIC DIVERSITY OF MAIZE DWARF MOSAIC POTYVIRUS (MDMV) IN HUNGARY

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The variation within viruses is what allows virus populations to adapt to changes in climate and other local environmental conditions. The ability of rapid adaptation is a viral feature, which is shown in the genetic flexibility and heterogeneity of the virus genome. The genetically diverse populations of RNA viruses are known as quasispecies. Important biological characteristics may be related to the levels of diversity in the quasispecies (quasispecies cloud size), including adaptability and host range. When a virus loses too many individuals, it becomes genetically uniform and far less adaptable and the chance of survival decreases. Studies of genetic diversity are a basic requirement to get to know the characteristics of a virus species and to estimate the risk of resistance breaking.
The maize dwarf mosaic potyvirus (MDMV) is the most common virus disease of corn and sorghum in Europe, and causes losses in grain quantity of up to 42 %. In our studies we collected samples from maize plants showing symptoms in 2006 and 2007 and investigated the interspecific and intraspecific genetic diversity of a 1318 bp long fragment using RT-PCR including the NIb, the CP region and the 3'UTR of MDMV. For phylogenetic analysis maize (Zea mays L. convar. saccharata), Johnson grass (Sorghum halepense (L.) Pers) and grain sorghum (Sorghum bicolor (L.) Moench) samples were collected in 2006 and 2007 from two areas in Hungary (Szeged (South-Hungary) and Martonvásár (Middle-Hungary)). The RNA was extracted from leaves and virus RNA was amplified using RT-PCR with a specific primer pair. After cloning and plasmid purification, the MDMV sequences were compared. To investigate the intraspecific genetic RNA population structure (quasispecies cloud size) of these MDMV isolates, we compared the sequence of 10 cDNA clones of three MDMV isolates. Two isolates (Mv2 and Sz12) showed an almost homologous base composition (99%-100%) in each of the 10 cDNA clones. However, the MDMV isolate Szgd5 showed differences in sequence up to 9%. Analysis of these 10 cDNA sequences indicates a mixed infection within the isolate.
However, phylogenetic analysis of the nucleic acid sequences of 35 MDMV isolates (31 isolates from Hungary and 4 isolates from the NCBI database) showed high divergence (up to 12%) concerning the narrow area investigated. This indicates that MDMV is very flexible and adaptable. Phylogenetic clusters did not show any correlation of geographical origin of the MDMV isolates.
Comparison of the amino acid sequence of the coat protein within the MDMV group consisting of 59 MDMV sequences the isolates Mv-0702-M, AJ563725-Hungary-M-Dallas and DQ973169-Argentinia-M-Arg were assigned to another subgroup because of an 11 amino acid long insertion in the C-terminus region of the coat protein. It is still unknown whether this insertion has a general impact on the biological function of MDMV.

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CHARACTERISATION OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS CASSELIFLAVUS* STRAINS ISOLATED FROM HEALTHY SLAUGHTERED CATTLE IN 2001

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We investigated the presence of the van genes in enterococci from cattle, depositing in the Hungarian resistance monitoring system from 2001 after three-year ban on the usage of avoparcin. Subsequently we identified the species of the van gene positive isolates as well as their genetic relatedness.
Enterococcus spp. were collected from intestinal samples of slaughtered cattle between January 2001 and December 2001. The vancomycin sensitivity was determined by disk diffusion method while the MIC values were obtained by VITEK2XL (bioMérieux) system. The presence of the van genes were detected by PCR. The strains carrying any van genes were identified by VITEK2XL system to species level and confirmed by PCR using genus-specific and species-specific primers. The origin of the samples was registered at county level. The relationship of these strains was determined by pulsed-field gel electrophoresis (PFGE) after digesting with SmaI enzyme and dendrograms were created.
Eleven (6%) of a total of 184 strains were vancomycin resistant by the disk diffusion method. By PCR only 4 (2%) van gene positive *Enterococcus casseliflavus* strains were found. These strains carried vanC2 gene. Three strains required MIC of vancomycin 8 mg/L and one strain 4 mg/L. The MICs of teicoplanin for these strains were less than 0.5 mg/L.
Disk diffusion method is not suitable for detecting the sensitivity to vancomycin even if it is less expensive and widely used. The van gene carrier strains originated from cattle were *E. casseliflavus* which is characteristic for poultry intestinal flora. These strains carried vanC2 gene and phylogeneticaly proved to be close to each other by PFGE. Avoparcin was allowed to use as growth promoter for cattle 1989 and was banned in 1998. Despite the prohibition of the usage of avoparcin, the vancomycin-resistant enterococci were still present in 2001.

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EFFECT OF HUMAN PAPILLOMAVIRUS ONCOGENES ON THE EXPRESSION OF CELLULAR GENES INVOLVED IN KERATINOCYTE DIFFERENTIATION

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Keratinocyte differentiation and human papillomavirus (HPV) life cycle are tightly linked. HPVs infect proliferating basal cells of the epidermis, while virus production is associated with terminally differentiated layers. Involucrin and transglutaminase 1 are widely used markers for keratinocyte differentiation. The expression of involucrin is activated in the spinous layer as a precursor of the keratinocyte cornified envelope. Transglutaminase 1 crosslinks a variety of glutamine-rich structural proteins of keratinocytes including involucrin. Rising the extracellular and intracellular calcium

stimulate keratinocyte differentiation and during this, the level of involucrin and transglutaminase1 increase. The purpose of the present study was to study the effects of HPV16 E6 and E7 oncogenes on the expression of involucrin and transglutaminase 1 in primary human foreskin keratinocytes. Primary keratinocytes were cultured in DK-SFM (Defined Keratinocyte - Serum Free Medium, low calcium) and infected with LXSN retrovirus vectors expressing HPV16 E6, HPV16 E7 or HPV16 E6/E7 genes. These cells were induced to differentiate by culture in DMEM (containing high calcium and serum) for 24h. Total RNA isolated from differentiating or non-differentiating infected cells was reverse transcribed, and the expression level of involucrin and transglutaminase 1 was estimated using real-time PCR with TaqMan Gene Expression Assays. The comparative Ct method was applied to evaluate the results. The differentiation of keratinocytes by serum/calcium highly increased the transcription level of involucrin and transglutaminase 1. The E6 and E7 oncogenes of HPV 16 together caused downregulation of the involucrin gene both in differentiating and non-differentiating cells. E6 alone reduced the expression of involucrin in non-differentiating cells, while E7 alone reduced involucrin expression in differentiating cells. The oncogenes of HPV16 had little effect on the level of transglutaminase 1 mRNA in differentiating cells, but together they reduced the transcription of this gene in non-differentiating cells.

COMPARISON OF *FRANCISELLA TULARENSIS* STRAINS ON THE BASIS OF CARBOHYDRATE UTILIZATION

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The intracellular bacterium *Francisella tularensis* is the causative agent of tularaemia, a highly contagious zoonosis. Fifteen *F. tularensis* strains, isolated from brown hares *(Lepus europaeus*), a patas monkey (*Erythrocebus patas*) and a vervet monkey (*Chlorocebus aethiops*) originating from different parts of Hungary, were characterised with the Biolog system. The system was already able to identify the strains after 4 hours of incubation, not only after the standard 24 hours. Results were confirmed with PCR and partial sequencing of the16S rRNA gene. After the analysis and comparison of the metabolic profiles of our strains with the Biolog database, we conclude that not all carbon sources indicated in the database were utilized by the studied isolates. We found that the Biolog software fails to distinguish the highly virulent *F. tularensis* ssp. *tularensis* (glycerol fermentation positive) and the moderately virulent *F. tularensis* ssp. *holarctica* (glycerol fermentation negative). As all our strains were unable to use glycerol as a sole carbon source they could be identified as *F. tularensis* ssp. *holarctica*. The dendrogram of the metabolic relationship between the fifteen strains shows that the isolates are very closely related to each other, which correlates with the conservative genetic character of *F. tularensis* ssp. *holarctica*.

CHANGES IN THE FATTY ACID COMPOSITION OF *BACILLUS* STRAINS IN CONTAMINATED SOILS

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Our aim was to point out the possible differences in the lipid composition in case of bacterium strains of certain bacterium species which are living in the four different and diversely polluted ecological

habitats. The environment can change and affect the bacterial lipid composition. Changes in fatty acid composition of bacteria can indicate the environmental load on soils. Soil microbiota on the basis of phospholipids, probably help to predict the changes in soils with complete certainty as well as to explore the changes of conditions inside the soil. Changes in bacterial fatty acid profile could generate a modification in function and virulence of the given bacteria species. These changes could be important for enabling the bacteria to partake in the transformation of inorganic and organic contaminants through utilizing new nutrient and/or energy sources and to adapt for the altered environmental conditions. FAME (fatty acid methyl-ester)-analysis can be effectively used for demodulation of environmental stress factors. Our sampling sites were pointed out near Beregdaróc and Beregsurány (villages east part of Hungary), on two previously cultivated areas which have been recently used as illegal dumpling sites. Samples were collected from a legal dumpling site in Gelénes (village east part of Hungary) for comparison, and an unpolluted area was chosen as a control site (forest near to the Hungarian-Ukrainian border).
According to our results, we can establish that different soil pollutions induce qualitative and quantitative changes in FAME composition of *Bacillus brevis, Bacillus cereus, Bacillus pumilis, Bacillus subtilis* strains, among others: 2-OH 10:0, C12:0, 2-OH 12:0, 3-OH 12:0, C14:1, C14:0 i-15:0, a-15:0 C15:1. The following fatty acid methyl-esters can not be found in *Bacillus* strains which were collected from the various sampling sites: C6:0, C8:0, C10:0, C11:0, C13:0, C21:0, C20:4n6. Moreover, C15:1, C17:1, C18:2n6t, C20:3n6, C20:2, C20:1 fatty acid methyl-esters can be found only in the samples of control sites. In contrast with the other sampling sites (Beregdaróc, Beregsurány, Gelénes), 2-OH 10:0 and C18:1(9)t FAMEs can not be found in the control site. Percentage of i-15:0 FAME is significantly higher in the samples of Gelénes and Beregsurány than it is on the control site, however, an inverse tendency can be observed in case of a-15:0 isomer, which occurs in significantly higher quantities on the control area than on the other sites.

THE DIAGNOSIS OF DANGEROUS BACTERIAL PATHOGENS PLAY AN IMPORTANT ROLE IN RAPID RESPONSE

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Main conditions of rapid response are: planning, preparedness and communication. The significancy of dangerous infections are increased because of urbanisation, globalisation, social changes, increasing of immunsupression and changing in usage the Earth and water base. The bacterial zoonosis and toxin production have a prominent significancy within A and B biological agents categories (i.e. *Francisella tularensis, Brucella* spp., *Burkholderia mallei, Burkholderia pseudomallei*). There are some characteristic features at present period: the incidency of dangerous infections are increased or will higher in the near future; new pathogens and/or re-emerging pathogens will cause infections in different ways; appearence of bioterrorism.

VITAMIN SUPPLEMENTATION OF LACTIC ACID FERMENTATION MEDIUM

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Nowdays a significantly increased need is noticeable to use of excess biomass or wastes from agriculture to produce energy, feed or food and other useful products, wich can be the solution of many economical and ecological problems. Lactic acid can be easily produced by fermentation from different raw materials, applying various technological ways and it can be an appropriate starting-point for several compounds. Our research group is working on a lactic acid producing technology and the main target of this project is to produce lactic acid from agricultural resources, namely from wheat. The starch content of wheat can cover the carbon source demand of many lactic acid bacteria, till the nitrogen source can derive from the protein content of the corn (gluten) as nitrogen source.
While the selected strain, a mesophilic, homofermentative lactic acid bacterium (Lactobacillus sp. MKT-LC878) utilizes starch after hydrolysis, a protein hydolysate from gluten arises as "by-product" of the hydrolysation process. However, beside glucose as carbon and hydrolysed wheat gluten as nitrogen source the strain requires a high level of further nutrient supplementation, including amino acids, vitamins and microelements. After a medium optimization experiment we found yeast extract the best nutrient source (with a productivity result of 2,41 g/l.h) and applying it in a minimal amount, it could cover all supplementation demand of the bacterium. This pointed at the fact, that yeast extract is not in a role of nitrogen source but it is a microelement, mainly vitamin source for lactic acid production. To decrease the operation cost of the technology, we tried to take out yeast extract by using vitamins. The role of vitamin supplementation in lactic acid fermentation is described widespread but it is specific for the producer strain. Since the vitamin need of our bacteria was not described yet, we performed some experiments to test several vitamins. We carried out shaking flask experiments to determine the essential vitamins using them in an equivalent amount with the vitamin content of 20 g/l yeast extract. The applied vitamins were: biotin, choline, cyanocobalamine, folic acid, inositol, nicotinic acid, PABA, panthothenic acid, pyridoxine, riboflavin, thiamine and thymidine. In a negative test we applied the vitamins together in the flasks but each flask missed one vitamin. As control we used a medium without vitamin and one with all vitamins. In this test we found three absolutely essential vitamins: choline, cobalamine and thiamine, without these supplements the lactic acid production was blocked. The lack of further three vitamins (biotin, panthothenic acid and pyridoxine) caused significant decline of lactic acid production. Applying these 6 vitamins in a positive test the results showed that they are actually essentials, while presence of the other 6 vitamins did not affect the lactic acid productivity. In both of the tests the lack of all vitamins blocked completely the fermentation.

SEASONAL CHANGES OF BACTERIAL COMMUNITIES IN DRINKING WATER NETWORK OF BUDAPEST INVESTIGATED BY MOLECULAR FINGERPRINTING

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Drinking water of Budapest originates from riverbank filtration wells located alongshore on the two banks of the Danube and on Szentendrei and Csepel Islands. High quality water of wells is treated

only by chlorine, and then distributed to the consumers. In recent study bacterial communities of discrete part of the drinking water network were investigated. Water samples were taken from 3 different bank filtration wells, 3 points of the collection network, 3 pump stations of the distribution network (containing chlorinated water) and the central pump station, which is located after chlorination. Water samples were taken 6 times from March 2007 to October 2007. DNA was isolated from 10 – 14 litres of water (MoBio Ultra™ Water DNA Kit), after partial amplification of 16S rRNA gene (TET-27F; 519R primers) T-RFLP analysis were performed (AluRI, BsuRI enzymes). Shannon's and Simpson's diversity indices were calculated, and samples were grouped by statistical analyses (Cluster Analysis, PCA) based on the number and relative quantity of TRFs. 16S rDNA clone libraries were constructed, TRF lengths and sequences of clones were determined to identify the peaks in community TRFLP-profile. Bacterial cell count of each water sample was determined using fluorescent microscopy.

Unchlorinated samples had 10^4-10^5 cell/ml values, chlorinated water samples contained 10^3-10^4 cell/ml, however in March chlorinated samples had similar values as unchlorinated ones. According to their molecular fingerprints, water samples before and after chlorination had different bacterial communities. Unchlorinated water in the wells and in the collecting system had diverse communities, dominated by oligo-heterotrophs (*Sphingomonas* spp., *Bradyrhizobium* spp.) and chemolitotrophs (*Gallionella* sp. *Nitrospira* sp.), while chlorinated water samples were characterised by lower diversity, and dominance of *Mycobacterium* spp. and *Methylocella* sp. This divergence could be observed during all sampling period, but some pump stations of the chlorinated part sometimes contained *Sphingomonas*-dominated community with high diversity, since these samples were similar to the unchlorinated ones. It might be caused by bacterial regroth or recolonisation in the distributing pipelines. However seasonal differences were also appeared. Communities' structures were changed when temperature of water was increased (spring) and decreased (autumn). In the middle of summer community of chlorinated water samples were changed, diversity increased, which may be explained by hot weather and high water temperature.

CHEMOMETRIC EVALUATION OF NEAR INFRARED SPECTROSCOPY MEASUREMENTS FOR BACTERIOLOGICAL SPOILAGE ASSESSMENT OF CHILLED BONELESS SLICE OF PORK MEAT

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There is a need for less labour intensive, rapid and reliable methods for monitoring microbiological quality in the food chain to identify hygienic and safety problems more rapidly, so that, corrective actions can be taken. Our intention was to study the utility of near infrared spectroscopy as a noninvasive technique to follow bacteriological deterioration of chilled boneless slices of pork during storage at 4 ,8 and 12 °C. Near infrared spectroscopy (diffused reflectance measurement) was performed on parallel meat samples in the wavelength range of 1000 – 1800 nm. Forming second derivative spectra and multiple scatter correction-treated spectra were made as data pre-treatments. Principal component analysis (PCA) and canonical discriminant analysis (CDA) were used for observation of segregation of the samples due to loss of freshness and onset of bacterial spoilage as a function of the storage time. The percentage of correctly classified samples decreased somewhat by increasing the storage temperature. Partial least squares (PLS) chemometric model was developed to predict and quantify bacterial loads from the 2nd derivative spectra. PLS evaluation (predicted versus

measured TAPC values), made when bacterial counts at all sampling days and storage temperatures were taken into account, resulted in a regression line with a correlation coefficient of 0.977, and a bias-corrected standard error of prediction: RMSEP = 0.438 log CFU/g. These preliminary results indicate the potential of utilizing near infrared diffuse reflectance spectroscopy in combination with multivariate statistical methods to monitor loss of freshness and detect bacterial spoilage of meat samples rapidly before deleterious microbial changes become apparent. Much larger number of samples should be studied yet to ascertain properly the prediction power of the spectroscopic method.

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

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

GENETIC VARIABILITY OF DOBRAVA HANTAVIRUSES CARRIED BY APODEMUS MICE IN HUNGARY AND CROATIA

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Dobrava hantavirus belongs to the genus Hantavirus, family Bunyaviridae and is carried mainly by yellow necked (*Apodemus flavicollis*) and striped field (*Apodemus agrarius*) mice. The virus may cause severe hemorrhagic fever with renal syndrome (HFRS) in many European countries. The goal of this study was to detect and genetically characterize Dobrava hantaviruses occurring in the Transdanubian region of Hungary and Northern Croatia.
Rodents were trapped in five different locations during the summer and autumn seasons of 2005-2007. Small mammals were dissected and lung tissues were used for Dobrva virus detection. The

viral RNA was extracted from lung suspensions with TRIzol reagent according to the manufacturer's recommendation. Dobrava hantaviruses were detected by SYBR Green-based real-time PCR, using newly designed virus specific primers. Positive samples were selected for sequence and phylogenetic analysis. In the present experiment a total of 125 *Apodemus* sp. (*A. agrarius* n=63, *A. flavicollis* n=62) were tested for the presence of Dobrava hantaviruses. Three (4.8%) *A. agrarius* and 7 (11.3%) *A. flavicollis* rodents were RT-PCR positive for Dobrava hantavirus. Phylogenetic and molecular sequence analyses showed, that at least two different genotypes of Dobrava hantaviruses occur in Hungary and Croatia according to the Apodemus host species. Viruses identified in the region were most closely related to those viruses detected in Slovenia. In this study, we provided comprehensive molecular data describing the occurrence of Dobrava hantaviruses in the Transdanubian region of Hungary as well as in Northern Croatia. Based on our new data from the region we concluded that extended reservoir studies would be necessary in the future.

COMPARATIVE STUDY OF CARBON SOURCE UTILIZATION OF 100 *HISTOPHILUS SOMNI* STRAINS ISOLATED FROM FARM ANIMALS

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Histophilus somni (former name: *Haemophilus somnus*) is a Gram-negative, fastidious, facultative pathogenic bacterium, that mainly occurs on the mucous membranes of the respiratory and genital tract of cattle and sheep. It can cause thromboembolic meningoencephalitis, pneumonia, reproductive problems and septicaemia in cattle. In sheep it is reported as a cause of orchitis and epidydimitis of rams, pneumonia, mastitis and septicaemia as well. Asymptomatic carriers can also occur in both animal species. *H. somni* was also described in goat, bighorn sheep and American bison.

The authors collected vaginal swabs from 5 and lung samples from dead calves in 12 cattle stocks, genital samples were taken in 7 sheep and 11 goat flocks of Hungary. Using the adequate culturing methods, they isolated 121 bacterial strains identified as H. somni on the basis of morphological, cultural and biochemical characteristics. The comparative study of the metabolic fingerprint of 100 different *H. somni* strains was carried out using the Biolog Microstation ™ ID System (Biolog, Ca). The system analyses the ability of the utilization of 95 single carbon sources simultaneously thus allows of detecting slight differences among the strains. Out of the 100 strains, 40 originated from calf lung, 20 strains from cattle vagina, 27 and 11 strains were isolated from ovine and caprine genitals respectively. They involved 2 *H. somni* type strains (ATCC 43625 – cattle, brain; ATCC 700025 – cattle, lung) into their examination.There were 50 carbon sources that could be utilized by at least one strain. Dextrin was metabolised by more than the 90% as well α-D-glucose could be utilized by 100% of the examined strains. A dendrogram was made on the basis of carbon source metabolism and the relationship was evaluated of the 100 *H. somni* strains.

FIRST ISOLATION OF *HISTOPHILUS SOMNI* FROM GOAT FLOCKS IN HUNGARY

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Histophilus somni (former name: *Haemophilus somnus*) is a Gram-negative, facultative pathogen bacterium that colonises the mucous membranes of cattle and sheep. It can cause local or generalised diseases and asymptomatic carriers can also occur in both animal species. It was also described in bighorn sheep and American bison. The presence and the etiological role of the microorganism have not been confirmed in any other domesticated species yet.
The purpose of this study was to prove the presence of *H. somni* in goats by bacterial isolation. Nasal, vaginal or praeputial swab samples were collected from 205 goats in 10 flocks. *H. somni* strains were isolated from 2 out of 10 flocks; in one flock 10 *H. somni* strains were isolated from the genital mucosa of 17 goats, while a single *H. somni* strain was cultured from a vagina of 26 animals in the other flock. Partial amplification and sequencing of the 16S rRNA gene (GenBank: EU708473) of three *H. somni* strains verified the identification. The comparative examination of carbon source metabolism using the Biolog Microstation™ ID System (Biolog, Ca) showed a close relationship of the caprine strains, while they were less related to *H. somni* type strain ATCC 43625 of cattle brain origin. *H. somni* strains were isolated only in the oestrus season from goat flocks with sheep contact. This is the first of *H. somni* from goats in the world.

NEWLY DISCOVERED HERPESVIRUSES FROM MAMMALIAN SAMPLES

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The number of new herpesviruses, recognized in various animals recently, is in a fast and steady increase thanks to the efficiency of novel PCR methods using degenerate consensus primers for the detection of the most conserved genome regions. With a nested PCR, targeting the DNA polymerase gene of herpesviruses, we carried out a screening on various animal samples originating from the Budapest Zoo and Botanical Garden and the Szent István University, Faculty of Agricultural and Environmental Sciences. The majority of these were pools of internal organs (including lungs, liver and intestines) taken from dead animals. The PCR products of the positive reactions were purified and sequenced with the PCR primers. The nucleotide sequences were identified using the blast programs of NCBI. Amino acid alignments were made by MUSCLE and phylogenetic calculations by the PHYLIP programs. The deduced short amino acid sequences were generally suitable for a preliminary genus classification of the detected viruses. The existence of four novel herpesviruses was hypothesized. The sample of an Egyptian fruit bat (Rousettus aegyptiacus) seemed to contain a novel betaherpesvirus, which, along with the herpesvirus previously reported from a lesser dawn bat (*Eonycteris spelaea*) represent a separate lineage (probably new genus) within the subfamily. Another sample from this species contained a gammaherpesvirus. The closest relatives of this virus are monkey herpesviruses from the Rhadinovirus genus. A putative novel rhadinovirus was found in an oriental small-clawed otter (*Amblonyx cinereus*). The closest relative has formerly been found in a Eurasian badger (*Meles meles*). Another gammaherpesvirus, demonstrated from a white-headed marmoset (*Callithrix geoffroyi*), was found to be closely related to other marmoset lymphocryptoviruses. We have found viruses that were described formerly, like badger herpesvirus 1 in an Eurasian badger (*Meles meles*) and the Eptesicus serotinus rhadinovirus 1 in a serotine bat (*Eptesicus serotinus*). These are the first known occurrence of these viruses from Hungary. Since no obvious relationship was recognized between the eventual pathological findings and positive PCR

results in each case, we assume that successful detection could rather be the consequence of recent reactivation of the virus from latency than that of primary infection. Our results support the theory on general co-evolution of herpesviruses and their vertebrate hosts. Further analysis of the newly detected herpesviruses is planned with the use of consensus PCR primers targeting the gene of glycoprotein B and the terminase of herpesviruses.

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GENOME ANALYSIS OF TWO HUNGARIAN AVIADENOVIRUS ISOLATES FROM GOOSE AND TURKEY

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The purpose of this work was to deepen our knowledge about the gene content of, and phylogenetic relationships among, members of the genus Aviadenovirus. Only four fully sequenced genomes from bird adenoviruses are available in the public databases. One is that of turkey hemorrhagic enteritis virus, which has recently been reclassified as a member of the genus Siadenovirus. Another is that of egg drop syndrome virus, which is attributed to ducks as the primary reservoir host and has been moved into the genus Atadenovirus. The remaining two sequences are those of the fowl adenoviruses (FAdVs) FAdV-1 and FAdV-9, which represent the genus Aviadenovirus.
We undertook a comparative genome sequence analysis of two aviadenoviruses isolated in Hungary. One of them is a goose adenovirus (GoAdV) and the other is a turkey adenovirus (TAdV). The GoAdV genome was sequenced by the shotgun method, and consists of 43,450 bp with an average nucleotide composition of 44.63% G+C. Genome sequence of the TAdV was determined by random cloning and primer walking method. Its genome size is 45,413 bp, and the G+C content is 68.1%. Preliminary analyses revealed that the central region of each genome has the characteristic adenovirus gene organization, containing the 16 genes conserved throughout the virus family. In addition, both of them have a second fiber gene.
The terminal regions of the genomes, which, in adenoviruses, generally contain genus-specific genes, are much more divergent. The left terminal region of GoAdV contains unidentified ORFs and the homologues of ORF1, ORF2, ORF12 and ORF24 from FAdVs. The homologues of ORF0, ORF1, ORF1A, ORF2, ORF12, ORF13, ORF14 and ORF24 are located in the same genomic region of TAdV. In the right terminal region, GoAdV contains homologues of ORF20, ORF20A and ORF22 from FAdV-1, two genes related to the lipase gene and a set of novel ORFs of unknown function, whereas TAdV has more homologues of several FAdV genes (lipase, ORF8, ORF9, ORF11, ORF17, ORF20, ORF20A, ORF22, ORF26, ORF28, and ORF29). Phylogenetic analyses performed with a number of different genes demonstrated that TAdV clusters with the FAdVs, whereas GoAdV represents a separate lineage within the genus Aviadenovirus**.**
Since adenoviruses are considered to have co-evolved with their hosts, this separation of waterfowl and fowl adenoviruses is probably a consequence of the ancient divergence of anseriform and galliform birds from the ancestral taxonomic group Galloanseri.

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THE SENSITIVITY OF TERT-BUTYL HYDROPEROXIDE TOLERANT *SCHIZOSACCHAROMYCES POMBE* MUTANT TO HYDROGEN PEROXIDE STRESS

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All organisms must have specific and precisely balanced internal conditions for optimal growth and function. The internal environment of the cell is maintained to promote proper operation of the cell; however fluctuations in the external milieu can result in a variety of cellular perturbations that can disrupt the internal environment. The *Schizosaccharomyces pombe* parental strain (ura4D18) was used to select a tert-butyl hydroperoxide (tBOOH) tolerant mutant by culturing cells in the presence of increasing concentration of the drug. The tBOOH is a glutathione (GSH) depleting (tBOOH reacts with GSH via GSH peroxidase generating GSSG), as well as lipid peroxidation (tBOOH is converted into free radicals /tert-butoxyl/ by iron-depending reaction thus initiating lipid peroxidation) and by this way reactive oxygen species (ROS) inducing agent. The monogenity of the mutation was demonstrated by tetrad analysis. The mutant exhibited increased tolerance to tBOOH, menadione (a peroxide inducing agent) and miconazole as well as increased sensitivity to H_2O_2 and amphotericin B in comparison to its parental strain. Mid-log phase cultures were used under noninduced conditions to measure the concentration of ROS and to determine the specific activity of antioxidant enzymes. The mutant strain in comparison with the parental one possessed (i) the same superoxide content and specific activity of catalase (CAT), (ii) significantly increased specific activities of glutathione peroxidases (GPx), glutathione reductase (GR) and gluthatione-S-transferases (GST), (iii) significantly decreased the peroxide concentration and the specific activity of glucose-6-phosphate-dehydrogenases (G6PD). (iv) We also measured the membrane fluidity of the strains and didn't find any differences. The mutant strain exhibited unbalanced oxido-reduction status of cells under noninduced condition which however defended them under tBOOH-induced stress condition. Results well explain the importance of upregulation of GPx, GR and GST in the tolerant mutant. To validate enzyme assays real-time PCR studies are currently in progress.

OPTIMIZATION OF MEASURING CELLULASE AND XYLANASE ACTIVITY IN OYSTER MUSHROOM SUBSTRATE

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The aim of the oyster mushroom substrate production, to prepare a partially decomposed substrate, which is free of mushroom-pathogens and enriched with microorganisms, which have a positive effect for the oyster mushroom. The production is based on the partial composting, pasteurizing and conditioning of wheat straw. One of the major change during the process is the breakdown of lignocellulose, especially of cellulose and hemicellulose. Following these processes one can get an indirect picture of the microbial activity. From the relevant enzymes the endo-1,4-β-glucanase (EC. 3.2.1.4.) and the endo-1,4-β-xylanase (EC. 3.2.1.8.) were chosen. These break down the cellulose and the hemicellulose to reducing sugar units, which are oxidized by the orange-coloured dinitrosalicyclic acid (DNSA). The concentration of the gained claret-coloured 3-amino-5-nitrosalicyclic acid was measured with spectrophotometer. The activity of the enzymes was defined as the amount of released

reducing sugar units per minute. The amount of the substrate, the concentration of the reagents, the incubation time and temperature of the measurement were optimized. Measuring the activity of the enzymes in one hour intervals for eight hours, we got a linear relationship between the activity and the amount of released reducing sugars in the first three hours. The enzyme showed its highest activity at 60 °C, but the temperature of the substrate is changing from 20 to 70 °C, so it is better to measure the activity at an intermediate temperature. Our further aims are to determine with the optimized measurements the endocellulase and endoxylanase activity of the oyster mushroom substrates, which derive from different production series and phases.

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OUTBREAK OF HAND, FOOT AND MOUTH DISEASE CAUSED BY COXSACKIEVIRUS A16 IN A CHILDRENS' COMMUNITY

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Epidemic hand, foot and mouth disease (HFMD) is most often caused by Coxsackievirus A16 which belongs to the enterovirus genus of Picornaviridae family. Descriptions of epidemics have shown a high attack rate among young children, transmission between siblings, and an increased risk of spread in overcrowded living accommodation. Although childrens' day-care centers clearly fulfill these conditions for the transmission of HFMD, there are few published reports of HFMD outbreaks in these institutions. We report the investigation and management of an outbreak of HFMD in a day-care home in Atkár, Hungary. The center provides day care for 53 children aged 3 to 6 years. On May 23, 2008 one child and five days later further 12 children were reported to have maculopapular or vesicular rashes, and mouth lesions. Some children had only mild sympthoms such as subfebrility. Altogether 21 children were affected by the disease. Swabs were taken for viral detection from throat and skin vesicle fluid and also stool samples were collected from the five most severely affected children. Laboratory examinations were done by diagnostic, enterovirus genus-specific nested RT-PCR. All of five stool specimens (5/5), two vesicle fluids (2/5) and four throat swabs (4/5) were found to be enterovirus RNA positive. The 317 basepairs long PCR products were sequenced. Data analysis confirmed the presence of Coxsackie A16 virus as an aetiological agent of outbreak. The clinical attack rate among children of different age groups was between: 33,3-46,1 %.
Although HFMD is generally a mild disorder, in young children mouth ulceration frequently leads to difficulty with feeding. Serious complications, which include myocarditis, are rare. Pregnant women among carreers or parents may also be placed at risk by an outbreak of HFMD since it has been suggested that maternal Coxsackievirus A16 infection may be associated with spontaneous miscarriage. Virus could be detected from the stools of patients with HFMD for some weeks after resolution of the exanthem. As faecal excretion seems to be a significant factor in the transmission of this disease, one would suggest that exclusion from the community restricted to the duration of the rash would not be an effective control measure because affected children may remain infectious for prolonged periods. Because of the significant public health implications of this infection in childrens' day-care facilities, in particular its ready communicability and potential for causing fetal loss in infected pregnant women, further studies are needed to establish the proper ways of the prevention of the spread of infection and management of HFMD in child day-care.

ANTIBIOTIC RESISTANCE PATTERN OF THERMOTOLERANT *CAMPYLOBACTER* ISOLATES FROM ENTERITIC PATIENTS IN HAJDÚ-BIHAR COUNTY, HUNGARY

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Recent increase in antibiotic resistance in thermotolerant campylobacters is alarming. Present work aims at investigating species distribution and antibiotic resistance of campylobacters isolated from human stool samples of enteritic patients above the age of one year in Hajdú-Bihar county.
The study was a part of a farm-to-fork survey of thermotolerant campylobacters from broilers to patients undertaken between March 2006 and February 2007. All *Campylobacter* isolates originating from stool samples of patients from Hajdú-Bihar county above the age of one year were collected and analyzed. Specimens were directly plated onto CCDA agar and incubated at 42 Cº in microaerophilic atmosphere. Campylobacters were identified using hippurate hydrolysis and results were confirmed by species-specific multiplex PCR. In case of isolates obtained between August 2006 and February 2007 MICs of erythromycin, tetracycline, nalidixic acid and the second generation veterinary quinolone enrofloxacin were determined using Etest. Enrofloxacin susceptibility corresponds well to and may be used to predict ciprofloxacin susceptibility. Results were interpreted according to NARMS breakpoints excepting enrofloxacin, for which the breakpoints used were as follows. MIC<=0.5 mg/l was interpreted as susceptible, MIC >=2 mg as resistant, and MIC between 0.5-2 mg/l as intermediate. Altogether 333 thermotolerant campylobacters were isolated out of 8044 stool specimens; 264 *C. jejuni* (79.3%) and 69 C. coli (20.7%). Other *Campylobacter* species were not found. MIC testing was performed on 143 isolates (113 *C. jejuni* and 30 *C. coli*). The proportions of isolates susceptible to erythromycin, tetracycline, nalidixic acid and enrofloxacin were 46.9%, 65.0%, 29.4% and 30.1%, respectively. Though resistance to erythromycin was relatively rare (11.2%), high proportion of isolates intermediately susceptible to erythromycin (42.0%) was notable. Decreased susceptibility to erythromycin was frequently associated with simultaneous resistance to tetracycline or enrofloxacin. As expected, *C. coli* was the more resistant species, its rates of susceptibility to erythromycin and enrofloxacin were as low as 30.0% and 13.3%, respectively. Corresponding rates in case of *C. jejuni* were 46.9% and 30.1%, respectively.
The observed frequencies of resistance are alarmingly high. Fluoroquinolones seem practically useless in the therapy of campylobacteriosis, while efficacy of the other drug of choice, erythromycin, is seriously threatened in the view of the extremely high proportion of intermediate isolates.

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CHLAMYDIA TRACHOMATIS NATIVE-MOMP INDUCES PARTIAL PROTECTION IN NON-HUMAN PRIMATES

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A vaccine is likely the most effective strategy to control human chlamydial infections. Recent studies have shown that the *Chlamydia muridarum* major outer membrane protein (MOMP) can induce significant protection against infection and disease in mice if its native trimeric structure is preserved. The objective of this study was to investigate the immunogenicity and vaccine efficacy of *C. trachomatis* native-MOMP (nMOMP) in a non-human primate model. Six cynomolgus monkeys (*Macaca fascicularis*) were immunized parenterally with nMOMP or ovalbumin emulsified in CpG-Montanide adjuvant. Immunization induced elevated serum IgG and IgA ELISA antibody titers with exceptionally high neutralizing activity. The PBMC of immunized monkeys produced a strong antigen-specific IFN-g response. Tear IgG and IgA antibodies were low titered or negative, respectively. Monkeys immunized with nMOMP exhibited a highly significant level of protection (98% reduction of infectious burden) during the early acute phase of the infection. However, this protection waned quickly and at later periods post-challenge had no effect on either the burden or duration of chlamydial shedding. There was no difference in disease severity between nMOMP and control immunized monkeys. These results demonstrate that systemically administered nMOMP is highly immunogenic in non-human primates but elicits only a transient partial protective immunity against ocular chlamydial challenge. Nevertheless, the significant reduction of chlamydial shedding early post-challenge, a time of maximum infectious burden, might have a dramatic effect on transmission as has been previously predicted by computer models.

PRESENCE OF HUMAN PATHOGEN VIRUSES IN HUNGARIAN SURFACE WATERS

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Water-borne viruses, such as adenoviruses, caliciviruses (norovirus) or enteroviruses, are the most frequent infectious agents of water-related illnesses. Human pathogen viruses are shed into sewage by the infected population and may be transferred to surface waters by treated or untreated waste-water. Surface waters may be the means of infection as natural bathing waters or as drinking water source. Detection of potential pathogenic viral presence in water is challenging as viral counts are generally very low. There is limited data on the prevalence of viruses in Hungarian surface waters. The aim of this study was to investigate the presence of human pathogen viruses (adenoviruses, norovirus, enteroviruses, hepatitis A virus and Torque Teno virus) in Tisza and Danube water samples. Methods for the concentration and detection of human pathogen viruses from surface waters, developed in the EU funded project Virobathe (http://www.virobathe.org), were implemented during the present study. Large volume (10 L) water samples were concentrated to 10 mL by glass-wool- or membrane-filtration an organic flocculation. Viral nucleic acid was extracted from the concentrates with magnetic silica bead extraction kit and analysed for the presence of target viruses by virus specific (RT-)PCR. All samples were tested in 3 dilutions. Viral presence was compared to bacterial faecal indicator counts and somatic coliphage titer. The performance of the methods was verified using tapwater and surface water samples spiked with adenovirus type 2 and ECHO11 virus. Test surface water samples were collected from the Danube and Tisza rivers and Danube dead-branches. A total of

35 samples were analyzed. Concentration and nucleic acid extraction were successful for each sample. Adenoviruses were detected at every sampling site (17 positive samples), noroviruses only at the Fadd-Dombori Danube branch (4 positives). None of the analysed water samples were positive for enterovirus, hepatitis A virus and Torque Teno virus. The PCR positive samples were confirmed by sequence analysis; water samples contained adenovirus type 2, 5, 12, 40, 41 and norovirus GGII according to the sequence similarity. Diluted samples (10 or 100-fold) were more frequently found positive than the undiluted parallels; this effect was more pronounced in case of highly contaminated samples. This phenomenon and the negative results are potentially due to chemical inactivation of the viruses or the presence of PCR inhibitors in the concentrate. The occurence of viruses did not correlate with the concentration of the bacterial indicators or somatic coliphages. None of the microbial water hygiene parameters were able to predict the presence of human pathogen viruses.

INVESTIGATION OF EBV PREVALENCE IN ORAL SQUAMOUS CELL CANCER AND IN ORAL LEUKOPLAKIA

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Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus present in more than 90% of the human population. It is associated with the development of both lymphoid and epithelial tumours, such as Burkitt’s lymphoma, Hodgkin disease, nasopharyngeal carcinoma and oral hairy leukoplakia. After primary infection, EBV establishes latent infection and is able to persist lifelong in the host. EBV has also been reported to be associated with oral squamous cell carcinoma (OSCC), but its role in the development of OSCC is unclear. We examined the prevalence of EBV DNA in OSCC and oral leukoplakia (OL) compared to an age-matched healthy control group. Besides the lesions, apparently healthy mucosa of all patients has also been investigated. EBV DNA was detected using nested PCR. Statistical comparison of prevalence data was performed by chi-square tests, the outcome of OSCC and the survival of patients were analyzed by Kaplan-Meier test. We also examined the interplay of EBV with different established risk factors of OSCC and some patient and disease characteristics in the risk of unfavourable outcome. We detected EBV DNA in 13/68 (19,1%) of control individuals. EBV was detected in 13/44 (29,5%) and 10/44 (22,7%) of the lesion and the apparently healthy mucosa, respectively, in OL patients. These data did not show statistically significant difference from the data of the control population. The tumour sample of 73.8% (48/65) of OSCC patients was EBV positive, healthy mucosa of OSCC patients showed an EBV prevalence of 66.2% (43/65). These data are significantly higher as compared to controls ($p<0.001$). Comparing prevalence of the virus on healthy mucosa of OSCC patients revealed no significant difference between patients with EBV positive and negative lesions (70.8% vs, 52.9%, respectively). EBV carriage was not associated with any of the analysed patient characteristics (age, sex), risk factors (smoking and alcohol consumption) and clinical data (T stage and histopathological grade) in OSCC patients. Survival was adversely affected only by the T stage of the tumour; but this could not be detected when examined only EBV negative patients. However, T stage of the tumour significantly decreased the survival in EBV positives ($p=0.010$), similarly to unfavourable localization of the tumour ($p=0.013$). Based on these data, the role of EBV as an etiologic factor could not be confirmed, it rather seems, putatively due to its frequent reactivation driven by the local immunosuppression in the oral mucosa of OSCC patients, to be an indicator of tumour development and a possible marker of unfavourable outcome.

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CHLAMYDOPHILA PNEUMONIAE PERSISTS IN HUMAN IMMATURE DENDRTITIC CELLS AND INDUCES THE MATURATION OF THE CELLS

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The link between human dendritic cells (DCs) present in atherosclerotic lesion and certain pathogens, possibly associated with the development of atheromas, is not fully clarified. We examined the interactions of human monocyte-derived immature DCs with Chlamydophila pneumoniae (Cpn). The exponential production of Cpn infectious elementary bodies in DCs was not observed. Cpn infection induced increased ratio of DCs expressing maturation markers (CD83, CD86 and HLA-DR) on the surface of the cells, and the Cpn antigens were present in all of the subpopulations during the maturation process. Functional maturation of DCs, e.g. antigen processing and presentation, was observed as measured by increased incorporation of BrdU in the autologous, CD4+ and CD8+ lymphocytes of Cpn-seropositive donors. Chlamydial transcripts of the 16S rRNA, groEL-1 and omcB genes were expressed continuously, as determined by quantitative real-time PCR, but expression of the ftsK gene was limited. DC cultures produced interferon-gamma (IFN-γ), but the presence of IFN-γ in the culture medium was not the major factor that limited the growth of Cpn, as was shown by neutralization of the IFN-γ. We show the firs time that cell population(s) identified as producing IFN-γ had no markers for T, B, natural killer, monocyte cells or macrophages, but displayed DC morphology and the expression of specific DC markers, such as CD11c and HLA-DR. The long-term presence of bacterial antigens in the infected DCs, restricted bacterial mRNA expression, occasional production of infectious EBs and IFN- γ production by DCs, indicate that the DCs play a major role in the development of chronic Cpn infections by providing a continuous antigenic stimulus to inflammatory cells co-localized with the DCs. The DCs may also serve as a source for the production of live bacteria, thereby contributing to the dissemination of the infection.
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ENZYMATIC HYDROLYSIS OF PECTINS FROM VARIOUS BERRIES

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In the processing of certain fruits (black and red currant, ..) pectin rich by-products are formed. These by-products can be utilised for manufacture of pectin and derivatives, like galacturonic acid, which are valuable compounds applied in various fields. In our experiment pectins were extracted from press-cake of blackcurrant and red currant. The extractions were carried out by hot water and pectins were then purified, concentrated by membranes and evaporation, finally they were precipitated by ethanol. The majority of the pectin was successfully recovered compared to the data found in literature. The degree of esterification of the new pectins was determined by FT-IR spectroscopy and titration methonde, respectively. Hydrolysis of the new, commercial not available pectins by a polygalacturonase (PG) enzyme from Aspergillus niger was studied from a kinetic point of view. Progress curves were measured and initial rates of the reactions were calculated. Strong product

inhibition occurs during the hydrolytic reactions. The process could be described by a competitive mechanism. Based on the reaction rate data, kinetic parameters (Michaelis-Menten constants [K_m], maximal reaction rates [v_{max}] and the inhibition constants [K_I]) were determined for each substrate pectins. To avoid product inhibition and improve productivity vacuum-assisted flat sheet membrane reactor was used. Higher productivity was managed to achieve for both pectin substrates compared to the traditional shaking flask, batch processes due to the elimination of product inhibition.

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EXPERIENCES OF MICROBIOLOGICAL HYGIENIC QUALITY CONTROL IN A FREEZE DRIED VEGETABLES PRODUCING FACTORY

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Contamination of *Escherichia coli* in freeze dried green peas were detected during the microbiological control of the product in a factory. In order to uncover the background the contamination, we conducted microbiological surface investigation at the critical points of the production line, based on HACCP. Furthermore, raw material of the product – deep frozen green peas – and hand samples of the member of production personnel were also examined. Depending on the areas examined, PCA and VRBL contact plate, contact slide and transport swab were used for the microbiological sampling. Altogether 118 samples have been examined. Swabs were streaked on BA, EM agar plates and Petrifilms. The isolated strains were identified using API E, API NE, API Staph tests; examination of antibiotic susceptibility was made by disc-agar-diffusion method: Antibiotics complying with international recommendations of Clinical Laboratory Standards Institute.
Among others, *E. coli* (11), *Enterobacter* spp. (20), *Pseudomonas* spp. (11), *Staphylococcus aureus* (3), coagulase negative *Staphylococcus* (8) and moulds (6) have been isolated from the surface samples taken at critical points of the production line. Deep-frozen row samples of green peas were contaminated with the same microbes. *S. aureus* (1), *E. coli* (2) and *Enterobacter* spp. (1) have been identified in the samples taken from the hands of the member of production personnel. Antibiotic susceptibility testing of 11 strains out of the isolated bacteria has been made.
During the production, contaminating microbs were found in the row materials, and some of them – in lower number – also were detectable on the hands of production personnel. Results of antibiotic susceptibility testing of the isolates showed that the occurrence of resistance to Ampicillin or Ampicillin and Augmentin was 6 among the examined Enterobacteriaceae strains. This kind of resistance is bound to the chromosomal Ampi C and occurs with a growing likelihood also among strains isolated from human samples. *Pseudomonas* spp. 2 strains were proved to be resistant to Carbenicillin, Ceftazidime, Imipenem, Gentamicin, Tobramycin, Amikacin, Cefepime, which is a general characteristic also in human isolates. Based on the findings, procedures of disinfection tidying, disinfection cleaning of the machinery line prior to the production, disinfection of the hands have been controlled. Rationing of the detergents and disinfectants, and keeping of the deadlines also fall within the scope of strict control. The personnel took part in repetition training. In order to ensure

the proper execution of the improvement measures and the microbiological safety of the product, the knowledge of the personnel has been controlled by tests. Furthermore, regular microbiological control of the whole production procedure based on HACCP has been set as an objective.

PREPARATION AND CHARACTERIZATION OF A LIVE-ATTENUATED VACCINE STRAIN OF *KLEBSIELLA PNEUMONIAE*

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The enterobacterium *Klebsiella pneumoniae* is able to cause urinary tract infection, pneumonia or septical infections. Multi-resistant nosocomial strains are particularly important as they may cause life-threatening infections that are difficult to threat. Vaccine development, therefore, appears to be a rational alternative to fight these infections.
In case of live-attenuated vaccine strains a key issue is the rational attenuation, which renders the mutated strain both safe and - at the same time - highly immunogenic. We assessed virulence attenuation and vaccine potential of the regulatory mutant with deletion of rfaH. It has been shown that RfaH is required for the expression of capsular polysaccharide and LPS. Consequently, virulence of the rfaH mutant was attenuated in mouse models of urinary tract infection and pneumonia.
On the other hand, expression of the affected surface virulence factors is only down-regulated but not entirely abolished (as in the structural mutant lacking WabG). This allows the rfaH mutant - in contrast to the wabG mutant - to adhere to cultured epithelial cells at a level similar to the parental wild-type strain. In the mouse lung model, the rfaH mutant induced high titres of specific serum IgG upon repeated intranasal immunizations. Vaccinated mice were protected against a subsequent challenge by the parental wild-type strain. As far as safety of the vaccine strain is concerned; we demonstrated that the rfaH mutant is hyper-sensitive to complement mediated killing. This phenotype originates from the loss of the long-chained LPS molecules with retained expression of short repeats of O-antigen subunits. The *K. pneumoniae* rfaH mutant appears to be highly attenuated, safe for mucosal administration and able to elicit immune protection. Further experiments are needed, however, to determine the spectrum and the exact immune-mechanisms of protection provided.

RELEVANCE OF HUMAN BETA DEFENSIN-1 IN *HELICOBACTER PYLORI*-INDUCED GASTRITIS

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Human beta defensins (HBDs) are antimicrobial peptides that are expressed by epithelia on mucosal surfaces. Recent reports have demonstrated that Helicobacter pylori infection induces HBD-2, whereas HBD-1 is thought to be expressed constitutively, and it's role in *H. pylori* infection is controversal. Therefore, the aim of the present study was to investigate the relevance of three SNPs of the HBD-1 gene in *H. pylori* induced chronic gastritis. We have also assessed mRNA expression of human beta-defensin 1 in by *H. pylori* infected AGS cells.
Three SNPs of the DEFB1 gene DEFB1 G-20A (rs11362), DEFB1 C-44G (rs1800972) and DEFB1 G-52A (rs1799946) were genotyped either by Custom TaqMan(R) SNP Genotyping Assays or by restriction fragment length polymorphisms (RFLP) in 150 patients with chronic active gastritis.

Patients were examined by gastroduodenoscopy. HP positivity was detected by 13C-UBT and hystopathology. 100 serologically *H. pylori*-positive subjects without gastric or duodenal symptoms served as controls. Human beta-defensin1 mRNA expression in AGS cells was measured by quantitative RT-PCR reaction. No significant difference was observed in the case of investigation the DEFB-1 SNPs at the region of -22 and -44. A definitive differences in the frequency of GA and AA genotypes of G-52 A SNP was observed between patients with gastritis and healthy controls.
Conversely, the wild type genotype (GG) was significantly more frequent (47%) among healthy subjects than in patients (29%). A dose dependent increase in HBD1 mRNA was observed in AGS cell line following infection with increasing number of *H. pylori.* We suppose, that the SNP in the -52 untranslated region of HBD-1 might be connected with a deficient function of human β-defensin. This could lead to an increased colonisation of *H. pylori* in the stomac, with an ineffective clearence, and a consequent inflammation. Considering the results of the genetic and in vitro experiments our results draw the attention that not only the inducible, but also the constitutive form of human beta defensin have importance in pathogenesis of *H. pylori* induced gastritis.

FREQUENCY OF TSST-1 AND EXFOLIATIVE TOXIN GENES IN RELATION TO ACCESSORY GENE REGULATOR TYPES IN HUNGARIAN, AUSTRIAN AND MACEDONIAN METHICILLIN-RESISTANT AND METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* STRAINS

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The purpose of this study was to examine the presence of genes responsible for toxic shock syndrome toxin-1 (TSST-1), exfoliative toxin and different accessory gene regulator (agr) types in *Staphylococcus aureus* strains isolated from invasive clinical samples, and compare according to country origin and methicillin resistance. The phenotypic identification of the strains was done by classical microbiological methods. The species level identification was verified by detecting the genes encoding thermostabile endonuclease (nucA) and 23S rRNA. According to the presence of the mecA gene 110 Hungarian (HU) methicillin-resistant S. aureus (MRSA) and 94 methicillin-sensitive *S. aureus* (MSSA), 48 Austrian (AT) MRSA and 128 MSSA, 73 Macedonian (MK) MRSA and 29 MSSA strains were studied. The presence of genes encoding TSST-1 (tst) and exfoliative toxin (eta, etb) was examined by simplex polymerase chain reaction (PCR). The four different agr types were detected by multiplex PCR. The pulsed-field gel electrophoresis of the strains was performed to confirm the heterogenic feature of the bacterial groups.
The tst gene was carried by HU, AT MRSA and MSSA strains in 1.8%, 2% and in 14.9%, 12%, respectively. The eta and etb genes were found in AT MSSA isolates in 5% and 1.5%. Both the HU MSSA and AT MRSA isolates harboured the eta gene in 1% and 2%. The HU, AT and MK MRSA strains in 22%, 40% and 97%, while the HU, AT and MK MSSA ones in 48%, 46% and 93% were classified as agr type I, respectively. The 72%, 58% and 3% of the HU, AT and MK MRSA isolates were proved to be agr class II. The 36%, 34% and 7% of the HU, AT and MK MSSA strains were found to be the agr type II. The agrIII-typed bacterial group was created by 6%, 2% and 13%, 15% of HU, AT MRSA and MSSA isolates, respectively. The HU and AT MSSA strains were classified as

agr class IV in 3% and 5%. The frequency of tst gene characterised significantly the agrIII-typed HU and AT MSSA strains in 50% and 63% compared to MRSA ones. The agrIV-typed AT MSSA isolates were featured by the presence of eta and etb genes in 50% and 33%, respectively.
The increased frequency of the agr type I was significant for the MK MRSA and MSSA strains compared to the AT and HU ones ($p<0.001$). Increased frequency of the agr class II characterised the HU MRSA strains ($p<0.01$). Comparing all the MSSA strains with MRSA ones the prevalence of agr type II was significant for the MRSA isolates ($p<0.001$). In case of the presence of TSST-1 and exfoliative toxin genes agr-type correlation was observed.

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UP-PCR BASED SCAR-PRIMERS FOR THE SPECIFIC DETECTION AND MONITORING OF A *TRICHODERMA HARZIANUM* ISOLATE SELECTED FOR THE BIOLOGICAL CONTROL OF RICE SHEATH BLIGHT

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Rice sheath blight caused by *Rhizoctonia solani* is one of the most important diseases worldwide. The disease is currently managed only through excessive application of chemical fungicides which are toxic and non-environmentally friendly. Therefore, greater emphasis is now given on biological control as safe and effective alternate strategy. *Trichoderma* species are ubiquitous fungi in soil that have antagonistic activity against several soil borne plant pathogens including *R. solani*.
More than 200 *Trichoderma* strains were isolated from soil, plant debris and phyllosphere in rice fields of Northern Iran. They were screened for their antagonistic ability against R. solani by several in vitro and in vivo antagonism tests. Two strains, *T. virens* AD1-3 and *T. harzianum* AS12-2, were the best in controlling rice sheath blight, even better than Tilt (Propiconazole), the commonly used fungicide in Iran. Molecular characterization of *T. harzianum* AS12-2 revealed that this isolate has a new ITS-type related with genotype 5 represented by strain TUBF771 from Nepal.
Searching for isolate-specific sequence regions, five UP(universally primed)-PCR primers were tested with strain AS12-2 and a series of other *T. harzianum* isolates, as well as strains from the species *T. virens, T. atroviride, T. hamatum, T. asperellum* and *T. brevicompactum*, that are also occurring in Iranian rice fields. Two out of five UP-primers, as well as their combination revealed a total of three isolate-specific fragments as endogenous markers for strain AS12-2. These fragments were cloned to vector and their sequence was determined. A total of 8 forward and 6 reverse SCAR (sequence characterized amplified regions) primers were designed based on the sequences of the fragments. Two primer combinations proved to be isolate specific, amplifying the characteristic fragment from strain AS12-2 but not from any other *Trichoderma* isolate from the rice fields. The optimized reaction was also negative with *R. solani*, other fungal isolates from rice fields as well as rice, suggesting that this method can be applied for the monitoring of the selected biocontrol *T. harzianum* strain.

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

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Fusarium head blight (FHB) is a disease complex of cereals in which several fungal species may cause symptoms. The species, found as the major cause of head blight of wheat are *F. graminearum* and *F. culmorum*. Less frequently isolated species are *F. acuminatum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides*. FHB can significantly reduce grain yield and quality.
Fusarium species are known as mycotoxin producers. The majority of mycotoxins produced by Fusaria are the trichothecenes, which can be formed in preharvest infected plants still standing in the fields, or in stored grain, as well. Mycotoxins in feed and food may cause chronic or acute mycotoxicoses in livestock and in humans. Species identification of mycotoxin-producing *Fusarium* species is of high importance in relation to FHB. The polymerase chain reaction (PCR) is a handy technique for the identification and differentiation of *Fusarium* species. Species-specific primers have been used for PCR detection or identification of several *Fusarium* species. The aim of this study was to apply species-specific PCR-based assay for the identification of *Fusarium* species from Hungarian wheat grains. In addition to the species identification we performed PCR reactions to reveal the presence/absence of genes responsible for the production of several toxins (DON, 3-ADON, 15-ADON, NIV, ENs) in the *Fusarium* isolates. After processing 30 wheat samples of different geographical origin we found 59 *Fusarium* isolates. Identification with species-specific PCR primers resulted the following distribution of species: *F. acuminatum* 5 %, *F. avenaceum* 30.5 %, *F. graminearum* 37.5 %, *F. poae* 22 %, *F. sporotrichioides* 5 %. The results were confirmed by morphological identification after culturing the isolates in Petri-dishes.

MODELLING THE MICROBIAL CONTAMINATION OF SPICE PAPRIKA

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The pods of the paprika (*Capsicum annuum* L.) have been widely used in various countries as spice and food-colouring additive. Mould contamination occurs in the field during vegetation season, but also during post-harvest ripening and storage if conditions are favourable. The first step of processing to powder is the washing of pods to remove debris and dirt. After drying low water content (below 11 %, a_w activity <0,75) should be maintained to prevent microbial and physiological deterioration during storage. Mould growth can be characterized with the colony diameter and the ergosterol content. Ergosterol is the primary sterol in the cell membranes of filamentous fungi and is either absent or present only as a minor component in the higher plants and yeast cells. Ergosterol is often used to estimate fungal biomass in various environments. The amount of ergosterol in fungi is not constant, depends i.e. on the fungal species, age of the culture, developmental stage (hyphae, conidia), and growth conditions (media, pH and temperature). Microbial growth models are traditionally applied for the description of bacterial growth and only recently also to moulds.
Our aim was to find correlation between the mould count and the ergosterol content in the spice paprika powder, and to apply the growth models for moulds using the ergosterol content as growth

indicator. Two Hungarian and one Brazilian spice paprika powders were incubated in a humidity chamber, at ambient temperature and at 30 °C. The number of colony forming units was determined by traditional microbial methods, and the total alkali ergosterol content with HPLC method over time.
There was a correlation between mould count and ergosterol content of the pods before, but not after drying. Drying at 60-90 ^{0}C decreases the mould count uniformly to 10^2-10^4 cfu g^{-1}. The growth of moulds on paprika could be characterized both by the cfu and ergosterol content, and both parameters were successfully applied in the growth models to describe the growth of mould community. The Gompertz- and the Baranyi-models fit significantly better, than the linear model. More data are necessary to characterize the lag phase, which is strongly influenced by the incubation temperature.

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THERE ARE REGIONAL DIFFERENCES REGARDING THE HISTONE ACETYLATIONS AND HISTONE H3-K4 METHYLATION AT THE LATENT EBV PROMOTER LMP-1

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Epstein-Barr virus (EBV) is a ubiquitous human gammaherpesvirus that is associated with numerous malignances. EBV latent membrane protein 1 (LMP-1) is essential for the EBV-induced immortalization of B lymphocytes in vitro. Earlier we examined the acetylation state of histones and the level of histone H3-K4 methylation at the regulatory region of LMP-1 on well characterised cell lines of type I, II and type III latency carrying strictly latent EBV genomes with the method of chromatin immunoprecipitation (ChIP) assay combined with real-time PCR. These ChIP results showed that the active LMP-1p contained more acetylated and H3-K4 methylated histones, than the inactive ones. We extended our study to the transcription initiation site and the coding region of LMP-1 as well. We found regional differences regarding the acetylation state of histone H3 and H4 and the level of histone H3-K4 methylation, respectively. In case of active LMP-1p we detected consequently higher level of histone acetylations and histone H3-K4 methylation in the regulatory region and translation initiation site of LMP-1 than in the coding region.

LITTER DECOMPOSITION INTENSITY AND SOIL ORGANIC MATTER ACCUMULATION IN SÍKFŐKÚT DIRT SITE

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The Síkfőkút DIRT Project (SIK) was established in 2000 as the part of US ILTER DIRT (Detritus Input and Removal Treatments) Network. The main goal of this project to asses how rates and sources of plant litter inputs control the accumulation and dynamics of soil organic matter (SOM) and nutrients in forest soil over decadal time scales. The soil organic matter is affected among others by the quality of litter input i.e. the kind of litter material, which is deposited on soil surface. SOM also strongly promotes soil aggregation, and retains water for use by plants. Extracellular enzymes of microbes also play an outstanding role in litter decomposition and nutrient cycling generally, that is

directly controlled by factors revealing to the given site such as temperature, moisture, nutrient availability and chemical properties of the litter. Soil C accumulation and turnover are important global processes: soils contain about 1.5 x 10^{18} g C, which is 2-3 times bigger than the total amount of carbon in vegetation. The carbon flux between soils and the atmosphere is huge, with soil respiration representing about 10 times the carbon flux due to fossil fuel combustion. Thus, any change in rates of soil carbon turnover has a remarkable effect to the global carbon cycle. The litter input quantity and quality are taken into account under most scenarios of global climate change, but the resulting effects on SOM stability and turnover cannot now be predicted accurately. The level of SOM is influenced by litter production and added soluble organic matter as input, and decomposition and leaching as output. The climate change affects on the input and output too. Plant litter inputs have been manipulated at the DIRT plots in the Síkfőkút forest since 2000. Six litter treatments (three replicates) were installed randomly at the site (Control, No Litter, No Input, No Root, Double Litter and Double Wood). Litter of NL plots was transferred to DL plots many times per year. New vegetation was permanently removed from the NR and NI plots. According to our litter manipulation field experiment, after a 5-year period, at the NL, NR and NI treatments the soil organic carbon, the soil pH, and soil respiration decreased. In the spring and summer months the soil temperature was higher in exclusion treatment (NI >NR >NL) as in the C. This situation was opposite to the winter mounts. In exclusion treatments (NI, NL), where the heat insulation litter layer was missed, the soil cooled down very fast, the temperature decreased below zero. In the winter mouths, the lowest temperature was measured in NI and the highest in DL. The plus organic matter input in treatments DL and DW that there was no more organic matter accumulation. Essentially the organic carbon and organic nitrogen content of the soil there was no difference in the C, DL and DW treatments after 5 years. From the 6th year by the white heat loss the organic matter of soil in the DL treatments there was significant difference at the C plots, so begin the soil organic matter accumulation in the soil.

MOLECULAR AND MORPHOLOGICAL STUDIES OF FOUR DESERT TRUFFLES SPECIES – ARE THEY GOOD TAXA?

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Fungi are usually associated with moist environments, however, they also play a fundamental role in arid areas. For example, ascomycetous mycorrhizal fungi are common in (semi)arid environments. Several ascomyceteous fungi develop hypogeous fruitbodies, and this strategy could be interpreted as an adaptation to arid environments. One well-known example is the group of the so-called desert truffles (Tirmania spp., Terfezia spp.), which have important market value in the Mediterranean region. The main aim of the study presented here was to carry out a comparative study of *Terfezia* species collected in (semi)arid areas of Spain. We aimed to test whether (i) there was any area specificity, (ii) the morphological characters of the species identification were correct, and (iii) the species of the genus were well supported by molecular phylogenetic analyses.

We studied the *Terfezia* specimens deposited to the Herbarium of the Real Jardín Botánico, Madrid, Spain. The anatomical characteristics (ornamentation and size of spores, peridial structure) of 110 specimens of four *Terfezia* species (*T. arenaria, T. claveryi, T. leptoderma, T. olbiensis*) were studied by light microscopy. The spore ornamentation of representatives of the species was studied by scanning electron microscopy. 72 specimens were chosen for molecular taxonomic analysis. Total DNA from the samples were extracted using an E.Z.N.A Fungal DNA extraction kit and the ITS

region of the nrDNA was amplified using a PCR bead kit. The ITS region of 68 samples was successfully amplified and 63 of them could be used in subsequent molecular phylogenetic analyses. *Terfezia arenaria* with warty spores and T. claveryi with reticulate spores formed well-supported distinct clades and these specimens could be unambiguously identified by the anatomical characters. The difference of *T. leptoderma* and *T. olbiensis*, namely the length of the spines of the spore ornamentation, is vague, as it is very variable even within a species depending on the developmental stage of the ascospore. On the other hand, sequences of *T. olbiensis* grouped among *T. leptoderma* sequences similarly to *T. trappei* sequences obtained from the GenBank. The ITS sequences of *T. leptoderma* formed several distinct clades and the presence of paralogue ITS types were also detected in several specimens; these results may indicate the presence of cryptic species within this taxon.

The research was supported by the SYNTHESYS Project EU (ES-TAF-3437) and by the Hungarian Research Fund (OTKA K72766).

UNIQUE STRUCTURE OF THE CCW7 CELL WALL PROTEINS AND THE ENCODING GENES OF 'TOKAJI SZAMORODNI' FILM-FORMING WINE YEAST STRAINS

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During maturation of the Hungarian botrytized wine 'Tokaji szamorodni' a buoyant yeast film develops on the wine surface, which is similar to the flor or velum formed during aging of sherry wines. In both cases the yeast films play an important role in the formation of aroma compounds that are responsible for the typical organoleptic characters of these wines. Our aims were the characterization of cell wall proteins of film-forming and non-film forming (sedimenting) wine yeast strains and finding protein candidates that were playing role in the establishment of special cell surface structure. As the first step of research the patterns of biotinylated cell wall proteins extracted from film-forming and non-film forming Saccharomyces cerevisiae strains were compared. It was found that all of the tested 23 film-forming 'Szamorodni' yeast strains had a decreased size of the Ccw7 protein, one of the members of the Pir-protein family. Sequencing of the encoding genes revealed that the strains were lacking three out of the eleven repeating sequences characteristic to this protein family. One of the film-forming strains contained CCW7 alleles of different length, what was generated by intragenic tandem duplication of a sequence containing two repetitive domains. Unlike the film-forming strains, sixteen non-film forming wine yeasts isolated from a different botrytized wine, 'Tokaji Aszu' showed pronounced polymorphism of the CCW7 locus. It is highly probable that the modified Ccw7 protein contribute to the stress adaptation during film-formation.

TWO NOVEL SIADENOVIRUSES FOUND IN FOUR BIRD SPECIES: A MOLECULAR AND PHYLOGENETIC STUDY

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Two novel adenovirus species were detected in birds. One was found in three different species of birds of prey – Bengal eagle owl (*Bubo bengalensis*), Verreaux's eagle owl (*B. lacteus*), and Harris

hawk (*Parabuteo unicinctus*) – kept in a private collection. Another was found in a dead great tit (*Parus major*). In both cases, partial genome characterization was carried out by sequence analysis of PCR amplified genome fragments. The full sequence of eleven adjacent genes in the raptor adenovirus was determined. These include the viral DNA polymerase, terminal protein precursor (pTP), 52K, pIIIa, III, pVII, pX, pVI, hexon, protease and DNA binding protein (DBP). Partial sequence of two additional genes, namely IVa2 and 100K, was also acquired.
It all adds up to approximately 16 kb sequence information in this virus, which, based on the two hitherto sequenced siadenoviruses, roughly comprises two-thirds of the genome. 12.8 kb sequence was obtained in the adenovirus found in a great tit. The full sequence of the following ORFs was determined: pTP, 52K, pIIIa, III, pVII, pX, pVI and hexon. Partial sequence of the viral DNA polymerase gene was also acquired. Both genomes exhibit an apparent A+T bias with a G+C content of as low as 38% each. Phylogenetical reconstruction based on the genes DNA polymerase and hexon suggests a common origin with turkey adenovirus 3 and the frog adenovirus, thereby doubling the number of siadenovirus species known to science.

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PROPHYLAXIS AND TREATMENT OF FIRE-BLIGHT BY BACTERIOPHAGES

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Bacteriophages against *Erwinia amylovora* were isolated from aerial tissues of healthy apple trees as well as from soil beneath apple trees displaying fire blight symptoms. 47 phage strains were characterized by electron microscopy and RFLPs. All viruses belong to Caudovirales, 34 strains are Myoviridae while 3 phages represent Siphoviridae. Their host specificity range against 6 *E. amylovora, Erwinia mallotivora* and *Erwinia raphontici* was determined. Dependency of phage-effect on growth-phases of *Erwinia amylovora* was investigated in case of four phage strains. All four phages reduced the living cell number of *E. amylovora* in liquid culture in a significant manner, during the investigated 48 hours period. Phage-impact was most significant in the mid-logarithmic phase of *E. amylovora* B.01960, when both MOI and virus-titer were the highest. In contrast, no growth-phase dependency was observed in strain G.255.
Resistance of *E. amylovora* against four of the original (isolated) phages were also determined during the growth of two E. amylovora strains. Resistance of E. amylovora B.01960 transitionally increased in the mid-logarithmic phase, most probably causing the limited effect of viruses on early-stationary phase bacterial culture. No significant virus resistance was observed in bacterium strain G.255.

SYMBIOTIC EFFECTIVENESS OF INOCULATION ON SOYBEAN PLANT (*GLYCINE MAX* L.)

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Symbiotic effectiveness of a simple (one *Bradyhizobium* strain) and a complex (6 *Bradyhizobium* strains) peat based microbial inoculum was studied. The purity of inoculums and the number of

viable cells were determined using plate count method in three replicats on YMA plates [1]. The effect of molasses (5 g/l) and trace element (2 ml/l) supplements on proliferation dynamics of *Bradyrhizobium* strains in YMB culture was studied as well. 80 kg of soybean seeds were inoculated with 0,5 kg of peat based inoculum. Six weeks after sowing, the height of plants; shoot and root dry weight; and the number of nodules were determined. Soil physical and chemical parameters were also analysed. Bacteria were isolated from root nodules of plants for further comparative studies. There was no significant difference in vitro between the two inocula in the number of colony forming units (CFU). The number of CFU's of both products were higher than 1011 without any contamination. At the end of the investigation period, at both inocula, the number of CFU's in the YMB medium supplemented with trace elements was significantly higher compared to the control. In the medium supplemented with molasses there were two orders of decrease in the number of CFU's of the simple inocula. The number of CFU's of complex inocula rose up to 109. As an effect of both supplements (molasses and trace elements) the maximum number of CFU's exceeded 1011; however, there was no significant difference between the effects of the two supplements used.
On the basis of field experiment it was established that complex inocula had a positive effect on soybean plants. The number of nodules ranged between 5 and 10 pieces on the taproots investigated. The height and shoot dry weight of the inoculated plants were 20 % higher compared to the control; the measured differences were significant. The trace element treatment enhanced the positive effect of inoculation, however, the increment was not significant. The simple inocula did not have positive effect on soybean plants. There were no nodules detected on the roots of the inoculated plants. The plant height was 30%, while shoot dry weight was 50 % lower compared to plot treated with complex inoculum. Results showed that despite of the high number of CFU's and the purity of both inocula, only strains originated from the complex inoculum were able to infect soybean plants.

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EFFECT OF FERMENTED WHEAT GERM EXTRACT ON FELINE VIRUSES (A PILOT STUDY)

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Several, differently processed wheat germ extracts available through pharmacies have immunostimulant effects. As dietary supplements in chronic diseases they improve life quality. A fermented wheat germ extract (FWGE) having Hungarian and FDA approvals increases cellular immunity, natural killer cell activity, IL-2 production, hematological parameters, results in weight gain, but decreases production of autoimmune antibodies. It facilitates programmed cell death of tumorous and leukemic cells. These results suggest its possible benevolent effects in virus infections. In the feline AIDS model, MBM feline lymphoid cells were infected with feline immunodeficiency virus (FIV) strains Petaluma (Pet) and Pisa-M2 (M2). HeLa human cervical cancer cells and CRFK normal feline kidney cells were infected with feline adenovirus (FeAdV) and treated with serial dilutions of FWGE subsequently cytopathic effects and virus titres were monitored. FL-4 feline lymphoid cells continuously producing FIV-Pet were similarly treated and followed. It was established that FWGE exerts cellular toxicity at ≥2000 microg/ml, but MBM cells are unusually sensitive to streptomycin. FWGE in a concentration dependent manner slightly increased replication of MBM cells, did not alter that of CRFK, but reduced both growth rate and viability of HeLa cells.

Upon treatment FL-4 cells rapidly died showing morphological signs of apoptosis and their virus production significanly diminished. A single dose of FWGE reduced apoptosis induction of both FIV strains in MBM cultures upto 17 days, and FIV-M2 showed higher sensitivity to FWGE. A single FWGE dose inhibited FeAdV production but facilitated destruction of infected cells upto 6 days postinfection. Among the same conditions its inhibitory effect on HeLa cells lasted upto 3 days, only. FeAdV produced in HeLa or CRFK cells also showed different sensitivity to FWGE. Preceding FIV infection also reduces replication of FeAdV. It is concluded that FWGE exerts its effect on the cells primarily, which are different in uninfected and infected cultures. Consequently the latter effects reduce their virus producing capacity. Exploration of the exact pathomechanism and immunological effects by FWGE might determine whether it could be applied along with antiviral drugs in human and feline AIDS to improve life quality for a longer period.

NEW AGRICULTURAL PESTS EMERGING: THE GREEN MOULD DISEASE OF CULTIVATED OYSTER MUSHROOM

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During the last two decades, several case reports have been published world-wide about green mould infections of cultivated *Agaricus bisporus*, and the causative agents were identified as *Trichoderma aggressivum*. In the latest years the green mould disease of oyster mushroom caused by *Trichoderma* has also been reported in several countries. *Pleurotus* ostreatus is the third most important commercially grown basidiomycete world-wide, and the production of it is getting increasingly affected by green mould infections resulting in great crop losses. The fungi responsible for the green mould disease of *P. ostreatus* proved to be different from *T. aggressivum* based on their cultural, morphological, physiological and molecular properties, and therefore have recently been described as the new species *Trichoderma pleurotum* and *Trichoderma pleuroticola*.
The aim of this presentation is to make an overview of the global situation of *Pleurotus* green mould disease, covering recent experimental data about extracellular enzyme production and epidemiology (potential sources and spreading) of the causative agents as well as possibilities for their fast diagnosis by specific PCR and biological control with antagonistic bacteria.

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CULTIVABLE SPECIES DIVERSITY OF THE SEDIMENT OF LAKE HÉVÍZ

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Lake Hévíz is the deepest, biologically active, peat bedded thermal lake of Europe. The mud of the

lake includes effusive and moorish components, and harbors special bacterial communities. Microbial communities may play an important role in the preservation of the natural state and the curative effect of the lake, although their species composition is hardly known yet. Gram-positive organisms with a high G+C content as members of phylum Actinobacteria are ubiquitous in soils and aquatic niches. Many of them, especially members of Streptomycetaceae produce different antibiotics for pharmaceutical industry and other metabolites of commercial interest. Others play important roles in decomposition of different organic compounds.

The aim of our work was to gain information about the presence, distribution and species diversity of bacterial communities, especially Actinobacteria of the sediment of Lake Hévíz by using cultivation-based molecular methods. Samples from two depths were taken in two different locations in October 2007. CFU values largely varied according to the sampling sites and the applied media. More than 500 aerobic bacterial strains were isolated from the nine different media. The DNA of pure cultures were extracted, 16S rRNA gene was amplified by means of PCR, and examined with ARDRA. Altogether 85 ARDRA groups were established and sequence analysis of a representative from each group was carried out. Most of the cultivated strains showed the highest sequence similarity with different species of Bacillus and related genera. Strains belonging to the phylum Actinobacteria were identified as members of the genera *Arthrobacter, Brevibacterium, Curtobacterium, Friedmanniella, Kocuria, Microbacterium, Micrococcus, Micromonospora, Mycobacterium, Rhodococcus, Streptomyces*. Clone library based investigations were carried out on the same samples concluding in a polyphasic study of the sediment microbiota.

EARLY-ONSET NEONATAL SEPSIS IN THE NEONATAL INTENSIVE CARE UNIT: A SEVEN-YEAR REVIEW

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Neonatal sepsis is a major cause of death in newborns despite sophisticated neonatal intensive care. In this retrospective study, the bacteriological patterns of early-onset neonatal sepsis (EOS) in two neonatal intensive centrums, Semmelweis University were analysed. Early-onset infection (vertically acquired) was defined when positive blood cultures were obtained within 72 hours following birth. Every case was consulted by clinicians. The major risk factors (gestational age, birth weight, APGAR, gender), also outcomes and results of the most useful surveillance methods were analysed. During the seven-year study period 1559 positive blood cultures were investigated, while 112 early-onsets and 333 late-onset culture-proven neonatal sepsis occurred. The vast majority of EOS was caused by Gram-positive organisms: *Streptococcus agalactiae* (GBS) 42%, coagulase-negative staphylococci 14%, *Staphylococcus aureus* 8%, *Escherichia coli* 8%, *Klebsiella* spp. 5%, *Enterobacter* spp. 5%. Two fungaemias and one sepsis caused by *Listeria monocytogenes* were identified. Every year, the rate of EOS roughly was the identical, while EOS caused by Gram-negative bacteria was not demonstrated in the last two year. GBS infection resulted in the highest mortality when the onset of sepsis was within the first 24 hours of life. Cultivation of the discharge from ear samples promptly after the delivery seems to be relevant surveillance methods for EOS. The investigated risk factors show no significant correlation with incidence of EOS. In conclusion, consensus is needed on definitions, surveillance protocols to detect EOS in time, and to develop management strategies.

FOOD-TRANSMITTED PARASITOSES

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Many parasites could be transmitted by contaminated food. Most of them are enteral protozoan, but some helminthes, first of al soil-transmitted helminthes (geohelminthes) could infect human also by contaminated food, but this way is not usual and only way to infect people for them (*Entamoeba, Giardia, Toxoplasma, Cryptosporidium, Ascaris, Ancylostoma, Necator, Trichuris, Strongyloides*, etc.). For some parasites the only way to penetrate to human organism is by ingestion of meat containing infective stage of parasites, like *Taenia solium, Taenai saginata, Trichinella* sp., which are common in our climate. Transmission of some exotic parasites, like flukes is also connected with ingestion of raw meat (fish): (*Paragonimus westermani, Opistorchis felineus, Clonorchis sinenesis, Heterophyes heterophyes, Metagonimus yokogawai*), which occurs mostly in Fare East, but they could be imported to Hungary. Toxoplasmosis: *Toxoplasma gondii* is the organism responsible for toxoplasmosis. *Toxoplasma* has worldwide distribution and 20%-75% of the population is seropositive without any symptomatic episode.

Human infection may be acquired in several ways, such as the ingestion of oocysts from contaminated soil or water, the direct contact or consumption of unwashed vegetables, the ingestion of raw or under-cooked meat containing viable tissue cysts, and less frequently, the direct recipient of tissue or blood from other contaminated humans and the vertical transmission from acutely infected mothers. Vertical transmission is responsible for congenital toxoplasmosis. Transplacentally acquired infection of the fetus may cause chorioretinitis, severe thrombocytopenia, intracranial calcification, hepatosplenomegaly and disturbances of head size, etc. In infected newborns, which appear normal at birth, retinal scars may develop slowly during the first 3–4 years of life, either with or without accompanying symptoms. The retinal lesion is probably the most frequent manifestation of congenital toxoplasmosis. In immunocompetent adults, toxoplasmosis may produce flu-like symptoms, sometimes associated with lymphadenopathy. In immunocompromised individuals, infection results in generalized parasitemia involvement of brain, liver lung and other organs, and often death. Laboratory methods greatly improve the diagnosis.

Routine *in vitro* diagnostic methods detect humoral immunity to *T. gondii* (ELISA IgA, IgM and IgG; IgG anti-*Toxoplasma* avidity test; *Toxoplasma* WB IgG/IgM Comparative Immunological Profile) and *Toxoplasma* DNA detection [*Toxoplasma gondii* nested PCR (B1 gene detection), *Toxoplasma gondii* real-time PCR (B1 gene detection)]. Taeniosis *(Taenia solium*, *T. saginata*): These cestodes have a worldwide distribution but incidence is higher in developing countries. A tapeworm larval cyst (cysticercus) is ingested with poorly cooked infected meat. Light infections remain asymptomatic, but heavier infections may produce abdominal discomfort, epigastric pain, vomiting and diarrhoea. *T. solium* eggs can also infect humans and cause cysticercosis. Antibodies are produced in cysticercosis and are useful epidemiological tools.

Diagnosis is based on the recovery of eggs or proglottids in stool or from the perianal area. Cysticercosis is confirmed by the presence of antibodies. Trichinellosis (*Trichinella* sp.): Trichinellosis is related to the quality of pork and consumption of poorly cooked meat. Infection occurs by ingestion of larvae, in poorly cooked meat. Man is the terminal host. Trichinellosis symptoms depend on the severity of infection: mild infections may be asymptomatic. A larger bolus of infection produces symptoms according to the severity and stage of infection and organs involved. Diagnosis is based on symptoms, recent history of eating raw or undercooked meat and laboratory findings (eosinophilia, antibodies to *T. spiralis*).

REVIEW OF CASES OF HUMAN DIROFILARIOSIS IN HUNGARY BETWEEN 2001 AND 2007

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At the Department of Parasitology, National Center for Epidemiology (Budapest, Hungary) in the period 2001–07 we diagnosed 30 cases (16 men; 14 women) of dirofilariosis caused by *D. repens*. Sixteen in 2001-2006 period and 14 in 2007! The mean age of the men was 52 years; the women had a mean age of 59 years. D. repens was identified on the basis of the morphological characteristics and measured microscopic parameters of the intact worm and in the histopathological section. Sixteen of the 30 cases had ocular localization, 13 were subcutaneous and one case was diagnosed in a histopathological section of removed axillary lymph node in patient with lymphoid leukemia. Eosinophilia was found in one case. We used the Knott concentration technique for detection of microfilariae in 21 cases, with 1 positive result! Sixteen of the other 30 patients were living in close or general proximity to dogs and/or cats; 14 patients had no evident contact with animals. In their history in terms of dirofilariosis no significant trips abroad had been recorded.

Analyses of the territorial distribution of these 30 cases showed that they were localized on the watershed of the Danube and Tisza River, and in one case in close proximity to Lake Balaton. Twenty-four of the 30 identified Dirofilariae were females and 6 were males.

The first autochthonous *D. repens* infection of a dog was described in Hungary in 1998 by Fok et al. Preliminary results of the epidemiological surveys started in 2005 by Fok et al showed that 116 of 826 (14%) samples of dog blood and 2 of 29 (7%) samples of cat blood tested positive for *D. repens* microfilariae. Territorial distribution showed that most of the animals that tested positive were found on the watershed of the Danube and Tisza River, with the exception of one animal found in close proximity to Lake Balaton. This result closely corresponds with our findings in human dirofilariosis cases. Visiting or living near riverbanks where mosquitoes are abundant appears to be a significant risk factor in contracting the infection. These veterinary reports complete and confirm our opinion that dirofilariosis is an emerging zoonosis in Hungary. Several factors may contribute to the apparent increase in observed cases of human and canine dirofilariosis recently reported in Hungary and in other European countries: better knowledge of distinctive features of the parasite in microscopical sections and of its clinical aspects; increased tourism with pets; increased number of dogs and cats kept as pets; a significant number of recent publications drawing the attention of the medical community to the diagnostic probability of dirofilariosis; and climatic change, the spread of the “greenhouse effect” leading to the extension of the Mediterranean climatic belt to the north, giving better opportunity for both vectors (mosquitoes) and filarias to thrive and spawn infection. The increasing number of diagnosed cases suggests that direct attention must be paid to this zoonosis, since its incidence may rise with the improvement of clinical diagnosis.

IMPROVEMENT OF COLD STRESS TOLERANT *AZOSPIRILLUM BRASILENSE* STRAINS FOR EFFECTIVE SOIL INOCULATION

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Environment-friendly agricultural farming – which allows preserving soil fertility and groundwater clearness – demands widespread use of microbial soil inoculants for nutrient supply of plants, both in intensive plant production and hobby-gardens. Utilization of chemical fertilizers can significantly be

reduced this way. Nitrogen fixing and plant growth promoting soil bacteria are crucial ingredients of the commercial microbial soil inoculants. Unlike chemical nitrogen fertilizers, these bacteria are able to provide well balanced nitrogen supply for the plant by biological nitrogen fixation, and stimulate plant growth by hormone-like substances, mostly by indole-3-acetic acid (IAA).
The associative nitrogen fixing and phytostimulator rhizosphere bacterium *Azospirillum brasilense* is key component of the microbial inoculants widely used in Hungary. Due to its tolerance to relatively high soil temperatures, it is effective under subtropical conditions. However, it hardly tolerates cold soils of temperate zone, and poorly survives in winter soil conditions of Hungary. Despite, only few efforts have gone to eliminate this drawback of the bacterium. The present work aimed at improving the cold tolerance of *A. brasilense* wild type strain by induced mutagenesis. Induced mutagenesis, using both physical and chemical mutagens, is a simple and rational approach for strain improvement since long. Thus, GMO-producing gene modification techniques can also be avoided. However, intensive selection and subculturing of the mutagenized cultures should be applied to get rid of segregation, and stabilize the improved features or phenomenon. The optimization of the mutagen treatment and the stabilization of the random mutagenized subcultures under continuous cold stress will be presented. Examples will demonstrate how the improvement of cold tolerance did affect the beneficial growth promoting capabilities of the wild type bacterium: the nitrogen fixing and IAA producing capacities of the subcultures will be shown. Effective strains with elevated cold tolerance were obtained, and would have been tested in plant inoculation assays.

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PREVALENCE OF ROTAVIRUS STRAINS IN HUNGARY, 2007

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After two decades of development, clinical testing and some unexpected setbacks, two rotavirus vaccines have been recently filed or already introduced in more than 100 countries. To monitor strain prevalence across countries, partly in association with the anticipated routine use of rotavirus vaccines in the pediatric population, an international strain surveillance network (EuroRotaNet) was launched in 2007 in Europe with the participation of Hungary. Rotavirus positive stool samples were collected from patients, mostly children <5 years of age with gastroenteritis in different regions of Hungary in 2007. The extracted genomic RNA was subjected to genotyping using multiplex RT-PCR assay. Type-specific primers included in our assay targeted G1 to G4, G6, G8 to G10, and G12 VP7 specificities, and P[4], P[6] and P[8] to P[11] VP4 specificities. A total of 489 rotavirus positive specimens was collected from 482 patients and 466 of these were successfully G typed. The prevalence data showed the predominance of G4 (33.7%) strains followed by G1 (30.5%), G2

(21.5%) and G9 (12.0%) rotaviruses. Minority G types included G6 (0.4%), G12 (0.4%), G3 (0.2%), and G8 (0.2%). Mixed G types were identified in 1.1% of samples and 3.8 % of strains remained G non-typeable. The P type could be determined for 474 strains. Genotype P[8] (76.6%) rotaviruses were the most prevalent followed by P[4] (20.9%) strains. Genotypes P[6] and P[9] were identified at low prevalence (1.7% and 0.6%, respectively). One strain was positive for more than one P type and 2.0% of the strains were P non-typeable. A total of 460 strains was fully G and P typed showing the circulation of the globally common antigen combinations (G1P[8], G2P[4], G4P[8], and G9P[8]) and minority strains endemic in Hungary (G1P[4], G2P[8], G3P[9], G4P[6], G6P[9], and G12P[8]), however, we identified three rare strains (i.e., G9P[6], G9P[4], and G8P[8]) for the first time in our country. The availability of rotavirus vaccines in the pharmaceutical private market of Hungary is a milestone in the effort to decrease the overall socio-economic burden of childhood infectious gastroenteritis. Depending on the vaccination coverage achievable in the forthcoming years, the post-vaccination rotavirus surveillance may allow us to gain comprehensive information on the impact of rotavirus vaccines on the prevalence of circulating rotavirus strains.

INVESTIGATIONS ON CULTIVABLE BACTERIAL COMMUNITIES OF THE DRINKING WATER NETWORK OF BUDAPEST

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The drinking water supply of Budapest is based on the River Danube's riverbank filtration wells. Since routine microbiological methods are mainly being used for the detection of pathogens and corrosive microbes in drinking water systems, the real microbial diversity is only partially known in such environment. Due to treatments (e. g. chlorination) as well as the network’s length and complexity the composition of bacterial communities may vary significantly in the collecting and distributing pipelines from the wells to the consumers.
The aim of the present study was to reveal and compare the bacterial communities of the water in 6 different points of the network (two riverbank filtration wells, one collecting tube and three pump stations of the chlorinated water distribution system) based on cultivation.
Water samples were taken in March 2007, filtered, and spread on oligotrophic agar plates (R2A, PYE, M27, Ravan media). Germ counts were estimated and bacteria were isolated from all media. Strains were grouped on the basis of their ARDRA and T-RFLP profiles, and partial 16S rRNA gene sequences of the selected representatives were analysed.
Estimated germ count numbers ranged between 10^0-10^2 CFU/ml and were 10-100 times lower in the chlorinated samples than in the unchlorinated ones. Cultivation from the unchlorinated samples resulted in the identification of *Brevundimonas, Acidovorax, Rhodoferax, Nocardioides, Sphingomonas, Sphingopyxis, Mycobacterium, Bradyrhizobium, Lysobacter, Caulobacter, Hydrogenophaga, Massilia, Polaromonas, Micrococcus, Methylibium, Asticcacaulis* and *Flavobacterium* genera, while chlorinated samples showed the dominance of non-pathogenic *Mycobacterium* species. According to low sequence similarities some strains may belong to novel species. Further detailed research is required for the identification of these oligotrophic bacteria.

EXAMINATION OF THE EFFECT OF DIFFERENT PREBIOTIC SUBSTANCES ON PROBIOTICS IN DIGESTION MODEL EXPERIMENTS

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A number of factors compromise the health of modern people: stressful lifestyle, unbalanced nourishment, excessive consumption of refined foods with a big measure, admission of different chemical agents into the human body. These factors harm directly or indirectly the intestinal activity, that forms a considerable part of the immune system, including the production of essential substances that have beneficial effects on the human body. The role of the so-called prebiotics (e.g. inulin, various oligosaccharides, raffinose, resistant starch and cyclodextrin) is to prevent and reduce the damage of useful microbes, which are termed as probiotics, as well. These substances selectively facilitate the propagation of probiotic bacteria (e.g. *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Enterococcus faecium*, *Lactobacillus acidophilus*), therefore increase the rate of the synthesis of vitamin B and of beneficial short chain fatty acids, improve the absorption of minerals, decrease the level of cholesterol, triglycerides, insulin, glucose, ammonia and uric acid and improve the functioning of the immune system. The majority of the examination results about prebiotics is based on clinical dietary and animal experiments. In contrast to this we simulated the process of digestion and the effect of prebiotics on probiotic and non-probiotic bacteria selected by us in an artificial digestion model. In this digestion model the different prebiotic substances get through the simulated oral cavity, stomach and intestine and may be modified in the course of this travel. The probiotic and non-probiotic bacterial strains were added to the model in the process of digestion in the simulated colon, then the digestion continued on the appropriate temperature (37°C), pH and under anaerobic atmosphere. The pH decreasing effect of lactic acid producing bacteria was compensated by pH buffer. The first sampling was carried out 24 hours after inoculation, whereas the second one was performed 36 hour after inoculation. At the same time preparation of dilution series and inoculation of the bacterial strains onto the adequate selective plates were performed. Based on the number of colonies counted on the selective plates we could see whether the prebiotics did help the propagation of probiotic strains against the non-probiotic strains. In another experiment we digested dairy products containing various probiotic strains together with different baking industrial products that were supplemented by various simple sugars and prebiotics, respectively. In the course of the examination we determined the germ number of the various dairy products at the start and at the end of the experiments. In addition we determined the rate of the decrease of germ number due to the digestion on low pH in the stomach. In the course of the digestion process in the colon we surveyed the effect of baking products containing prebiotic materials on the survival and propagation rate of bacteria. To perform this survey dilution series were prepared after 24 and 48 hours cultivation then the bacteria were inoculated onto selective media and the growing colonies were counted.

THE BIOLOGICAL FEATURES OF THE CLASSICAL SWINE FEVER VIRUS: COMPARING A VACCINE CANDIDATE WITH OTHER STRAINS IN DOMESTIC PIGS

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The classical swine fever (CSF) is a disease of domestic pigs which causes great loss for the economy. Wild boars (Sus scrofa) as reservoir organisms play an important role in the epidemiology of the CSF. The goal of our scientific project cooperating with several European institutes is to develop a marker vaccine against this disease which can be feasible for orally immunisation of wild boars. In these presented studies a vaccine candidate strain was compared with virulent and traditional vaccine strains. During the three experiments domestic piglets were challenged oronasally with a wild boar isolate called '11722-WIL' and with the CP7_E2alf vaccine candidate and with a reference C-strain vaccine. Our goal was to explore the biological attributions and the distribution of the vaccine strains. The same protocol was followed in each study. The rectal temperatures of the animals were measured every day during the experiment and their general health status was observed evaluated by a score system. Blood samples were collected from the live animals and blood and organs from the exterminated 2-2 piglets on the sampling days.
The viral antigenes in the tonsils were detected with the help of virus isolation (VI) on PK15 cell culture and the Real-time RT-PCR technique. The humoral immune response was traced with antibody-ELISA. The organ samples were stained with hematoxilyn-eosin and were observed under a stereomicroscope. The occurrent patogene in the organs was detected with immunohistochemistry method.
The wild boar isolate caused the typical clinical symptoms of the CSF in the piglets. We confirmed that the tonsil is the most reliable organ for the early stock CSF diagnosis. The CP7_E2alf candidate and the C-strain caused no CSF-like symptoms so these vaccines were safe on the animals. The CP7_E2alf candidate induced neutralizing antibodies early as the C-strain did. The C-strain was detectable in the tonsils with VI and Real-time RT-PCR and we pointed out a short period of viremia. The antigenes of the candidate were hardly detected with the help of these techniques and we found no viremia period. The immunohistochemistry proved to be a reliable diagnostic tool of early virus detection in all the three cases and a practical method to study and compare the vaccine candidates.

Our studies were carried out in frame of FP6 research project 'CSFV and wild boar' (SSPE-CT-2003-501599)

GENETIC CHARACTERIZATION OF HUNGARIAN AND SERBIAN VANCOMYCIN RESISTANT ENTEROCOCCAL ISOLATES

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63 vancomycin resistant enterococcal clinical isolates from Hungary and Serbia have been tested for the carriage of the van gene and representative strains were further characterized by a variety of molecular typing techniques. Most of the isolates harboured vanA or vanB genes, however in two Enterococcus gallinarum blood culture strains from Serbia vanC resistance gene was detected. Pulsed-field gel electrophoresis (PFGE) proved very useful in demonstrating relatedness in outbreak isolates while variable -number tandem repeat analysis (VNTR) and multi locus sequence typing (MLST) unequivocally established clonality. All tested strains in both Hungary and Serbia belonged to the international nosocomial clone CC17.

"HELIOTROPE" PHOTOBIOREACTOR FOR HYDROGEN PRODUCING *CHLAMYDOMONAS* STRAINS

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Hydrogen is one of the most promising renewable energy sources. It is clean and available in almost unlimited quantities as the component of the water. Under specific conditions some microorganisms, like the green algal Chlamydomonas species are able to split the water and release the hydrogen in gaseous form. They produce hydrogen gas via biophotolysis using only the energy of the sun.
Chlamydomonas reinhardtii possess a reversible hydrogenase enzyme which is responsible for the hydrogen production. Photobioreactors for hydrogen production should be able to ensure the requirements of the cultivated *Chlamydomonas* strain concerning light intensity, temperature, composition of the media, sedimentation of the cells and aeration. Hydrogenase enzymes are very sensitive to oxygen, therefore a cultivation system designed for *Chlamydomonas* strains should include anaerobic conditions as well. Biological capabilities like the size of antenne pigments, activity and oxygen tolerance of the hydrogenase enzyme and growth rate of the selected algal strain determine the hydogen productivity. Due to the special conditions of the hydrogen production unusual photobioreactors have to be constructed with separated aerobic and anaerobic modules which are using different nutrient media and are working simultaneously. As the energy balance in this hydrogen producing system is essential, the optimal light absorbance in the aerobic modul and the thermal regulation of the whole phorobioreactor should be achieved with minimum energy consumption. Considering the above mentioned potentials and limitations 9 *Chlamydomonas* strains are investigated in laboratory experiments to measure and optimize their hydrogen production. The results of In vivo and In vitro experiments are used to select the best strain for the mass production. The strains are evaluated on basis of their biomass production, enzyme activity and gene expression analysis. A microcontroller regulated automatic photobioreactor is developed for hydrogen production, which can operate in artificial and sunlight as well. It includes an aerobic and an anaerobic module and is equipped with a turning mechanism to ensure the optimal light intensity and temperature in the culture suspension. The reactor can operate both in outdoor and laboratory conditions. The optimal conditions are maintained by evaluating the values of pH, temperature and turbidity, which are measured by integrated sensors.
The present paper demonstrates a newly developed photobioreactor and some preliminary results concerning the hydrogen production of selected *Chlamydomonas* strains.

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EXAMINATION OF FUNCTIONAL HOMOLOGY AND COMPLEMENTATION OF *SCHIZOSACCHAROMYCES POMBE* MEDIATOR SUBUNITS

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Mediator complex has an important role in the transcriptional regulation. At least 20 Sch. pombe Mediator subunits are known and the majority is conserved. However, the exact functions of the

subunits are not yet clear. Two subunits (sep15/Med8 and sep10/Med31) of the Sch. pombe Mediator complex were earlier investigated. Their point mutations impaired the stress response, the separation process of the cells and caused hyphal morphology [1, 2]. We also identified the genes dependent on these subunits [3]. Here we report on the further examination of the sep10/Med31 and sep15/Med8 subunits. The functional homology of sep10/Med31 was investigated by interspecific complementation with Saccharomyces cerevisiae gene. To reveal the role of different domains of the sep15/Med8 gene, mini-deletions were created in it. The mutant alleles were cloned into Sch. pombe vectors and transformed into the point mutant strain. Morphology, cell separation and stress response were tested in the transformant cells.

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BLACK ASPERGILLI CAUSING CORNEAL INFECTIONS IN SOUTH INDIA

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Aspergillus species are frequently involved in human corneal infections. *Aspergillus* keratitis commonly occurs among agricultural workers living in hot, humid, tropical or semi-tropical climates. The main risk factor for the infection is trauma by vegetable matter during agricultural activities, but the prolonged use of corticosteroids, inappropriate use of antibiotics, wearing of contact lenses, diabetes mellitus and other ocular diseases also predispose to infection.
Certain *Aspergillus* species, mainly *A. flavus, A. terreus* and *A. fumigatus* have long been regarded as important pathogens in eye infections, especially keratitis. *A. niger* is also recognized as a cause of keratitis in an increasing number of cases. Unfortunately, most of the retrospective studies of fungal keratitis report the causative agents at the genus level only, e.g. as *Aspergillus* spp., and only some of them provide details at the species level. Furthermore, the isolated black aspergilli are described simply as *A. niger* in the available retrospective studies, however, other species of *Aspergillus* section *Nigri*, that are hard to differentiate from *A. niger* based on morphological and cultural characteristics alone, may also be involved in fungal infections of the cornea. We applied the sequence analysis of a fragment of the β-tubulin gene to identify 7 black aspergilli isolated from keratitis cases in the Aravind Eye Hospital, Coimbatore, South India, and only three of them proved to be due to *A. niger*. Two isolates were identified as *A. tubingensis*, while further 2 proved to belong to *A. brasiliensis*, a recently described new species isolated from soil from Brazil, Australia, USA and the Netherlands, and from grape berries from Portugal. Neither of these two black *Aspergillus* species have been previously reported from keratitis, furthermore *A. brasiliensis* is not yet known as the causative agent of any other human disease. Based on the relatively high occurrence frequency (28.6%) of these two species in our sample set, it can be presumed that they have been occurring previously in corneal infections, but they were just identified and reported as *A. niger*.

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SPOILAGE AND PATHOGENIC FUNGI IN FOODS

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The overview covers five topics. 1. Place of yeasts and molds in the system of fungi. Neither group can be considered as a taxonomic unit; both yeasts and molds may belong to three phyla, respectively. Either one is united by common morphological and physiological characters and from practical point of view. 2. Ecology of food spoilage. Activity of yeasts and molds are determined by intrinsic and extrinsic factors of food and its environment permitting the expression of appropriate properties. 3. Practical roles. They play a significant role in the spoilage of foods. However, it is far counterbalanced by their productive use in food fermentation and industrial biotechnology. 4. Detection methods. For their diverse and differing properties, no unified methodology can be applied for studying foodborne yeasts and molds. The use of 'total yeast and mold count' is particularly misleading and erroneous in assessing the quality and stability of foods. 5. Mycological food safety. Although there are some obligate and opportunistic species of molds and yeasts, the production of mycotoxins is considered the greatest food-related risk.

CHANGES IN PHOSPHOMONOESTERASE ACTIVITY IN RESPONSE TO NITROGEN FERTILIZATION ON A CALCAREOUS SANDY SOIL

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N fertilization plays a substantial role in the growth of plants and microorganisms. Studies were carried out on the effects of long-term nitrogen fertilization on the acid and alkaline phosphatase activity of a calcareous sandy soil. Phosphomonoesterases are essential enzymes of the phosphorus cycle. Various soil properties and fertilization influence the enzyme activity.
Soil samples were taken from the 0-20 cm layer in late autumn (22 November 2007) in Őrbottyán (Hungary). The long-term field experiment was set up with the following N fertilizer doses: 0 (control), 75, 150 and 225 kg $N \cdot ha^{-1}$ applied in autumn. Uniformly all treatments received 100 $kg \cdot ha^{-1}$ P_2O_5 and K_2O fertilizers. The phosphatase activity was measured by the colorimetric method, using p-nitrophenyl phosphate. pH dependence of phosphatase activity was determined in the range from pH 4.0 to pH 12. The results demonstrate significant differences between the control and N-treated soils in the autumn period. It was found that elevated N supply stimulated not only plant growth, but microbial processes, as well. N fertilization significantly increased the humus content and enhanced the phosphatase activity of soil. In the calcareous sandy soil alkaline phosphatase activity was higher than that of acid phosphatase. Soil phosphatase activity markedly differed in the soil samples originating from different N application levels. N treatment had a greater influence on the enhancement of alkaline phosphatase activity than that of acid phosphatase. The relationship between pH and the ratio of alkaline and acid phosphatase activity could be described by a linear equation.

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CHANGE OF SOME PLANT AND SOIL PARAMETERS IN A PHYTOREMEDIATION EXPERIMENT

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Metals and metalloids are the most common contaminants in the World. Waste materials containing toxic elements (As, Cd, Pb, Zn) of the historic Pb-Zn mine at Gyöngyösoroszi (North-East-Hungary) pollute neighboring area. Heavy metals inhibit plant development and growth and affect elemental content of plants and affect on the soil microbial parameters, as microbial biomass, microbial respiration, enzyme activities, and AM fungi infection. The aim of present work determined the effect of contamination at a sediment area of the Toka valley on the previous parameters of maize (*Zea mays* L.) plant and maize rhizosphere. Soil contamination resulted in a marked difference of maize growth and heavy metal content of plants. Stem and leaf biomass of maize plants sampled in flowering stage was reduced by 29 % and 53 %, respectively in the polluted soil compared to the unpolluted soil. Cd, Pb and Zn content of maize grown in polluted soil were enhanced typically.
Influence of soil contamination resulted in change of some microbial parameter, too. Microbial biomass C and the basal respiration rate were significantly higher in unpolluted soil than in polluted soil. Conversely a significant increase of acid phosphomonoesterase activity and AMF infection due to the pollution were determined in the rhizosphere soil.

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PREVALENCE OF ANTIRETROVIRAL DRUG RESISTANCE-ASSOCIATED MUTATIONS IN NEWLY DIAGNOSED HIV-1 INFECTED PATIENTS IN HUNGARY

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Highly active antiretroviral therapy (HAART), based mainly on combinations of reverse transcriptase (RT) and protease (PR) inhibitors is widely used to threat HIV-1 infections. Whenever the virus is able to continue replication in the presence of these drugs drug resistant variant emerge and the response to therapy is lost. The mutations resulting in amino acid subtitutions associated with resistance to reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs) can be classified as major (or primary) and secondary (accesory or compensatory) mutations. Major mutations lead to a several fold decrease in sensitivity to one or more antiretroviral drugs whereas secondary mutations may not result in a significant decrease in sensitivity, but are associated with an increase in viral fitness (replication capacity). Drug resistant strains can also be transmitted between individuals. The result of an international study (Wensing et al., 2005) in Europe showed that 10.4% of patients who had never been exposed to antiretroviral therapy carried HIV with ≥1 drug resistance mutations.

In the present study we examined for the first time the prevalance of antiretroviral drug resistance mutations in drug naive HIV-1 infected patients in Hungary.
A total of 25 HIV infected patients without prior antiretroviral treatment with documented seroconversion during 2004-2008 were included in this study. Viral RNA was extracted from patients plasma samples and was used as template for PR-RT amplification by reverse transcription coupled with nested PCR and sequencing. Stanford HIV Sequence Database was used for interpretation of resistance data. Among the 25 newly diagnosed HIV-1 infected patients enrolled in the study no subject displayed major resistance mutations in PR. Minor mutations that might contribute to protease inhibitor (PI) resistance were detected in 13 of 25 (52%) Hungarian drug-naive patients. The most frequent subtitutions were L10I in 6/25 (24%), L10V in 1/25 (4%), L33I in 1/25 (4%), A71T in 1/25 (4%) and A71V in 4/25 (16%). One of 25 (4%) patients had RT mutations, M41V and T215E associated with reduced susceptibility to nucleoside reverse transcriptase inhibitors (NRTIs). Two other mutations including L210V and L210F were detected in two patients, but these mutations do not reduce NRTI susceptibility. This is the first study reporting the prevalence of drug resistance-associated mutations in naive HIV-1 infected patients from Hungary. These data underline the importance of genotyping resistance testing of chronically HIV-1 infected patients before initiating treatment in order to select the most suitable drug regimen.

EPIGENOTYPES OF LATENT EPSTEIN-BARR VIRUS GENOMES

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My research group is focusing on the mechanisms regulating the expression of growth-transformation-associated Epstein-Barr virus (EBV) genes. EBV is a human herpesvirus that contributes to the development of a wide variety of neoplasms. The viral genomes persist as circular episomes in latently infected tumor cells or *in vitro* immortalized lymphoblastoid B cell lines (LCLs). Depending on the activity of the latent viral promoters, different sets of latent gene products are expressed in a host cell phenotype-dependent manner. These products define the major latency types of EBV (type I, II, and III latency, also called viral latency programs).
Epigenotypes are modified cellular or viral genotypes which differ in transcriptional activity in spite of having an identical (or nearly identical) DNA sequence. Cell type specific viral epigenotypes are generated by the major cellular epigenetic regulatory mechanisms (DNA methylation, binding of regulatory proteins, histone modifications) that leave distinct marks on the regulatory regions of latent EBV promoters.We established that in Burkitt's lymphoma (BL) cells (type I latency) and nasopharyngeal carcinoma (NPC) cells (type II latency) the inactive C promoter (Cp, where transcripts encoding the nuclear antigens EBNA 1-6 are initiated) was highly methylated at CpG dinucleotides. In contrast, in LCLs (type III latency) the active Cp is unmethylated and its regulatory region is associated with the nuclear proteins CBF1 and 2 (C promoter binding factor 1 and 2). We also found that the active Cp is located in a chromatin domain enriched in diacetylated histone H3 and tetraacetylated histone H4 („acetylation island"). BL and NPC cells use an alternative promoter, Qp, where transcripts for EBNA 1 (but not EBNA 2-6) are initiated. In LCLs the invariably unmethylated Qp was silent and bound by a putative repressor protein. Similarly to active Cp, active (but not silent) Q promoters were also situated in „acetylation islands". Type I and II latency differ in the expression of latent membrane proteins (LMPs) encoded by EBV. We found that the LMP1 promoter is regulated by DNA methylation whereas the activity of LMP2A promoter is modulated by the combinatorial effects of DNA methylation and histone modifications (H3 and H4 acetylation, and the level of histone H3 dimethylated on lysine 4). Two non-translated small RNA molecules (EBER

1 and 2, transcribed from internal promoters by RNA polymerase III) are invariably expressed in the major EBV latency programs. We found that the EBER 1 and 2 transcription units were hypomethylated. We also observed that c-Myc, an oncoprotein deregulated in BLs, bound to the 5' regulatory region of the EBER 1 promoter. We speculated that binding of c-Myc, that acts as a chromatin-remodelling nuclear matrix-attachment factor and global transcriptional regulator, may contribute to the survival of BL founder cells by facilitating expression of the anti-apoptotic EBER 1.

BACTERIAL COMMUNITIES IN SULFUROUS THERMAL WELLS OF HARKÁNY SPA

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Bacterial communities from the karstic, sulfide containing thermal-waters of Harkány Spa were investigated by cultivation independent molecular biological methods. Samples from lukewarm and thermal wells were collected seasonally in 2006 and 2007. Following the filtering of the water samples, DNA was extracted from the compressed biomass. 16S rDNA was amplified with PCR using universal eubacterial primers. Denaturing Gradient Gel Electrophoresis (DGGE) was applied to compare the community structures and the seasonal changes; clone library analysis was used to reveal the phylogenetic diversity. The DGGE profiles of the tepid wells showed the highest similarity, and variability mainly among the spring samples of different origin was found. From the clone libraries 16 different species or genera were identified by sequence analysis. The majority of the clones of tepid wells belonged to ε-Proteobacteria (45%), *Desulfocapsa* sp. (25%) and *Thiothrix* spp. (5%), while dominant clones of the thermal well were affiliated with the thermophilic *Sulfurihydrogenibium* and *Thiomonas*. The identified taxa were related to obligate and facultative chemolithotrophic bacteria, accordingly the investigated karstic waters can be characterized with seasonally variable bacterial populations participating in the sulfur-cycle.

THE ROLE OF INTERLEUKIN-17 DURING *CHLAMYDOPHIIA PNEUMONIAE* INFECTION IN BALB/C MICE

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Infection with *Chlamydophila pneumoniae* (*C. pneumoniae*) will occur at least once in >50% of the human population world wide, causing pneumonia, sinusitis, and bronchitis. Persistent or recurrent infection with this pathogen has been associated with chronic inflammatory diseases such as asthma and coronary heart diseases. The immunological mechanisms by which *C. pneumoniae* induces neutrophil influx into lung tissues remain poorly understood. According to our hypothesis the recently discovered interleukin-17 produced mainly by Th17 cells plays important role during acute chlamydial infection. Using experimental mouse model we examined the kinetics of the IL-17 production on mRNA and protein level after *C. pneumoniae* infection. The mRNA expression was detected by real-time PCR and the protein level was determined in ELISA assay. Expression of IL-17 gene was markedly increased on the second day and IL-17 protein level was increased on the third

day also in the lung suspension. Interestingly, four weeks after infection the mRNA and the protein level of IL-17 was still elevated compared to the non-infected control lungs. The kinetics of the IL-23 expression - which induces IL-17 production - showed similar course during infection. We detected no correlation between IL-15 and IL-17 mRNA expression. Seven days post-infection high number of IL-17 producing spleen cells was detected after in vitro re-stimulation in ELISPOT assay. The phenotype of the IL-17-producing cells was determined, and the IL-17-secreting cells were CD4 positive. To clarify the role of IL-17 during *C. pneumoniae* infection an in vivo neutralization experiment was carried out. Groups of mice were treated intraperitoneally with anti-IL-17 or isotype control antibodies. In the lung suspensions of the anti-IL-17 treated group the level of the inflammatory cytokines such as macrophage-inflammatory protein-2 (MIP-2), keratinocyte-derived chemokine (KC) and LPS-induced C-X-C chemokine (LIX) were reduced, suggesting an impaired inflammatory response due to the absence of IL-17. In the anti-IL-17 treated group the *C. pneumoniae* clearance was also reduced compared to isotype antibody treated group on the third day.
The results demonstrate that IL-17 has important role in acute *C. pneumoniae* infection and contributes to the defence mechanisms against the infection in the lung by modulating neutrophil recruitment and activity.

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ANALYSIS OF RELATIONSHIP BETWEEN HUMAN PAPILLOMAVIRUS AND LIVIN APOPTOSIS INHIBITOR GENE

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The inhibitor of apoptosis proteins (IAPs) are a family of highly conserved cell apoptosis inhibitors. In mammalian cells the basal level of apoptosis is tightly controlled by endogenous IAPs by direct binding and inhibition of specific intracellular proteases, primarily caspases 3, 7 and 9. Intensive research has proved that IAPs play a major role in tumor initiation and progression and resistance to treatment. Livin is one of the novel human IAPs family members which is not detectable in most normal adult tissues with the exception of the placenta, testes, spinal cord and lymph node, but is present in developing tissues and in several cancers, including cervix carcinoma.
The E6 and E7 oncogenes of high-risk human papillomaviruses (HPVs) due to their interfering effect on cell cycle can induce the immortalization of the host cell. Associate with this ability these viral genes can disturb functions of numerous apoptosis inhibitor proteins, e.g. the Livin.
The effects of papillomaviral oncogenes on the Livin expression and endogenous mRNA level have not been studied extensively yet, so our aim is to examine these using transfection assays and Taqman gene expression assays. For studying of Livin expression we established stable transfected keratinocyte cells with the E6 and E7 oncogenes of HPV16. We examined the mRNA expression with real-time RT-PCR in these stable transfected keratinocytes and in several HPV negative or positive cell cultures. To investigate whether the HPV 16 E6 and E7 genes have a direct effect on the activity of the Livin promoter, we cloned the Livin promoter into a transcriptional reporter vector and measured its transcriptional activity in the presence of these viral oncogenes.

HIGLIGHTS OF BACTERIAL FOOD SAFETY

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Food safety has several aspects deeply affecting almost all sectors of life, and today we witness the growing importance of bacterial food safety issues in economy and trade, in policy and social life. Consequently this is an area of a growing and exciting challenges for microbiologists worldwide. Science is one of the driving forces that should help to understand the actual problems of bacterial contamination of food. Due to mass production of, and due to the use of mass channels of national and international trade for distribution of food of animal origin, a special attention should be paid to zoonotic foodborne bacteria such as *Salmonella, Campylobacter* and verotoxigenic *E. coli* (VTEC). Besides, antimicrobial resistance of foodborne pathogenic and commensal bacteria and the spread of resistance determinants within and between populations of animals and man, represent special challenge for a wide range of professionals on the areas from molecular genetics, microbiology, to animal-, and food production up to legislation. Different options, actual scientific challenges, present and future trends in monitoring and appropriate control for most of the above bacterial food safety problems (at national and international level) are the topics offered for discussion in frame of this lecture saluting to the memory of late Prof. Elek Farkas who was one of the pioneers representing the modern concept of “one health”.

SPECIES DETERMINATION OF STRAINS BELONGING TO GENUS *BACTEROIDES* (SENSO STRICTO) BY CONVENTIONAL AND PROTEIN-BASED MOLECULAR TECHNIQUES; CHANGING TAXONOMY

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Bacteroides fragilis and related species are important commensalists in the lower intestinal tract of mammals and are also important opportunistic anaerobic pathogens causing sever infections including intra-abdominal, pelvic, lung and brain abscesses, peritonitis and sepsis. Correct identification is necessary as resistance to different anti-anaerobic drugs may differ according to the species. Several taxonomic changes have occurred in this group of anaerobic bacteria during the past years and new species were found and accepted to belong to the genus. The phenotypic identification of members of genus *Bacteroides* similarly to other anaerobic bacteria is difficult not only because many anaerobes are rather inactive in biochemical tests, but also because the slow growth of bacteria is influencing their identification, especially by commercially available identification kits such as API 20A or ATB ID 32A (BioMerieux). Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry has been shown to be a useful and simple method for rapid identification of microorganisms associated with infectious diseases and also to discriminate among different subtypes of pathogens. In this study our aim was to set up a database for the most frequently isolated anaerobic bacterial species belonging to the genus *Bacteroides*. 222 clinical isolates identified in routine laboratories as different species of the genus *Bacteroides*, collected from different European countries (Sweden, France, Croatia, Belgium, Germany and Hungary), were identified by MALDI-TOF MS and the dedicated BioTyper software using ATCC reference strains and sequenced clinical isolates as references. The phenotypic identification was carried out by classical biochemical tests and by rapid ID 32A (ATB) and API20 ANA (BioMerieux). *B. fragilis*

could be clearly identified with both methods whereas subtype differentiation were detected by mass spectrometry. The discriminatory power and identification accuracy of MALDI-TOF was superior to biochemical tests in the case of *B. theaiotaomicron, B. ovatus* and *B. uniformis*, which was confirmed by the 16S rRNA sequencing of some selected isolates. Newly accepted species as *B. salyersae* and *B. nordii* (which were earlier misidentified in routine laboratories as *B. stercoris* or *B. uniformis*) could be identified by the MALDI-TOF correctly. Clear differentiation could be achieved for less frequent clinical isolates the taxonomic place of which was recently established.. This lecture will evaluate the value of different nucleic acid-based and protein-based molecular methods for the identification of *Bacteroides* strains with clinical interest.

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DEVELOPMENT OF VACCINES AGAINST ENTEROBACTERIAL PATHOGENS

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Members of the family Enterobacteriaceae are among the most common human pathogens. Besides being a major cause of mortality in the third world, enteric bacteria are responsible for the majority of urinary tract infections, bacterial diarrhea, and sepsis in developed countries as well. Moreover, some species are prone to develop resistance to numerous antibiotics, and hence are able to cause severe nosocomial infections or even outbreaks difficult to treat. Vaccines preventing some of these infections are anticipated to have a positive impact on fighting these infections and epidemics. Vaccine development against enteric bacteria is hindered by the huge number of serovars. Serotype/groups are distinguished according to the extremely variable and highly immunogenic surface antigens (i.e., capsular, flagellar and LPS O-antigens) expressed. On the other hand, these antigenically highly variable structural elements cover and shield a rather “conserved” panel of surface antigens. An attractive approach would be to construct live vaccine strains lacking this multiform external cover and hence expose the shared antigens, which otherwise bear minor immunogenic potential. The highly variable outermost surface structures, however, appear to be indispensable for the virulent phenotype. Consequently, structural mutants lacking these virulence factors tend to be over-attenuated and hence inappropriate as live vaccine candidates. Here we show, that rational down-regulation, rather than total elimination of surface virulence factors appears to find the optimal balance between virulence attenuation and retained immunogenic potential. Moreover, down-regulation of the serotype-determining antigens enhances immunogenicity of conserved minor antigens. A panel of outer membrane proteins shared by various members of Enterobacteriaceae was identified by immuno-purification followed by mass spectrometry. The potential to apply attenuated live strains as well as shared purified proteins as broad-protective vaccine candidates is discussed.

THE COMPARISON OF BIOFILMS EVOLVED ON DIFFERENT CARRIERS IN AMMONIUM-RICH WASTEWATER USING MOLECULAR METHODS

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The treatment of wastewater containing high amount of ammonium – inter alia sludge digester liquids – is not solved nowadays. An experimental fixed-bed nitrification system for treating these kinds of sewage was constructed. The bacterial community of this system was examined with molecular methods based on DNA. The wastewater treatment columns were operated in parallel and had zeolite and ceramic beads respectively as biofilm carrier. Biofilm grown on the two different carrier was analyzed by Polymerase Chain Reaction (PCR) – for the gene of 16S rRNA and for gene amoA – and Terminal Restriction Fragment Length Polymorphism (**T-RFLP**). The principal component analysis of the gained fingerprints showed that the bacterial community of the inlet water changed afterwards getting into the columns, and this alteration happened in a different way in the two systems. There was variation between the TRFLP patterns of the liquid phase's biomass and the biofilm either, in both of the two columns. The zeolite carrier was able to form a special micro-environment that made remarkable differences between the attached and the floating bacterial community structure. In the case of the ceramic beads the microbiota showed more similarity in the two phases.
On the surface of the zeolite carrier and in its surrounding liquid, ammonia-oxidizing bacteria (AOB) were present in every level. In the ceramic beads column, AOB were detectable only on the surface of the carrier in the middle levels. AOB community proved to be more diverse on zeolite than on the ceramic beads: in every sample *Nitrosomonas* spp. were dominant, while on the zeolite *Nitrosospira* spp. appeared as well. The former results show that the more economic zeolite carrier provided much more favorable circumstances for a stable microbe community that is able to eliminate nitrogen rather than the ceramic beads carrier.

PRODUCTION AND APPLICATION OF THE ENZYMES ON THE ANAEROBIC GLYCEROL UTILIZATION PATHWAY

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After a several year long research and development work we patented a new enzymatic process for 1,3-propandiol (PD, valuable raw material for polymer industry) production utilizing the biodiesel by-product glycerol. Although the first biological production of PD by DuPont started with real economical and ecological success, an enzymatic process has usually further advantages, like higher product yield, which is not decreased with biomass formation, lower cost of reusable biocatalysts (for example immobilized enzymes), and avoiding further metabolite (i.e. by-product) formation. Applying the most appropriate enzymes together with coenzyme retention and regeneration the costly coenzyme addition can also be avoided in an enzymatic bioconversion.
To realize these advantages, we produced three key-enzymes (Glycerol-dehydratase, GDHt; 1,3-propandiol-oxydoreductase, PDOR; Glycerol-dehydrogenase, GDH) by fermentation and ultrasonic disintegration of Clostridium butyricum cells. Applying the crude cell-extract as enzyme solution in the presence of NAD^+, glycerol was dehydrated to 3-hydroxypropionaldehyde (HPA), and further reduced with $NADH_2$, wich was obtained from the glycerol oxidation into 1,3-dihydroxiacetone (DHA). Although, the two main products had to be the PD and DHA (which is a valuable raw-material for cosmetic industry), the second one could be further converted in a series of consecutive reactions to butyric acid for the use of crude enzyme solution. We already presented our complex mathematical description of this byproduct formation. We found both in silico and in vitro, that

butyric acid formation can be inhibited. After successful bioconversion experiments we started an optimization process to make this strategy more cost effective. The first step was to reach a cost-effective enzyme production, thus we started a media optimization. According to the literature, for fermentation 2YT media is needed, which contains very high concentration of the expensive tryptone and yeast extract. For this reason, we tried to lower their applied amount. Different kinetic models and process simulations were adapted and examined to describe the enzyme fermentations on the original as well as the modified media. The second step was to test theoretically and experimentally the possibility of the separation of the two main products (PD and DHA).

PRODUCTION OF INULINASE BY *THERMOMYCES LANUGINOSUS*

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Inulinases are β-fructan fructanohydrolases (EC 3.2.1.7, EC 3.2.1.26) that hydrolyze inulin to produce fructose and fructooligosaccharides. Both products are important ingredients in food and pharmaceutical industry. Inulin is naturally accumulated polysaccharide in the underground organs of chicory, dahlia and Jerusalem artichoke (JA). Fructooligosaccharides (FOS) are recognized as prebiotic and its positive effects on human health have been widely acknowledged. Endoinulinase (EC 3.2.1.7) is responsible for the partial hydrolysis of inulin-type polymers to FOS. Fructose can obtain by acid hydrolysis of the inulin, but due to degradation of fructose at low pH and process gives rise to discolouration of the hydrolysates and by product formation in the form of difructose anhydrides. Fructose syrup can also be produced from starch by enzymatic methods involving α-amylase, glucoamylase and glucose isomerase resulting mixture consisting of 42 % of fructose, 50 % of glucose and 8 % of oligosaccharides. To produce high fructose syrup (95 %) applying in pharmaceutical industry, HPLC technique is used and thus makes this method uneconomical. Alternative procedure is the use of exoinulinase (3.2.1.26) in one step hydrolysis of inulin yielding about 95 % pure fructose syrup. Industrial inulin hydrolysis is carried out at 60 ^{o}C in order to prevent microbial contamination and also because it permits to use of higher inulin substrate concentration due to increase solubility. Thus, a thermostable inulinolytic enzyme that may be produced by thermophillic organisms would be expected to play an important role in hydrolysis of inulin for food and pharmaceutical purposes. In this study, screening of thermophilic fungus *Thermomyces lanuginosus* strains was carried out to produce inulinases. Moreover media compositions were optimized for production of inulinase using the selected strain.

Ten *T. lanuginosus* strains were cultured in shaken flask using dahlia inulin as inducer and inulinase activity in extracellular fraction were measured. Seven strains showed inulinase activity higher than 0.5 U/ml. The strain IMI 140524 was selected for further studies, because this strain had shown about 0.6 U/ml enzyme titer in ferment broth. Highest inulinase activity occurred between 24 and 48 hours of fermentation. Effects of various carbon and nitrogen sources on inulinase activity were investigated using the selected strain. Fungus grew well and produced inulinase activity on all tested carbon sources. Best inulinase activity was detected in the cases of glucose, inulin and Jerusalem artichoke. Because of inulin content in further studies, JA was applied as main carbon source. Among 9 nitrogen sources, peptone seemed to be the best one. Response Surface Method (RSM) experimental design technique was applied to optimize the amounts of nitrogen and carbon sources of fermentation medium for enhancing the production of inulinase. After numerous optimization and checking steps, 1 % of JA and 0.6 % of peptone were found to be optimum concentration of main carbon and nitrogen sources. In this case, about 6.5 U/ml enzyme titer was measured at 48th hour of

fermentation. Effects of surfactants (Tween 20, Tween 40, Tween 60, Tween 65, Tween 80, Tween 85, SDS and Triton X) on enzyme secretion were investigated. Best result (10 U/ml) was obtained in the case of adding 1 % of Tween 80 to fermentation medium, but supplementing with SDS or Triton X also gave outstanding inulinase activity. Thermophilic fungus *T. lanuginosus* is able to secrete extracellular inulinase. This enzyme has potential application in hydrolysis of inulin to produce oligosaccharides or fructose syrups that are important ingredients in food and pharmaceutical industries.

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SEWAGE SLUDGE TOLERANCE AND LIGNOCELLULASE ACTIVITY OF THERMOPHILIC FUNGI ISOLATED FROM COMPOSTED SEWAGE SLUDGE AND PLANT DEBRIS MIXTURES

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Fifteen thermophilic fungi were isolated from composted sewage sludge and plant debris mixtures. The fungi identified by morphological and molecular techniques belonged to five species, namely *Aspergillus versicolor, Rhizomucor pusillus, Thermoascus aurantiacus, Talaromyces thermophilus*, and *Thermomyces lanuginosus. T. lanuginosus* and *T. versicolor* were the two most frequently occurring species, represented by eight and four isolates respectively. Most thermofilic isolates grew equally well at 50°C, but their growth rate greatly varied at 55°C. Sewage sludge tolerance of these fungi was assessed on potato agar amended with 0.1, 0.5, 1, 5 and 10 % (w/v) sludge. Thirteen of the 15 isolates could tolerate even 10 % sludge concentration, furthermore the growth of several strains of *T. lanuginosus, T thermophilus*, and *R. pusillus* was stimulated by extreme concentration of the sewage sludge. The toxic effect of sewage sludge was due to its high Cr, Cu, Ni, Pb and Zn content. On the other hand, sludge samples contain ample amounts of nitrogen, phosphate and potassium and these compounds, together with easily utilized organic materials could stimulate the growth of several strains that acquired increased heavy metal tolerance. The lignocellulase degrading capability of these fungi was assessed by photometric determination of their lignin peroxidase, manganese-dependent peroxidase and laccase activities [1, 2]. The RET-TT2 strain of T. thermophilus showed outstanding activities for all three enzymes. Strains of *T. lanuginosus* excerted similar levels of laccase activity, but their lignin perioxidase and manganese-dependent peroxidase activities varied greatly. The present study demonstrated that compost inhabiting thermophilic fungi can maintain their highly efficient lignocelluloses degrading enzyme systems even under extreme environments. The composting technologies should be adjusted to support the colonization activity of these fungi or, alternatively, bulk cultures can be used to inoculate xenobiotics containing compost piles.

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PHAGE-LADEN PRODUCT AGAINST *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is a Gram-positive, facultative intracellular pathogen. It is widespread in nature, able to grow at extreme low temperatures (-18°C), represents a threat to pregnant women, the fetus, patients treated with immunosuppressants, and to the old aged people; it causes septicaemia and meningitis. The introduction of cold storage has opened an ecological niche for its growth. *Listeria monocytogenes* can be present in all food types that have not been exposed to listericidal treatments during production. Similar to other causative agents, the bacterium will presumably show arising resistance to aminopenicillines used against it. In 30% of the cases, it does not react to antibiotic treatment. These facts led us to aim the development of a product containing phages which can lower the living cell numbers of the bacterium in food, especially which is going through longer cold storage periods. Samples were taken from wastewater, meat products and from infected humans. The phage strains were tested to host-specificity, and were analyzed both morphologically and genetically.

QUANTIFICATION OF PROBIOTIC BACTERIA, CULTIVATED IN PREBIOTIC-CONTAINING LIQUID MEDIUM, BY MOLECULAR METHOD

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Though the beneficial effects of probiotic bacteria were discovered by Metchnikoff in the first third of the last century, a couple of decades elapsed until the industrial production of probiotic products started. Later on it turned out that there are a couple of substances, the so-called prebiotics that help the preservation of human health via their beneficial impact on bacteria.
At the beginning of the work our aim was to elaborate such a molecular method that makes the verification of the presence and the quantification of probiotic bacteria faster and simpler. Traditionally quantification of and presence verification of bacteria are made on selective media but this method is time consuming, especially in case of slowly growing bacteria. Contrary to the traditional method there are molecular methods, like PCR (polymerase chain reaction) and real-time PCR, used by us that allow us to realize the investigations within a few hours. In our cultivation experiments we use three probiotic bacteria (*Bifidobacterium bifidum, Enterococcus faecium, Lactobacillus acidophilus*) and the *Escherichia coli* as indicators, to investigate the impact of prebiotic materials on these bacteria. We isolated DNA from the bacteria by the use of three different kits: with one of them the DNA is precipitated by isopropanol, whereas in the other two kits the DNA is bound to the filters. The precipitation based kit can be used efficiently in those cases where solid materials (e.g. flour) are present in the liquid cultivation medium, whereas the filter based kits are efficient if bacteria are cultivated in a medium that does not contain solid materials.
For the PCR based identification of bacteria we used primers that were collected from the literature. We had full success in the real-time PCR based identification and quantification of *E. coli*, but we need to do more experiments and optimization to get the same result with the other three bacteria, however we have some reassuring results. The expected result of our work is the elaboration of such a quick and reliable practical method that can replace the work-, tool- and time-consuming microbiological methods in this kind of examinations.

LH-PCR AND CLONE LIBRARY BASED COMMUNITY ANALYSIS OF THE SEDIMENT MICROBIOTA OF LAKE HÉVÍZ

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Lake Hévíz is the largest thermal lake in Europe, showing unique hidrological properties and being renowned for its curative effect. Its sediment is giving habitat for a diverse microbiota, even endemic species, but this diversity is yet unassessed by modern molecular community analysis methods. Two sediment samples were taken by Hargrave sediment sampler from 2-3 m water depth, close to the thermal well crater. Total community DNA was isolated from the top layer and from -20 cm layer in both samples. DNA was PCR amplified by universal eubacterial primers targeting the first third of the 16S rDNA gene (positions: 63-519). Community fingerprints were made by length heterogeneity PCR, using TET labeled forward primes and capillary electrophoresis. The most diverse sample was cloned into pGEM (Promega) and TOPO (Invitrogen) vectors in parallel. Two ~300 CFU large clone libraries were constructed, thus providing a methodological comparison of the two vector systems, and giving a wider and more even coverage on the community members and structure. Clones were grouped by ARDRA and all OTU's insert length was measured by capillary electrophoresis. This allowed us to compare the community structure shown by the LH-PCR fingerprints and the two libraries. The samples showed enormous diversity, Simpson index values 0,976 and 0,984 for the pGEM and the TOPO library respectively. Major OTUs (58 groups including more than one clone) were sequenced. Parallel investigations on the same samples by culture-based molecular methods have led to an in depth description of the lake's microbial diversity.

MOLECULAR DETECTION AND EPIDEMIOLOGY OF TYPE A AND B RESPIRATORY SYNCYTIAL VIRUSES IN CHILDHOOD RESPIRATORY INFECTIONS

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Human respiratory syncytial virus (hRSV) is one of the major causes of respiratory infection of infants and children worldwide. The molecular epidemiology of hRSV is unknown in Hungary. Our aims were the molecular detection and genetic analysis of hRSV from childhood respiratory infections in Hungary. Samples, which were collected from children under the age of 10 years with acute respiratory infections were provided by Children's Hospital of Mosdós. Samples were taken from 15 October to 15 May in 2005/2006 and 2006/2007 seasons. The clinical and epidemiological data were collected with prospective method. The amplification of the surface fusion glycoprotein (F) and the attachment glycoprotein (G) genes of viral RNA was made by RT-PCR method. PCR-products were sequenced and analyzed. Nasopharyngeal aspirates of 104 children were examined out of which 23 (22.1%) samples – 16 males (69.6%) and 7 females (30.4%) – (first season: 1/49, 2%; second season: 22/55, 40%) contained hRSV. The hRSV infections were taking place from December to March. The average age was 2.1 years (1 month to 8 years). The leading symptoms were dropping nose, fever, cough and hard-breathing. Forty percent of the hRSV infected children had underlying disease. Based upon the F region 22 (96%) viruses genetically belonged to the type A and 1 (4%) was classified as type B hRSV. Based upon the G region out of the 11 type A viruses 8 (72.7%) belonged to group GA5 and 3 (27.3%) to group GA2. In several cases the nucleotide sequence of viruses was

identical. To our knowledge, this is the first report on molecular detection and genetic analysis of the two types (A and B) of hRSV of children under the age of ten with respiratory infections in Hungary. In winter and spring hRSV is an important cause of childhood respiratory infections particularly in infants, which require hospitalization.

ANALYSIS OF COMMUNITY STRUCTURE CHANGES IN SLUDGE DIGESTER MODEL SYSTEMS DURING CELLULOSE AND CELLULASE FEEDING VIA CHEMOTAXONOMICAL METHODS

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Community structures of microbes living in biogas reactors are an important field of research. In our work effect of feeding with milled grass and MethaPlus cellulase enzime product on communities producing biogas from wastewater sludge was studied. The aim of the present work was to follow the changes in the structure of eubacterial communities in the digesters via chemotaxonomical methods. Cultivation of 0.5 dm^3 microcosm cultures (derived from the operating digesters of the South-Pest Wastewater Treatment Plant) was performed at 35 °C, with 10 days exchange rate and continuous stirring. Basic feeding material was the same sterilized raw sludge through on the whole experiment. Thereby disturbing effect of daily change in contain of raw sludge was avoidable. Feeding was performed every two days. Produced gas was led through liquid filled glass pipes capable of detecting the volume of bubbles passing through. Manual evaluation of video records of bubbles provided information about gas yield.

Two of the eight microcosm digesters (signed as B and F) was fed in all occasions by 0.5 g dry, milled grass together with the sludge. Another two digesters (D and H) were fed with grass and MethaPlus enzyme product besides sludge. Two ones (C and G) as controls were fed only with MethaPlus and sludge while another two ones (A and B) only with raw sludge. Samples were taken in every four days. Analyses of total menaquinones and fatty acids of the samples were carried out.

Dominant menaquinone of our samples always was MK5(H2), which is associated to *Desulfobulbus* spp. Producers of this quinone were in dominant position all the while. In spite of this, in D and H bottles this dominance seemed to moderate. Several quinone species present in the beginning of the process (particularly many long chain, saturated forms produced probably by actinobacteria) disappeared at the end. Reason of this may be that community structure got simplified in time due to the one-sided feeding (in contrast with the varying composition of sludges in the plant). Specialists of the given constitution might crowed out the other guilds. Changes in abundance of fatty acid markers show larger difference between the states of same microcosms in different times than between several microcosms at the same time. At the second half of the experiment branched chain fatty acids show a complementer pattern with that of sulphate reducer markers (15:1, 17:1 fatty acids). Producers may be affected by the same factors. Ratio of cyclopropil fatty acids (presumably produced by fermentative bacteria) was minimal also in D and H bottles. As regard sulphate reducers, profiles of fatty acids and quinones hang together. Ratio of 15:1 and 17:1 fatty acids as well as that of MK5(H2) quinone decreased in D and H bottles compared to the other microcosms. Since sulphate reducers are considerable hidrogene competitors of methanogenes, this change is positive.

OCCURRENCE OF *STAPHYLOCOCCUS AUREUS* IN MILK SAMPLES OF COWS WITH MASTITIS

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Staphylococcus aureus is the most prevalent and economically significant pathogen causing mastitis in dairy cows. The objective of this research was to determine and help reduce the occurrence of mastitis caused by S. aureus on Hungarian dairy farms.
Six farms (F1 to F6) were enrolled in the study carried out from June 2005 through June 2008. The farms were located in Hajdú-Bihar County, at a distance of 15 km to 100 km from one another. Udder quarter milk samples were plated on Columbia Blood Agar and Baird-Parker Agar, and a coagulase test was used to confirm the presence of *S. aureus*. A total of 2299 milk samples were collected and examined. The occurrence rate of S. aureus in mastitic bovine milk samples ranged from as low as 0% to 15.1%, with an overall mean of 7.1%. On farms F1, F2, F5, and F6 less than 10% of the samples tested were positive for S. aureus. It is worth noting that no *S. aureus* positive sample was found on farm F1. By contrast, 14.9% and 15.1% of the samples collected on farms F3 and F4, respectively, had detectable counts of the pathogen. The incidence of *S. aureus* in mastitic milk was monitored over a 4-year period on farm F4. The percentage of *S. aureus* positive milk samples was found to progressively decrease from 22.5 to 4.7 on this farm. The most plausible explanation for this beneficial trend is that, as a result of regular bacteriological and resistance monitoring of the herd, infected cows were identified and segregated, and antibiotics were used appropriately and judiciously to treat cows for clinical and sub-clinical mastitis caused by *S. aureus*. In conclusion, it is recommended that a mastitis control program be in existence at farm level. This program should contain the following elements: good record keeping by the farmer; regular bacteriological and resistance monitoring of the herd; regular visits by a veterinarian to advise on optimum methods of control and to monitor antibacterial usage; and the monitoring of milk somatic cell counts and regular maintenance of the milking machine. *Staphylococcus* aureus is hard to eradicate, however, its occurrence should be reduced to less than 5% of the cows in the herd by practicing proper milking hygiene, administering dry cow therapy, and by culling chronically infected cows.

ACTIVITY SENSOR-EXPRESSING AUJESZKY'S DISEASE VIRUSES FOR NEURAL CIRCUIT ANALYSIS

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Currently available transsynaptic virus tracers are only applicable for mapping the anatomical architecture but not for the physiological characterization of a neural circuitry. In addition, techniques for the analysis of a particular circuit of multiple neurons at the same time within a region are not yet available. In order to attack these problems we have constructed Aujeszky's virus (AyV)-based transsynaptic tracers expressing troponeon, a fluorescence calcium sensor, which, beside labeling synaptically connected nerve cells, permits assaying the dynamics of neural activity in neurons located several synapses away from the inoculation site. A limitation of using viral-based methods for transsynaptic gene delivery is the cytotoxic effects of the virus especially at late phase of infection. To this end, we have generated timer viruses by inserting a red fluorescent protein gene expression cassette (DsRed2) to the genome of activity sensor PRV. DsRed2 served as indicator of late phase of virus cycle since it exhibits a slow maturation time, and it was inserted to a transcriptionally less permissive DNA region than the troponeon gene.

ANALYSIS OF GENOME STABILISATION IN *SACCHAROMYCES CEREVISIAE* X *SACCHAROMYCES UVARUM* INTERSPECIES HYBRIDS

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In a previous work we reported on the isolation and genetic analysis of a fertile interspecific hybrid of the yeast species, *Saccharomyces cerevisiae* and *Saccharomyces uvarum*, which are also important yeasts in wine fermentation [1]. Now we present novel data about the hybrid genome which we obtained from the analysis of additional hybrids.

To examine the hybrids and their meiotic progenies (spore clones) we used PCR-RFLP of genes and chromosomal regions of several chromosomes (such as HIS4, YCL008c, LEU2, MET2, MET10, URA3, ITS1-5,8S rDNS-ITS2) and the so-called delta-sequences which are specific for S. cerevisiae.

Our findings indicate that the transformation (reduction) of the hybrid allotetraploid genome into allodiploid is a gradual, complex process, in which we detected significant variance considering the origin of certain genes even in progeny clones coming from the same tetrad.

For other genes only alleles from one certain species (this was mainly *S. cerevisiae*) were found in the progeny generations after the relative stabilisation of their hereditary material. A very high variability of spores sharing the same origin was observed in the analysis of the delta-sequences, which flank the Ty1 transposons of *S. cerevisiae*. Our results give introspection to a theoretically interesting subject, that comes up frequently but yet is examined seldom: the emerging of interspecific hybrids and the stabilisation of their genomes. However, considering the role in the wine industry of the two yeast species, our results may also be important in a practical viewpoint; they may be applied to the production of new hybrid starter cultures for wine industry.

[1] Antunovics, Z. et al. (2005): FEMS Yeast Res., **5**, 1141-1150.

BIODEGRADATION OF PACKAGING MATERIALS

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The rate of packaging materials in the communal solid waste is cca 20-40 % worldwide (910-1840 thousand tons), and they mostly get into the dumps, and only less than 30 % is recycled (the EU demand is the 50 % recycling of the packaging waste). Selective garbage collection is partly working for papers, tin cans, PET and glass bottles, but not for plastic bags. The use of plastic for packaging is around 30-40 % of all materials. Synthetic polymers are difficult to degrade in the environment, and the use of biodegradable materials would be preferred. Photodegradation is a possible way, however it means first of all physical disruption and after that a very slow chemical/biological degradation. The tree symbol on packaging means compostability/ biodegradability.

Paper, plastic, paper/plastic composite, “compostable” commercial plastic bags and experimental starch-based packaging material were investigated for their biodegradation in soil applying embedding and respirometric methods and using Avicel® as reference material. Starch-based material and paper were readily biodegradable, while composits only up to their paper ratio. “Compostable”

commercial plastic bags treated or non-treated with UV illumination were stable within the 50 days of investigations.

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PRELIMINARY MOLECULAR STUDY OF NITROGEN FIXING BATERIAL POPULATIONS IN DETERIORATED SOILS

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Besides industrial pollution agricultural chemicals also cause great environmental damage. New methods allowing environment-friendly farming should be found, in order to preserve the fertility of soil and clearness of water. One of these methods is to decrease the usage of chemical fertilizers in intensive plant production. The resident bacterial populations greatly change due to acidification or secondary salinization of the soil. Only the most adaptive microbes can survive. This microflora is unique and highly adapted to the stress conditions.

The beneficial microbe-plant associations have overriding importance in the recultivation of deteriorated agricultural soils. Diazotroph bacteria are able to reduce air nitrogen to ammonia in the course of biological nitrogen fixation. Thus, nitrogen fixing bacteria play a significant role in nourishing plants in the deteriorated soils. Our primary aim was to develop a PCR based technique that enables us to detect the variability of the resident diazotrophs in deteriorated acidic, secondary saline soils in Hungary. Soil samples were obtained from intensively cultivated wheat field and secondary saline, deteriorated field out of cultivation. Aerobic and microaerophile nitrogen fixers were enriched from the rizosphere populations in nitrogen free medium. Nitrogen fixing ability was confirmed by acetylene reduction assay. Effective nitrogen fixing and salt tolerant isolates were obtained. Genus-based sorting of the isolates was approached by preliminary genetic polymorphism studies of particular genomic DNA region - Intergenic Transcirbed Spacer (ITS) between DNA sequences coding ribosomal RNA subunits 16S and 23S.

ITS sequences available from genomic DNA databases of prokaryotes were aligned (http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html) to get the corresponding ITS consensus sequence. Primer pair suitable for reliable getting of ITS regions was designed on the basis of the consensus. Amplifications from total DNA preparations of the isolates and reference strains were performed. DNA fragments of approx. 1500 bps were obtained, which overlap the total ITS regions and little parts of the neighboring 16S and 23S rDNA sequences. RFLP patterns of the fragments were produced and compared. Restriction endonucleases suitable for genus-based distinction among the tested isolates were proposed.

Our method will most probably be suitable for fast survey of main diazotrophic genera both in deteriorated soils and in overcultivated agricultural regions.

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ISOLATION AND CHARACTERIZATION OF AN IMPORTED PER-1 ESBL PRODUCER *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATE

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Pseudomonas aeruginosa is one of the most frequently isolated nosocomial pathogens, causing life-threatening infections, such as pneumonia, bacteraemia, and wound infections. It exhibits intrinsic resistance to several β-lactams and acquire easily additional resistance mechanisms, including the production of extended spectrum β-lactamases (ESBLs). PER (*Pseudomonas* extended resistance) enzymes are one of the rarely detected β-lactamases, although the importance of these enzymes is increasing. PER-1 confers clear resistance to oxyimino-β-lactams, especially ceftazidime. The bla_{PER-1} gene is usually located within a specific transposon, Tn1213. The PER-1 was recovered in 1991 from France. Later, PER-1 producers were recognised to be widespread in Turkey, and disseminated in Italy, Poland, Japan, and Romania. From Hungary, three PER-1 producer *P. aeruginosa* isolates have been detected, interestingly two of them from a Romanian citizen.
During the period of 2004 to 2008 we isolated 27 ceftazidime resistant, non-mucoid *P. aeruginosa* isolates from different non-cystic fibrosis patients, hospitalized in nine different hospital wards of South-Hungary. Identification by VITEK 2 system and susceptibility test by disk-diffusion method was performed, using CLSI breakpoints. The genes of the extended spectrum β-lactamases (PER-1, PER-2, TEM, SHV, GES, VEB-1, OXA I. group) were looked for by PCR methods. The strains were typed by pulsed-field gel electrophoresis, the clonality was detected by multi-locus-sequence typing (MLST). The eBURST algorithm was used for phylogenetic analysis. The iso-electric focusing of the β-lactamases was performed, the enzymes were visualized with nitrocefin. To investigate the location of the β-lactamase gene plasmid purification, S1 nuclease analysis and PCR detection of the Tn1213 specific IS element were performed. PCR experiments revealed the presence of bla_{PER} in one isolate. Sequencing of the coding region identified the PER-1 gene. According to the MLST analyses, this strain belongs to a clonal complex, previously identified in VIM metallo-β-lactamase producers in Hungary, namely CC11. Interestingly, the PER-1 producer strain was isolated from a polytraumatized Romanian citizen on admission to the hospital of Szeged. This suggests the possibility, that this strain was imported to Hungary from abroad.

COMPARISON OF DIFFERENT METHODS FOR THE MOLECULAR TYPING OF *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa is a Gram-negative rod causing life-threatening nosocomial infections, often isolated from outbreaks. Several methods for the typing have been developed to determine the relatedness of these nosocomial pathogens, such as bacteriophage typing, serotyping, plasmid fingerprinting, ribotyping, or PCR based methods. For epidemiologists a reliable typing method is essential for measuring the effectiveness of the infection controll, and in case of the increasing number of resistant *P. aeruginosa* isolates, the ability to decide if it is due to patient-to-patient transmission. During the period of 2004 to 2008 we isolated 28 carbapenem resistant non-mucoid *P.*

aeruginosa isolates from different non-cystic fibrosis patients, hospitalized in nine different hospital wards of South-Hungary. Identification by VITEK 2 system and susceptibility test by disk-diffusion method was performed. We compared the discriminatory power of different typing methods for these *P. aeruginosa* isolates, namely pulse-field gel-electrophoresis (PFGE), multilocus sequence typing (MLST), and the DiversiLab typing system (BioMériux), which is based on repetitive element-based PCR (rep-PCR). Rep-PCR method uses primers targeting highly conserved repetitive elements in the bacterial genome, and suitable to determine the genetic diversity of *P. aeruginosa*. PFGE is considered to be the "gold-standard" method for molecular typing of *P. aeruginosa,* commonly used and has a high discriminatory ability. However it is limited by technical complexity, expense and time. In 2004 Curran and colleagues (JCM, 2004) developed the MLST scheme for *P. aeruginosa*, which is based on the allelic differences in certain housekeeping genes (acsA, nuoD, trpE, mutL, guaA, aroE, ppsA), and becoming more and more popular bacterial typing method.
We determined ten different pulso-types with the PFGE. Twenty-one of the 28 isolates were members of three different outbreaks observed in the intensive care unit, and according to the MLST analysis they belong to clonal complexes. Our experiences suggest, that the PFGE had a higher discriminatory power than the MLST, but the latter method provides more data about the clonal relationship of the isolates. Rep-PCR is suitable as a rapid epidemiologocal surveillance tool, however, it is not able to discriminate highly related isolates.

GENETIC DRIFT AND PANDEMIC POTENTIAL OF GENOTYPE GII4 NOROVIRUS STRAIN IN HUMANS IN SEVEN CONSECUTIVE EPIDEMIC SEASONS AND THE FIRST DETECTION OF CALICIVIRUSES IN ANIMALS (SWINE AND CATTLE) IN HUNGARY

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Caliciviruses are belong to a genetically diverse group of RNA viruses with 5 genera and multiple genogroups (G) and genotypes. Viruses in both Norovirus and Sapovirus genera are known to infect human and animal species. In humans, norovirus genotype GII4 is the predominant agent in gastroenteritis outbreaks all over the World including Hungary. However, there are no data about the detection of animal caliciviruses in the country. The author's aim was to investigate the genetic variation of the epidemic GII4 strains in seven epidemic seasons from November 2000 to June 2007 and the molecular detection of animal caliciviruses in swine and cattle in Hungary. Based upon the prospective molecular epidemiological surveillance of norovirus outbreaks in Hungary, GII4 outbreak strains were selected for genetic and antigenic analysis for RNA-polymerase (ORF1), capsid (ORF2 including N, S P1A, P2 and P1B domains) and ORF1/ORF2 junction regions by RT-PCR, sequencing and phylogenetic analysis. Fecal samples from swine (in 9 farms, 436 samples) and cattle (in 2 farms, 73 samples) in different age-group were also tested by RT-PCR and sequencing for caliciviruses. Three hundred and seventy seven (76.8%) of 491 confirmed norovirus outbreaks were caused by genotype GII4. GII4 was the predominant genotype in 6 of the 7 epidemic seasons. Four main GII4 variants - epidemic point mutants - (GII4-2000, GII4-2002, GII4-2004 and GII4-2006b) were detected each of them circulating dominantly in 2 consecutive epidemic seasons associated with outbreaks in hospitals, residential institutions and elderly homes. GII4-2006b strain was also identified in the largest waterborne outbreak ever seen in Hungary in Miskolc in June 2006 which

had a major impact on the norovirus epidemic season of 2006/2007. Nine sapovirus (2.1%) and 1 (0.2%) norovirus (GII) were detected in young domestic pigs in 3 farms. Four (5.5%) noroviruses, three genetically identical GIII2 and a GIII1 strains were detected in young cattle. Genotype GII4 confirmed as a predominant genetic type in epidemic norovirus seasons. Genetic drift is one of the potential factors which promotes the re-emergence of GII4 variants in the population. The elevated number of norovirus outbreaks in the population predict the emergence of new GII4 genetic variants as part of an international epidemic. Major public health catastrophe can also influence of the norovirus seasonality. This study confirms the circulation of caliciviruses in swine and cattle in Hungary (and in Europe) and give additional information of their genetic diversity and relationship to viruses referred to as human caliciviruses, too.

PRODUCTION OF FUNGAL CELL-WALL DEGRADING ENZYMES OF *PLEUROTUS* PATHOGENIC *PSEUDOMONAS* STRAINS

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Various species belonging to the *Pseudomonas* genus play key role in the growing and differentiation of mushrooms, but depending on the environmental conditions - such as temperature, relative humidity and other factors - some strains could become pathogenic and cause different diseases. The yellowing of *Pleurotus ostreatus* and the brown blotch disease of Agaricus bisporus, caused by *Pseudomonas tolaasii* is well known. The bacterium produces the toxins tolaasins that disrupt the cellular membrane by forming pores. *P. tolaasii* can be identify easily, with tolaasin toxin gene specific primers. Besides the tolaasins there are various membrane and cell degradative enzymes - produced by the members of the *Pseudomonas* genus - which could increase the mushroom destruction.The aim of this study was to find correlation between the enzyme production and the pathogenicity of the pseudomonads. We collected samples from the different steps of the production, in an infected oyster mushroom (Pleurotus ostreatus) farm in Hungary and we isolated *Pseudomonas* strains. Sixty *Pseudomonas* strains, belonging either to the fluorescent or to the non-fluorescent groups, were isolated on the *Pseudomonas* selective S-1 medium, from infected, deformed fruit bodies of the oyster mushroom, water and straw samples. For the molecular experiments DNA samples were isolated from the strains, and specific PCR reactions were performed with special primers. Fungal membrane and cell-wall degradative enzyme producing abilities were examined by classical and modern chromogenic and fluorogenic substrate methods. The enzymes of β-1,3-glucanase, chitinase, protease and lipase enzyme systems were investigated. On the basis of molecular investigations and in vitro antagonism tests the strains were divided into a pathogenic and an apathogenic group, and their enzyme production abilities were investigated. Strong correlations were found between the pathogenicity and the levels of glucanases and chitinases of the strains.

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DIVERSITY OF ARBUSCULAR MYCORRHIZA FUNGI COLONIZING MAIZE AT DIFFERENT PLANT DENSITIES

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Arable crops can be colonised extensively by arbuscular mycorrhiza (AM) fungi and crop production benefits from this mutualistic plant–fungus interaction. We assessed the diversity of AM fungi associated with maize in a long-term crop production experiment established at Martonvásár to understand the effect of different fertilization practices and plant densities (70.000 and 100.000 plants ha^{-1}) on species diversity and community structure of these organisms [1].
Differences in small subunit ribosomal RNA genes amplified by nested PCR were used to identify groups of arbuscular mycorrhiza fungi actively colonizing maize roots [2]. Amplified products were cloned into *Escherichia coli* DH5α and subjected to RFLP analysis. The phylogenetic diversity of AMF subgroups revealed by PCR-RFLP was estimated by using the Shannon-Weiner diversity index. The *Glomus* clade dominated at both plant densities, but there were significant differences in the phylogenetic group composition of AM fungi demonstrating the impact of plant densities on AMF subgroups in the soil. Members of the *Glomus*-Aa subgroup occurred more frequently at low plant density, whereas fungi belonging to the *Glomus*-Ad subgroup were showed more intense colonization activity at high plant density. Besides *Glomus*–A fungi, members of the Glomus-B group also occurred at both plant densities, but at significantly lower frequency. According to previous research AM fungi contribute less efficiently to phosphorus supply of plants under high plant density due to the extensive overlap of root and hyphae phosphorus depletion zones. The present survey revealed that plant density substantially affects of composition of AM microflora and this may also influence the nutrient uptake of plants.

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PRODUCTION OF BIOLOGICALLY ACTIVE LIGNANS WITH FORSYTHIA CELL CULTURES IN BIOREACTOR

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The literature contains many examples for the production of natural compounds by plant cell cultures. The capability of cultivating plant tissues as single cells or small clumps of cells has provided the opportunity for development of large scale processes for production of plant cells and their products by methods analogue to those used in microbial production.
The central problem in the industrial application of plant cell cultures is scaling up the laboratory methods to large-scale fermentation. The reason for this is the specific characteristics of plant cells. These are the following: under mechanical agitation the plant cells easily break, low growth rate of plant cells in culture, and optimal conditions for growth and secondary product synthesis are significantly different. The cytodifferentiation is the key of secondary metabolite production. In most cell cultures accumulation of metabolites occurs maximally, when the growth rate of the cultures decreases or starts to decrease and/or the cultures exhibit some structural differentiation.
The ideal kinetic pattern for growth and metabolite accumulation is so-called growth associated product synthesis. This kinetic pattern qualifies the cell cultures for an industrial use in a large-scale fermentation technology. In our laboratory cell cultures of the plant *Forsythia* were established. This

plant accumulates biologically active lignans (arctigenin, matairesinol, pinoresinol and phillygenin) in high quantities. These lignans have come to the fore in research due to their significant pharmaceutical effects. They possess antitumor activity against various types of cancer and also have anti-HIV, antiinflammatory, hepatoprotective and neuroprotective and antioxidant activity. In our present work the establishment of *Forsythia x intermedia* in vitro cultures, optimalization of culture media and conditions are performed. Callus tissues were induced from leaves of *Forsythia x intermedia* on Gamborg B5 medium supplemented with 0.5 mg/l 2,4-D. Calli were placed on Murashige & Skoog (MS) solid media containing different types of hormones. After three subcultures they were placed into liquid media, and agitated in a flask. A version of MS medium supplemented with 2 mg/l naftilacetilacid and 0.2 mg/g kinetin was proved to be the best, providing high lignan content (3.9 mg arctigenin/g dried matter), in suspension culture maintained under light. Besides the medium's hormone content, the lightning (10-15 µmol/m2/sec) played a great role in the high lignan production. Furthermore the biomass production of this suspension culture (grown in this medium) was also high. It means that the product formation is nearly parallel to the growth rate of the biomass. Since the amount of product per mg cell is constant, it seems to be the best choice for a large-scale fermentation technology and hopefully for scale-up too. At present the fermentation experiments on laboratory level are under way.

CHARACTERISATION OF AVIAN *PASTEURELLA MULTOCIDA* ISOLATES WITH MOLECULAR METHODS

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Pasteurella multocida is a Gram-negative bacterial pathogen, which is known to cause a big variety of diseases in mammals and birds worldwide including fowl cholera, bronchopneumonia and hemorrhagic septicaemia in cattle and buffaloes, atrophic rhinitis in swine and snuffles in rabbit. Human cases are also described. Fowl cholera is a severe systematic disease of different avian species that results in significant economic losses to poultry industries. *P multocida* has been isolated from more than 100 different wild and domestic avian species. This wide host-spectrum provides *P multocida* with high surface exposed antigen variability. In our study we investigated 61 *P multocida* strains isolated from geese, domesticated and Muscovy ducks, turkeys, chickens and pheasants in Hungary. The strains were examined with molecular fingerprint method to detect relationships between the strains and distinguish isolates from different hosts. ERIC-PCR (enterobacterial repetitive intergenic consensus sequencer polymerase chain reaction) analysis showed considerable correlation with the geographical origin and the host species. It gave a possibility to detailed examination of host adaptation of our strains. The bacterial surface is the point of the interaction between the bacterium and the host cells. The molecules within the outer membrane that promote adherence have a potential role in colonization and infection of the host cells. Therefore we selected some genes coding of variant surface exposed proteins and studied them with PCR-RFLP (PCR-restriction fragment length polymorphism). These proteins represent different kind of cell surface molecules: capsule (hyaD-hyaC), porins (ompH, ompA), fimbriae (ptfA) and other outer membrane proteins (oma87). The PCR-RFLP technique is able to identify DNA sequence variation in the target gene region in two steps by amplification with PCR then digestion with a restriction endonuclease. The DNA diversity of these genes refers to the changes in the surface exposed proteins, which could be putative responsible factors of host-pathogen interaction in fowl cholera.

A NOVEL ANAMORPH GENUS THAT REPRESENTS A POTENTIAL LINK CONNECTING MICROSTROMATALES, EXOBASIDIALES AND ENTYLOMATALES

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Microstromatales, Exobasidiales and Entylomatales are related orders of the class Exobasidiomycetes that comprise species which are usually dimorphic having both yeast and hyphal phases. Recently, we described a novel genus, *Jaminaea*, that formed a basal branch of Microstromatales in molecular phylogenetic analysis of the chromosomal regions coding for the D1/D2 domain of the LSU 26S rRNA, the SSU 18S rRNA and ITS1-5.8S rRNA-ITS2. The chromosomal region encoding the 18S rRNA contains an S943 nuclear small subunit rRNA group IB intron similar in location and sequence to introns found in certain species of Exobasidiales and Entylomatales. Since no similar introns have been detected in Microstromatales, the new genus may represent a phylogenetic link connecting these three orders. The type species *Jaminaea angkorensis* (isolated in Cambodia) produces smooth, slightly pink yeast colonies on culture media, which upon prolonged incubation form slowly growing invasive mycelium extending towards nutrient-rich parts of the medium.

INVESTIGATION OF THE CARBAPENEM AND CEFOXITIN RESISTANCE MECHANISMS OF *BACTEROIDES*: DETECTION AND ANALYSIS OF HETERORESISTANT STRAINS

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During our regular antibiotic resistance surveillances and investigations of the resistance mechanisms of *Bacteroides* spp., strains heteroresistant for cefoxitin and carbapenems were isolated. Heteroresistant phenotypes are already known for methicillin and vancomycin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. Our aim was to document and characterize the heteroresistant phenotypes and the underlining resistance mechanisms of these *Bacteroides* strains by antimicrobial susceptibility testing and molecular methods.

In previous antibiotic susceptibility surveys of *Bacteroides*, 8 *B. fragilis* strains proved heteroresistant to carbapenems in Etest susceptibility measurements. The phenotypes in these cases involved the appearance of resistant colonies in the inhibition zones (borders of continuous growth 0.25-4 μg/ml with resistant colonies up to 16-32 μg/ml). All of them were cfiA-positive, but they did not harbor insertion sequence elements in the upstream region of the resistance genes. One strain from a previous pan-European susceptibility survey, with an imipenem MIC of 16 μg/ml determined by agar dilution, was able to grow in the presence of 100 μg/ml imipenem, probably due to the presence of more resistant subpopulations of cells. Analysis of the resistance mechanisms of carbpenem-resistant strains from a current European *Bacteroides* antibiotic susceptibility study is presented. Among 100 *Bacteroides* isolates from 2007, 21 strains proved to be heteroresistant to cefoxitin. Their Etest patterns generally displayed continuous growth of the less susceptible subpopulation from 8-128 μg/ml up to 256 μg/ml. Of these 21 strains, 11 harbored cfxA genes and their upstream regions were usually altered to the common 1.2 kb fragment, as seen in our previous studies.

Population analysis profiles demonstrated the presence of more resistant subpopulations in the cultures of strains corresponding to the more resistant colonies in the Etest ellipse zones. Heteroresistance to important β-lactam antibiotics appears among *Bacteroides* strains, but the phenotype can not yet be linked to any particular genetic constitution. Additionally, the significance of such strains should be examined from the aspect of more clinical detail.

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SINGLE-TUBE ASSAY FOR DETECTION OF MICROORGANISMS FROM BIOLOGICAL FLUIDS

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Early detection and adequate treatment of infections are critical for successful outcomes for patients with systemic infections. Application of blood cultures are time consuming and often yield false-negative results due to low sensitivity. Non-culture methods including polymerase chain reaction (PCR) being developed for detection systemic infections. Previously we have reported on the PCR-based system which can detect and differentiate of fungal pathogens. Further experiments have been carried out to find proper approaches for the species-specific detection of bacterial DNA, too. Klaschik and co-workers have described a LightCycler based system to detect and differentiate a Gram-positive and Gram-negative bacteria. This method is used the upper channels (640 and 705 nm) of the LC 1.5, while our method uses the lower one (580 nm). Both methods take an advantage of the melting point analysis. In addition, the annealing temperatures of the primers and the amplicon length are similar. Based on these facts o aim of the present study was to combine the two methods to detect and differentiate the most common pathogens in one step. Further advantages of this method are its rapidity and the fact, that the tests can be performed without species specific probe sets.

MICROBIOLOGICAL RISK MANAGEMENT (MRM)

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Diseases caused by foodborne microbial hazards1 constitute a world-wide public health concern. During the past several decades, the incidence of foodborne diseases has increased in many parts of the world. Foodborne threats occur for a number of reasons. These include microbial adaptation, changes in the food production systems, including new feeding practices, changes in animal husbandry, agronomic process and food technology, increase in international trade, susceptible populations and travel, change in lifestyle and consumers demands, changes in human demographics and behaviour. The globalisation of food markets has increased the challenge to manage these risks. Effective management of risks arising from microbial hazards is technically complex. Food safety has been traditionally, and will continue to be, the responsibility of industry operating an array of control measures relating to the food hygiene within an overall regulatory framework. Recently, risk analysis, involving its component parts of risk assessment, risk management and risk communication, has been introduced as a new approach in evaluating and controlling microbial hazards to help protecting the health of consumers and ensure fair practices in food trade. It could also facilitate the

judgement of equivalence of food safety control systems. Government decisions and recommendations have as their primary objective the protection of the health of consumers. The definitions of risk analysis terms related to food safety incorporated in the Procedural Manual of the Codex Alimentarius Commissions. This new definitions: hazard, risk, risk analysis, risk assessment, hazard identification, hazard characterisation, dose-response assessment, exposure assessment, risk characterisation, risk management, risk communication, risk assessment policy, risk profile, risk estimate, food safety objective (FSO), performance objective (PO), performance criterion (PC), traceability/product tracing and equivalence. Risk manager8 is defined as follows: a national or international governmental organisation with responsibility for microbiological risk management.
The definition of the appropriate level of protection (**ALOP**) is the one included in the WTO Agreement on the Application of sanitary and phytosanitary measures (SPS agreement), Annex A, para 5. In the MRM process, the ALOP is a key concept, as it is a reflection of a particular country's expressed public health goals for foodborne risks. According the approved Codex Guidelines (CAC/GL 63 – 2007) the main principles of MRM are PRINCIPLE 1: Protection of human health is the primary objective in MRM. PRINCIPLE 2: MRM should take into account the whole food chain. PRINCIPLE 3: MRM should follow a structured approach. PRINCIPLE 4: MRM process should be transparent, consistent and fully documented. PRINCIPLE 5: Risk managers should ensure effective consultations with relevant interested parties. PRINCIPLE 6: Risk managers should ensure effective interaction with risk assessors. PRINCIPLE 7: Risk managers should take account of risks resulting from regional differences in hazards in the food chain and regional differences in available risk management options. PRINCIPLE 8: MRM decisions should be subject to monitoring and review and, if necessary, revision. MRM should address the food chains as individual continuums, when considering means for controlling the public health risks associated with food. This should typically include primary production (including feeds, agricultural practices, and environmental conditions leading to the contamination of crops and animals), product design and processing, transport, storage, distribution, marketing, preparation, and consumption.

COMPARISON OF KILLING ACTIVITY OF CASPOFUNGIN AGAINST *CANDIDA PARAPSILOSIS*, *C. ORTHOPSILOSIS* AND *C. METAPSILOSIS*

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Killing activity of caspofungin was determined against seven *C. parapsilosis*, three *C. orthopsilosis* and four *C. metapsilosis* strains. Minimum fungicidal concentration (MFC) was determined using the standard broth microdilution method with elevated (105 cells/mL) starting inocula in RPMI-1640 and antibiotic medium 3 (AM3). In time-kill tests all strains were tested at 0.06-16 mg/L caspofungin concentrations in RPMI-1640 and AM3. In RPMI-1640 MFC ranges for *C. parapsilosis* and *C. orthopsilosis* were 4->8, while for *C. metapsilosis* were only 0.5-2 mg/L. In AM3 MFC values, with the exception of a single *C. parapsilosis* isolate, were lower by at least two two-fold dilutions for all three species. In the killing studies, caspofungin showed fungistatic effect in case of *C. parapsilosis* and *C. orthopsilosis* in RPMI-1640 even after 48 h. However, two *C. parapsilosis* and two *C. orthopsilosis* isolates were killed at a single concentration (2 and 16 mg/L in case of each species). Caspofungin was fungicidal at 1-8 mg/L concentrations against all but a single *C. metapsilosis* isolate. In AM3 four out of the seven *C. parapsilosis* strains were killed at ≥0.5 mg/L caspofungin concentrations; 3 isolates showed paradoxical growth. In AM3 all *C. orthopsilosis* and *C. metapsilosis* strains were killed at 1 and 0.06 mg/L caspofungin concentrations, respectively.

Caspofungin both in RPMI-1640 and AM3 showed better in vitro killing activity against the newly described C. orthopsilosis and *C. metapsilosis* species, than against *C. parapsilosis*.

LINEAGE-SPECIFIC SILENCING OF HUMAN IL-10 GENE EXPRESSION IN NORMAL CERVICAL EPITHELIAL CELLS AND IN CERVICAL CANCER CELLS

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Epigenetic analysis was performed to demonstrate that the normal and neoplastic epithelial cells do not serve as the source of the locally elevated IL-10 production during cervical carcinogenesis. Bisulfite sequencing was used to correlate promoter CpG methylation with the transcription of the gene. Lack of IL-10 transcription in HeLa, SiHa, Caski, HT-3, C33-A, HaCaT cell lines and in primary human keratinocytes correlated consistently with the methylated state of the proximal CpG residues, particularly with the two most proximal CpGs at positions –185 and –110. On the other hand, IL-10 producing peripheral blood mononuclear cells had unmethylated CpG residues in the proximal promoter associated with acetylated H3 and H4 histones as determined by chromatin immunoprecipitation. To prove that promoter methylation is not induced during extensive culturing of the epithelial cell lines,we determined the methylation status of the two CpG sites in normal exfoliated cervical cells (n = 3) and in cervical cancer tissue specimens (n = 10). CpG site -110was uniformly methylated in cervical cancer biopsies and in normal cervical epithelial cells. CpG site -185 also tended to be highly methylated in the clinical samples, although partial demethylation at this site was found in two cervical cancer biopsies. In conclusion, the CpG methylation pattern of the proximal promoter is implicated as a major determinant of transcriptional silencing of human IL-10 expression in cells of cervical epithelial origin.

INVESTIGATIONS ON THE ROLE OF HUMAN PAPILLOMAVIRUSES IN ORAL CARCINOGENESIS

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The role of HPVs in malignant tumours of the head and neck is controversial. Their etiological role in oral and laryngeal cancer has been suggested by numerous authors, this has remained unproven. Our aim was to investigate the potential role of HPVs in oral squamous cell cancer (OSCC) and its premalignancies oral leukoplakia (OL) and oral lichen planus (OLP). Tumour tissue samples were collected from excised OSCC lesions, OL and OLP was sampled using cytobrush. Exfoliated cells collected from apparently healthy mucosa accompanied each lesion sample. Exfoliated buccal epithelial cells were collected from an age-matched group of healthy individuals and used as a control. HPV was detected using the widely used MY-GP nested PCR, genotyping of HPVs was performed by restriction analysis of GP amplimers. A gradual increase in HPV prevalence was observed in lesions with increasing severity, together with low positivity rate in control population (3/72; 4.2%). In OLP, OL and OSCC lesions we found 31.9% (37/116), 40.9% (18/44) and 46.7%

(28/60) HPV positives, respectively. Patients with HPV positive lesions carried HPVs in their healthy mucosa at lower prevalence, but in these samples similar tendency was observed (positive samples in OLP, OL and OSCC were 11/37, 29.7%; 8/18, 44.4% and 14/28, 50.0%, respectively). HPV genotypes were mainly high-risk genotypes (HPV16 and HPV18). HPV genotypes of patients with HPV positive lesions were the same found in the lesion with the exception of one HPV16 positive OSCC patient carrying HPV11 in the normal mucosa. All lesions carried HPV significantly more frequently than the healthy controls ($p<0.001$ in all comparisons), but we did not find significant difference between any two patient groups ($p>0.5$). The gradual increase of HPV prevalence we report here strongly suggests an association of HPVs with increasing dysplasia severity and malignant transformation, supporting that HPVs may be involved in carcinogenesis in the oral mucosa.

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TRANSCRIPTIONAL REGULATORS IN *SCHIZOSACCHAROMYCES POMBE*

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In eukaryotes, transcriptional regulation of protein-coding genes depends on a complex interplay between signal-transduction pathways, gene-specific regulators, general and special transcription factors and RNA Polymerase II. The Mediator complex is also essential for the regulated gene expression. It acts as a bridge between the control elements and transcriptional machinery. To learn more about this regulation, two subunits of the *Sch. pombe* Mediator have been investigated. Here we report on the results of genetic interactions between *Sch. pombe* med10, med8 genes and other regulator genes. We also show the bioinformatics analysis of the genes and their promoter regions, which are regulated by the above Mediator subunits. We show the tagging of the med10, med8 genes and their integrative transformation.

INVESTIGATION OF THE FUMONISIN MYCOTOXINS PRODUCED BY HUNGARIAN *FUSARIUM VERTICILLIOIDES* ISOLATES

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Mycotoxins are toxic secondary metabolites produced by certain fungi growing on agricultural commodities in the field and/or during storage. Fumonisins are produced by several species of *Fusarium*; however *F. moniliforme, F. proliferatum*, and *F. nygami* are the principal fumonisin-producing strains. The toxicity of the B series of fumonisins has been extensively studied, and a variety of species-specific toxicities have been reported. The 28 fumonisin analogues that have been characterized from 1988 to the middle of 2006 can be classified into four main groups, identified as

the fumonisin series A, B, C, and P. The fumonisin B (FB) analogues are the most abundant naturally occurring fumonisins, with FB1 being the predominant and usually being found at the highest levels. FB1 typically accounts for 70 to 80% of the total fumonisin produced, while FB2 usually makes up 15 to 25% and FB3 from 3 to 8% when cultured on maize, rice or in liquid medium. Apart from the FB series, some of the other analogues may also occur in naturally contaminated maize at relatively low levels (<5% of the total fumonisin content). In the last few years, 60 F. verticillioides isolates were collected about originating from different plants grown in Hungary. The strains were purified and the rice cultures were infected with them for the fumonisin fermentation. The secreted mycotoxins where analysed by reversed-phase (RP) high performance liquid chromatography (HPLC)/electrospray ionization (ESI) ion trap (IT) multistage mass spectrometry (MS) after the simple sample extraction step. Characteristically different fumonisin profiles were obtained within some of the culture extracts analysed. In most samples the fumonisin profile was similar to the formerly published results, i.e. FB_1 was found in the highest amount. However, some of the culture extracts did not contain FB_1, and at the same time these samples contained high amounts of FB_2 and FB_3. The experiments furnished the result that, besides well-known mycotoxins of fumonisin type, some of the culture extracts also contained numerous novel developed fumonisin analogues and fumonisin-like compounds, which were published earlier by Bartók et al. in 2006.

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CHARACTERISATION OF LIVE ATTENUATED *SHIGELLA FLEXNERI* VACCINE CANDIDATES

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Bacillary dysentery caused by *Shigella* pathogens elicits more than 1 million fatal cases worldwide, mostly among children under the age of 5. Furthermore, *Shigella* spp. tend to become resistant to most of the antibiotics used in the therapy of bacillary dysentery. These facts would highly justify development of vaccines against *Shigella* pathogens. In spite of extensive studies, however, no licensed vaccines are currently available mainly due to the lack of cross-protection against the numerous serotypes of *Shigella* spp. Moreover, the nature of antigens required for protection is not fully elucidated. There are two main factors playing significant role in the pathomechanism of shigellosis; the LPS (which determines the serotypes) and the virulence plasmid responsible for the invasive phenotype. We have examined the immunogenicity and the role of these antigens in the development of protection. We constructed an isogenic auxotrophic (ΔaroC) and LPS rough mutant (ΔrfbF) of *S. flexneri* 2a strain 2457T by non-polar deletions. Furthermore, we selected variants of both the auxotrophic and LPS mutants as well as their parental strain, which had spontaneously lost the virulence plasmid (resulting in the loss of the invasive phenotype). The virulence and the immunogenic potencial of the mutants was tested in the mouse lung model. We demonstrated that inactivation of aroC and rfbF resulted in moderate attenuation, whereas lack of the virulence plasmid caused a much higher elevation of 50% lethality dose (LD_{50}). Mucosal immunization with both the invasive and the non-invasive mutants elicited significant protection against a challenge by the virulent parental strain. However the histological examination of the lungs of mice infected with the invasive and non-invasive mutants proved qualitative difference in the cellular immune response provoked. The humoral immune response was assessed by the determination of the serum and mucosal Ig levels. We attested that the immunisation with the rough non-invasive double-mutant provoked significant anti-*Shigella* titers. Furthermore, it was shown that conserved antigens shared by

heterologous serotypes are more immunogenic in the double mutant background compared to the parental strain. As this double-mutant elicited good protection against both homologous and heterologous strains, existence of protective antigens other than the O-antigens or plasmid encoded antigens can be supposed. Identification of these antigens is of major importance in the development of a broad protective vaccine strategy against shigellosis.

COMPREHENSIVE STUDY OF OXYGEN SUPPLY DURING LYSINE PRODUCTION IN 420 M^3 TOTAL VOLUME FERMENTERS

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Repeated fed batch lysine fermentation technology was adapted and developed in 420 m^3 total volume fully instrumented automatic fermenter systems. The effects of the aeration, agitation, pressure, working volume and the antifoam- and sugar feedings on the productivity were analysed. Beside the controlled environmental parameters and substrate feedings dissolved oxygen (DO) concentrations and redox potentials (ORP) were measured simultaneously at three different levels of the fermenters and controlled at the bottom impellers. O_2 & CO_2 contents of the effluent gases were also monitored. Oxygen limitation and the correlations of the DO and ORP were investigated. Theoretical oxygen saturation concentrations at the different levels were calculated. The ratios of sugar uptake/oxygen uptake were also determined. No any oxygen limitation was observed in case of positive DO partial pressure. Close correlations are existing between the DO and ORP data. At very low DO concentrations the ORP measurements are more reliable than the DO. The multilevel measurements of DO and ORP are very useful tools to investigate the oxygen avaibility at the different levels of a high volume production fermenter. It can be concluded that we are not able to control the fermentations by the DO or ORP but we are able to control the DO and ORP by the fermentations. No inhibitory effect was observed even at high CO_2 concentration in the effluent gases. Using the above mentioned results and taking the sugar uptake/oxygen uptake ratios as very useful parameter of the oxygen utilization we are able to optimize the oxygen supply not only for the requirements of the metabolism but according to the economical aspects too.

COMPARISON OF SOME SOIL MICROBIAL AND BIOCHEMICAL CHARACTERISTICS IN SOME LONG-TERM EXPERIMENTAL FIELDS

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Soil quality assessment is based on physical, chemical and biological properties of soils. It is crucial to appoint a relevant reference, the range and temporal variability of each microbiological and biochemical variables. Our aim was to study soils originating from long-term experimental fields having a wide range of texture, organic matter and pH and without treatment e.g. control or undisturbed field which might be used as a local reference. The range of microbiological and biochemical properties and correlations between them and with soil physical and chemical properties

were evaluated. We found that most of the investigated microbial biomass and activity indicators differed significantly among soils. We found significant correlations between microbial C and microbial N (r=0.90), microbial C and substrate induced respiration (r=0.99). Fluorescens diacetate hydrolysing activity was correlated with phosphatase activity (r=0.79). Soil texture was correlated with the counts of aerobic heterotrophic bacteria and also with the counts of cellulose decomposing bacteria. Soil pH was not correlated with any investigated microbial properties. Humus content was in correlation with the counts of heterotrophic bacteria, fungi and aerobic cellulose decomposing bacteria, microbial biomass C and substrate induced respiration. Soil texture and humus content was also significantly correlated. Fine texture soils contained more organic matter.

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STUDY OF BACTERIAL BIOFILMS IN AN ULTRA PURE WATER INDUSTRIAL SYSTEM USING CULTIVATION

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Ultra pure water, that contains only traces of organic and inorganic solute matter, is widely used as industrial cooling water: a nutrient deficient environment with low osmotic pressure, however it's microbial contamination may occur. Due to the sedentation of microorganisms, biofilms can arose on different surfaces of the system. This immobilised biomass clogs the heat transfer, the water flow, and subjects the pipelines to microbially-influenced corrosion. As part of a polyphasic investigation of a power plant's cooling water system, the biofilms developed on the inner surface of the pipes in fornt of (sample 1), and behind (sample 2) an ion-exchange resin were studied by cultivation, using R2A, M27 and TSA media. The 138 (sample 1) and 117 (sample 2) strains we got after random isolation and purification were grouped according to their ARDRA pattern (with AluI and Hin6I enzymes) in case of sample 1, and their membrane fatty-acid composition in case of sample 2. The phenon-representatives were identified by partial 16S rRNA gene sequence analysis.
Our research revealed a diverse bacterial community in both samples, with a significant difference between the cultivable microbiota of the biofilms in front of, and behind the ion-exchange resin. From sample 1, we isolated α- (*Mesorhizobium huakuii, Rhizobium rhizogenes/lustianum*) and β-proteobacteria (*Variovorax paradoxus, Pandoraea norimbergensis, Ralstonia insidiosa*), low G+C Gram-positive bacteria (*Bacillus* sp., *Staphylococcus epidermidis*) and Actinobacteria (*Tsukamurella spumae/pseudospumae, Tsukamurella pulmonis/poriferae, Kocuria kristinae, Kocuria carniphila, Rhodococcus erythropolis, Micrococcus chenggongense, Brevibacterium casei, Microbacterium xylanilyticum*). In sample 2, we were able to identify α- (*Bradyrhizobium* sp., Blastobacter denitrificans) and β-proteobacteria (*Ralstonia insidiosa, Ralstonia pickettii, Variovorax paradoxus*), members of the CFB group (*Elizabethkingia meningoseptica*), low G+C Gram-positive bacteria (*Bacillus* sp., *Staphylococcus epidermidis, Lactococcus lactis*) and Actinobacteria (*Leifsonia xyli, Microbacterium paraoxydans, Brevibacterium casei*). As shown above, the ion-exchange resin may induces a notable change in the pipeline biofilms, several genera are detected in sample 1, but cannot be found in sample 2. Genus *Bacillus, Ralstonia, Variovorax, Staphylococcus, Brevibacterium* and *Microbacterium* are present in both samples, the first two being the most abundant member of the cultivable biofilm community behind the resin. Most of the bacteria isolated from the two samples have an aerob respirative chemoorganotroph, or facultative chemolithotroph metabolism, minor part of them are fermentative organisms. Several of the identified microbes are commonly isolated from oligotrophyic enviroments, and from ultra pure water industrial systems (members of genera

Ralstonia, Bradyrhizobium, Staphylococcus, Microbacterium, Bacillus). Certain strains of genera *Ralstonia, Blastobacter* and *Variovorax* are known of their ability to grow chemolithotrophycally with H_2 utilization, therefore they might influence a corrosive process.

DETECTION OF HUMAN BOCAVIRUS FROM STOOL SAMPLES OF HUNGARIAN CHILDREN WITH ACUTE GASTROENTERITIS

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Human bocavirus (HBoV) was identified by non-specific genome amplification methods in 2005. The virus was assigned to the Parvoviridae family, and was associated with respiratory diseases and acute gastroenteritis mostly among young children. Two variants of the virus with different geographical distribution are described. So far, nothing is known about differences in biological characteristics and clinical aspects of the genotype groups. HBoV infections show a seasonal distribution with the winter months as peak season in the temperate zone. In a retrospective study, thirty-five throat swabs from children under 5 years with acute respiratory symptoms (ARS) and 61 stool samples from children (>5 years) with acute gastroenteritis were collected in the period of October 2007 - March 2008. A HBoV specific polymerase chain reaction for detection of the virus, and sequence analysis for identification of virus variants were performed. Restriction fragment lenght polymorphism (RFLP) may be a possible tool for further identification of genotypes, as the digestion of PCR products with BstAPI enzyme in silico was also successful in differentiation of virus variants. Although respiratory samples were all negative, the 3,3% of stool samples (2/61) prooved to be positive for human bocavirus. The virus carrier children were 3 and 5 years old. The ratio of HBoV positive samples is similar to the international results (2,1-5,5%). Stool samples were screened for other gastroenteric pathogens such as rota-, adeno-, and/or noroviruses. Fifty-two out of 61 samples were screened also for rota-, and adenoviruses. Eleven of them (21%) proved to be positive for rotavirus and in 2 of them (3,8%) both rota-, and adenoviruses were detected (as coinfection). Fourty-seven of 61 samples were screened also for norovirus, and 14,9% (7/47) of them were positive. Thirty-nine out of 47 stool samples were tested for rota-, adeno-, and noroviruses, coinfections were not detected. Based on the results of sequence analysis and in silico RFLP, human bocaviruses isolated in Hungary belong to the '2' genotype. The aetiological agents of the gastroenteritis outbreaks remain unidentified in about 25-40%. Our results provide a further evidence that a wide range of viral pathogens play a role in acute gastroenteritises.

FATTY ACID PRODUCTION OF BACTERIA CULTIVATED IN DIGESTIVE MODEL IN CASE OF DIFFERENT PREBIOTICS

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Prebiotics are thought to improve the human's health by inducing favorable changes in intestinal microbiota. These would be food additives whose favourable stimulate the growth and activity of some strains of the native microflora or microflora introduced with the ingested food.
Principal end products of prebiotics bacterial fermentation in the colon are: CO_2, H_2, CH_4, short chain fatty acids (SCFA) and other organic acids. Reduction of gut pH through short chain fatty acids

formation inhibits growth of pathogenic colon bacteria. Using gas and short-chain fatty acid production as endpoints, Wang and Gibson showed that fecal slurries fermented oligofructose, along with a wide range of other carbohydrates, but that oligofructose and inulin selectively stimulated growth of bifidobacteria. Oligosaccharides are not hydrolyzed in the human small intestine, but degraded by resident flora in the colon. They mainly increase the growth of endogenous intestinal lactobacilli and bifidobacteria in humans and animals, which make them, part of the prebiotics complex. In addition, its fermentation decreases colonic pH, produces short-chain fatty acids and lactate, and increases the proportion of butyrate. The prebiotic potential of native chicory inulin was assessed by monitoring microbial community from the colon compartments, its metabolic activity and community structure. Inulin addition selected for a higher short chain fatty acid production with shifts towards propionic and butyric acid. In our study the digestion process were simulated by an artificial model and the effects of some prebiotics (inulin, cyclodextrin, oligosaccharides, raffinose) were examined by microbiological and analytical methods. Total bacterial numbers were estimated by microbiological methods and fatty acid composition was determined by analytically. Fatty acid production was determined on samples in duplicate from each fermentor at each sampled time point by gas chromatography after chloroformed extraction and esterification.

ISOLATION OF NITROGEN FIXING BACTERIA FROM INTENSELY GRASS-GROWN AREAS

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Nitrogen is one of the most important nutrient for plants, animals and humans. Although the air contains ~78 % N_2 (nitrogen gas), it is not available for higher organisms. It is transformed to ammonia mostly by the nitrogen fixing soil bacteria, e.g. *Klebsiella, Azotobacter, Rhizobium*, etc. Biomass production of intensely grown grass fields is strongly affected by nitrogen supply of the plants. Chemical nitrogen fertilization is not preferred because of environment pollution aspects. However, a well-balanced N-supply – by the biological nitrogen fixation of the diazotroph soil microflora – makes possible to reduce the applied nitrogen fertilizers significantly. Thus, our aim was to search for highly effective nitrogen fixing bacteria in the rhizosphere zones of intensely grown grass fields. These isolates can be promising components of new microbial soil inoculants which support grass growing by nitrogen fixation and by secretion of different plant growth stimulants. Soil samples were collected in Europe from fields where grass growing is opulent, first of all from meadows. The first step was to isolate nitrogen fixing bacteria from the soil samples. Isolation procedure was preformed in nitrogen free medium (A2). The promising isolates were tested for nitrogen fixation and indol-3-acetic acid production, two key features of the plant growth promoting effect. Simple and representative test was applied to estimate the in situ effect of the isolates on early growth of grass plants. It was carried out in triplicate, in glass Petri-dishes containing sterilized common, black mould soil. Soil was inoculated with liquid culture of the isolates and equal number of grass seeds was planted per dish, subsequently. Most effective isolates were selected and applied in pilot experiments to test their capabilities for batch fermentor culturing and culture processing.

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FOOD-BORNE VIRUSES

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Significance of food-borne viruses has received particular attention in the last decade. Unlike bacteria, viruses do not replicate in foods, however, the viruses spreading via fecal-oral route can spread by food or drinking water. Detecting of the viruses in food is still poorly developed. Food itself may contain viruses. For example shells living in contaminated water may contain caliciviruses or hepatitis viruses, goats infected by tick-borne encephalitis virus spread the virus via milk. However, most of the viruses infecting via fecal-oral route get into the food due to careless treatment. Food-borne viruses may be grouped by the symptoms they cause. Gastroenteritis viruses are the most common among the food-borne viruses (Calicivirus, Astrovirus, Rotavirus, Adenovirus 40,41). Enteric hepatitis viruses (hepatitis A and E) can spread by food and contaminated drinking water. Enteroviruses may cause a variety of symptoms ranging from rashes to myocarditis, meningitis and paralysis. Anelloviruses have not been linked to any specific disease so far. Certain viruses spreading characteristically via non-enteral way can cause food-borne epidemic. Recently tick-borne encephalitis epidemic was caused by raw goat milk.

Prions may spread by foods as well. Problem of the bovine spongiform encephalopathy (BSE, also known as "mad cow disease") attracted attention to the food safety. The presentation summarizes the significance of food-borne viruses and the prevention of the diseases they cause.

A FUNCTIONAL GENE TARGETED SINGLE NUCLEOTIDE PRIMER EXTENSION ASSAY FOR THE DETECTION AND TYPING OF BTEX-DEGRADING *RHODOCOCCUS* SPECIES

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Aromatic hydrocarbons are frequent groundwater pollutants associated with petroleum product releases. All BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) can be degraded by aerobic microorganisms. To carry out a successful bioremediation process it is important to have knowledge on the diversity and the degradation potential of the microbial communities involved in the degradation of the contaminants. Catechol 1, 2 -dioxygenase plays a key role in the aromatic ring cleavage and can be used as a marker gene to monitor functions and activities in bacterial communities of BTEX contaminated environments. In the Nocardiaceae family *Rhodococcus* species are well known BTEX-degrading bacteria, possessing catechol 1, 2 -dioxygenase (catA) genes. Our previous study has shown that the catA gene sequence of these bacteria carries phylogenetic information due to the lack of recent lateral gene transfer among *Rhodococcus* species in the case of catA gene. From this reason in contaminated environments BTEX-degrading *Rhodococcus* populations can be identified based upon the detection and sequence analysis of the catA gene. Furthermore the *Rhodococcus* related catA genes can be divided into groups and subgroups. The three major groups are the so called *erythropolis*, *opacus*, and the *Rhodochrous*, which later group contains two subgroups, the *Rhodochrous* I. and the *Rhodochrous* II. To monitor microbial subpopulations of samples from ongoing bioremediation processes rapid and sensitive molecular techniques are needed The method of single nucleotide primer extension (SNuPE) makes unnecessary

the further cloning and sequencing of the *Rhodococcus* catA PCR product. This study demonstrates a four primer SNuPE assay to reveal diversity of BTEX-degrading Rhodococcus populations through the detection and typing of their catA gene.

PCR-BASED DETECTION OF SELECTED PATHOGENIC BACTERIA IN THE DRINKING WATER SYSTEM OF BUDAPEST

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The major resource of the drinking water of the Hungarian capital is River Danube. Riverbank filtration serves a cheap and effective tool for acquire water with high quality through natural physical, chemical and biological processes that occur during ground passage. The drinking water is such a natural element which can not be substituted, therefore continuous monitoring of its microbiological quality is especially important. If the number of the pathogen bacteria increase people may expose to various waterborne infections. In our study we investigated the drinking water supply system (DWSS) of Budapest with PCR-based molecular methods.
Samples were taken from ten points of the DWSS from the wells to different points of the distributions system. We examined some selected common pathogens (Pseudomonas aeruginosa, Legionella sp., Enterococcus faecalis etc.) with taxon-specific PCRs. The positivity of samples were confirmed by sequence analysis. We also compared our molecular findings with the results of conventional culture-based microbiological water quality monitoring techniques. Our results showed that: (1) the applied taxon-specific PCR-based method is more effective than the cultivation-based method, since we were able to detect the selected pathogens in samples with no plating potential, (2) increased temperature in summer may lead to higher number of pathogenic bacteria in the DWSS as it was indicated with the elevated number of positive samples (3) addition of free chlorine is an important and effective method for reduction of the number pathogenic bacteria from the DWSS, since no positive signal was observed in the sample taken directly after chlorination (4) farther after chlorination pathogenic bacteria could also be detectable in some cases due to the reduced effect of chlorine or due to other factors associated with the distribution system (condition of pipelines, microbial biofilm etc.). Further improvements for our PCR method are required to determine if we detect viable cells or the DNA of dead cells and to quantify the number of pathogenic bacteria.

CONNECTION BETWEEN THE SULFUR METABOLISM AND THE HYN HYDROGENASE IN *THIOCAPSA ROSEOPERSICINA*

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Hydrogenases are metalloenzymes capable to oxidize molecular hydrogen or reduce protons. They can be classified into three major classes according to the metal content of the active centre: [Fe]-, [FeFe] and [NiFe] hydrogenases. NiFe hydrogenases have at least two subunits, the large contains the catalytic centre, while the small subunit responsible for the electron transfer [1].*Thiocapsa roseopersicina* BBS is a phototrophic sulfur bacterium. Four NiFe hydrogenases are coded in the genome of T. roseopersicina (Hup, Hyn, Hox1, Hox2). Two of them are cytoplasmic NAD^+ reducing

hydrogenases (Hox1, Hox2), which mainly produce hydrogen. The membrane bound Hup hydrogenase is an uptake enzyme [2]. Downstream from the hup operon, the gene of the sulfide quinon reductase was identifed. The product of this gene can be important in the sulfide-dependent anoxygenic photosynthesis [3]. Hyn is a remarkable stabile enzyme; it is active under extreme conditions. The genes of the small and the large subunit are separated by an intergenic region which code for two proteins: Isp1 and Isp2. It was shown by in silico analysis that Isp1 is a b type heme binding transmembrane protein while Isp2 seems to be a cytoplasmic protein, belonging to the heterodisufhide reductase family [4]. The substrate of this reductase is still unknown, but according to our hypothesis the enzyme convert the trisulfide form of the glutathion-amide (GASSSAG). Mutation of the Isp2 had dramatic effect on the activity of the Hyn hydrogenase in vivo in T. roseopersicina. The flavin containting glutation-amide reductase [5] produces GASSAG from glutathione amide (GASH) hypothetically produced from the trisulfide form by Isp2. The cycle seems to have further components: the above mentioned sulfide quinone reductase and/or flavocytocrome c (FCC). Under phototrophic conditions the hydrogen production of the Hyn hydrogenase increases when the media contain elevated amount of sodium-thiosulfate while the hydrogen oxidation rate does not change. The hydrogen production of the Hyn hydrogenase also increases when cells are grown on elementary sulphur, while in the absence of thiosulfate hydrogen is not produced even in the presence of elementary sulfur. The connection between the hydrogen and sulfur metabolism was examined and an integrated – but still hypothetical - sulfur-hydrogen metabolism model was outlined.

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MOLECULAR EPIDEMIOLOGY OF COMMUNITY-ACQUIRED AND NOSOCOMIAL *CLOSTRIDIUM DIFFICILE* INFECTIONS IN HUNGARY

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The recent emergence of an hipervirulent *C. difficile* (PCR ribotype 027) in the majority of European countries and North America has emphasized the importance and the necessity of epidemiological investigations worldwide. Therefore our aims were to continue our previous investigations, in which the presence of the major toxin genes (toxin A and B) and binary toxin genes of *C. difficile* isolated from diarrhoeal patients was determined, and the possible emergence of more virulent ribotypes was sought; the obtained results were compared with the results of the previous study periods. We also characterized the tcdC gene responsible for negative regulation of the major toxin genes, and we determined antibiotic susceptibility of the examined strains. 150 *C. difficile* strains isolated in various Hungarian laboratories from diarrhoeal faeces of both inpatients and outpatients were analyzed. The presence of toxin genes (tcdB, cdtB and the 3'end of the tcdA), and tcdC gene among binary toxin positive isolates were detected by PCR in the Anaerobe Reference Laboratory (Szeged, Hungary). The ribotypes of binary toxin positive strains were detected by PCR ribotyping method. Antibiotic

susceptibility of 100 strains was determined by E test (Solna, Sweden).
During the study period, 120 (80%) of 150 *C. difficile* isolated from diarrhoeal specimens were positive for both toxin A and B using PCR. No deletion in the 3'-end of the tcdA gene could be detected. 8 strains (5.3%) proved to be binary toxin positive; all of them harboured toxin A and B genes. Among binary toxin positive isolates, tcdC gene PCR showed various deletions in most isolates. PCR ribotyping of binary toxin positive strains revealed that one of the tested strain belonged to PCR-ribotype 027. Other binary toxin positive strains belonged to PCR-ribotypes 078 and 131. All of the tested 100 isolates were sensitive to metronidazole, 20 (25%) of 80 strains isolated in 2006-2007 were resistant to moxifloxacin, while moxifloxacin resistant isolates were not seen among strains isolated in 2003. The prevalence of erythromycin resistant isolates was 23.7%, while 37 (46.3%) of 80 isolates proved to be resistant to clindamycin. Moxifloxacin and erythromycin resistant strains were not found among strains isolated in 2003, and only 2 isolates showed resistance to clindamycin. 5 (6.3%) of 80 isolates were resistant to rifampicin, all of these showed high level resistance to clindamycin, moxifloxacin and erythromycin. In comparison of the given results with our data from a previous study, in Hungary, the prevalence of toxin A and B positive isolates and binary toxin positive strains has been increasing.

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INVESTIGATION OF VIRULENCE FACTORS OF *KLEBSIELLA* SPECIES FROM URINARY TRACT AND BLOOD STREAM INFECTIONS

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Klebsiella spp. account for up to 8% of all nosocomial infections in the developed countries, placing them among the eight most frequent infectious agents in hospitals. *Klebsiella pneumoniae* is the medically most important species of the genus followed by *K. oxytoca*. They are opportunistic pathogens commonly associated with hospital-acquired urinary tract infections (UTI), pneumonia, septicaemia (blood stream infection, BSI), and wound infections. Previously, we have already reported preliminary results of a study on phenotypic characterisation of Klebsiella isolates at this forum. The aims of our present study were to extend the number of isolates involved in the study to obtain statistically more relevant results, and broaden the spectrum of studied virulence characters. The isolates of BSI originated from blood cultures of septic patients and UTI isolates originated from urine cultures of patients having significant bacteriuria (10^5cfu/ml) with *Klebsiella* as the only pathogen in the cultures. All clinical specimens were collected at different clinical wards of Pécs University. Both groups contained 97 isolates and encompassed 81 (85.5%) Klebsiella pneumoniae and 16 (16.5%) K. oxytoca isolates, respectively. This ratio approximately reflects their occurrence in infections. We examined the presence of fimbriae, production of three different iron acquisition systems, the frequency of magA gene as one of the genetic determinants of K 1 capsule type, the biofilm production capacity and serum resistance of the isolates. Type 1 fimbria (mannose-sensitive haemagglutinin) was expressed by 72.1% and 74.2% of the UTI and BSI isolates, respectively. Type 3 fimbria (mannose-resistant and *Klebsiella*-like haemagglutinin) could be shown on 65.9% and 74.3% of the urinary and blood culture strains, respectively.
Enterobactin was produced by 92.8% and 83,5% while aerobactin was produced by 1.1 % and 8.2% of the UTI and BSI isolates. There was a considerable difference between frequency of yersiniabactin production of blood borne (89.7%) and urinary strains (53.7%), respectively. The capacity to

produce biofilm was considerably higher for urinary isolates (75,2%) than for blood culture strains (54,6%). The rate of serum resistance was surprisingly low in both groups. We could not show the presence of magA gene by PCR technique in both groups of isolates pointing to the absence of K 1 capsule type. The above data will be discussed in detail and substantiated by statistical dissection. Further phenotypic and genotypic analysis of potential virulence factors of the isolates is in progress.

TRANSCRIPTIONAL ANALYSIS OF AUJESZKY'S DISEASE VIRUS BY REAL-TIME RT-PCR

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In this study we examined the expression kinetics of Aujeszky's disease virus (AyV) genes by quantitative RT-PCR using strand-specific primers for the reverse transcription. We infected porcine kidney-15 (PK-15) cell line with various titers of AyV. Cells were either treated or untreated prior to infection with cycloheximide (blocker of the protein synthesis) or PAA (blocker of the replication of the virus DNA). Samples were taken at various time points (1, 2, 4, 6 and 8 hours) for total RNA isolation, followed by cDNA synthesis using Superscript III. This approach for the analysis of transcriptome of viruses with large genome sizes (including Herpes viruses) has not been used until now. Furthermore, this is the first report on the genome-wide analysis of AyV transcription. We classified the AyV genes according to their expression kinetics. In addition, in this study we detected the expression of complementary DNA strand of each gene.

COMPARATIVE GENETIC ANALYSIS OF BOVINE VTEC AND NON-VTEC *ESCHERICHIA COLI* O157

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Earlier we revealed that *E. coli* O157 Hungarian bovine strains belong to diverse (enterohaemorrhagic, enteropathogenic, and atypical) pathogroups. In the present study we aimed to further analyse and compare eleven EHEC (stx+, eae+), eleven EPEC (eae+) *E. coli* (EPEC) and nine atypical *E. coli* O157 (stx- and eae-negative) strains. EHEC and EPEC O157 strains uniformly carried gamma type of eae and gamma type of tir genes and tccp and paa. Further virulence genes located on pO157 virulence plasmid and different O-islands (OI-43 and OI-122) also characterised the EPEC and EHEC O157 strains with similar incidence. On the other hand none of these virulence genes were detected by PCR in atypical O157 strains, but five out of nine atypical O157 strains produced cytolethal distending toxin-V (CDT). Macro restriction enzyme analysis (PFGE) revealed that these *E. coli* O157 strains belong to three main clusters. In one group only EHEC, in one group only atypical strains occurred while in the third group both EHEC and EPEC strains occurred. Multi locus sequence typing (MLST) analysis revealed that the investigated five housekeeping genes were identical in EHEC and EPEC O157 strains, but showed diversity in the atypical O157 strains. These results suggest that the Hungarian bovine *E. coli* O157 strains represent at least two main clones: EHEC/EPEC and atypical O157. The atypical O157 strains represent not only novel genotypes but might be a novel pathogenetic group, but the pathogenic potential of the strains has to be elucidated.

REGULATORY FUNCTION OR TRANSCRIPTIONAL NOISE? – ANTISENSE RNAS IN AUJESZKY'S DISEASE VIRUS

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In this study we analyzed the transcriptome of Aujeszky's disease virus (AyV) by means of quantitative real-time RT-PCR. Using gene-specific primers for the reverse transcription we revealed the existence of genome-wide expression of the antisense DNA strand. We classified the expression kinetics of antisense transcripts in comparison with the corresponding messenger RNAs. The function, if any of these antisense RNAs remains to be ascertained.

CHANGES OF BACTERIAL COMMUNITIES IN COLLECTING AND DISTRIBUTING NETWORK OF DRINKING WATER SYSTEM OF BUDAPEST

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Although potable water is usually studied in public health aspect, composition and diversity of bacterial communities in drinking water systems is barely documented. In recent study diversity and changes of bacterial community of a discrete part of drinking water collecting and distribution system of Budapest was investigated by cultivation-independent methods.
Ten water samples were collected (from three riverbank filtration wells, from three points of collecting tubes in Szentendrei Island, four points of chlorinated drinking water distribution system), for molecular investigations 10-14 litres of water from each sample were filtered. DNA of was isolated from the filter (MoBio Ultra Clean™ Water DNA Kit), and after partial 16S rRNA gene amplification T-RFLP analysis was performed. In order to identify TRF peaks, partial 16S rDNA clone library was constructed. Clones were grouped by ARDRA, sequence and TRF lengths of representing clones were determined. To compare each bacterial communities statistical analyses were carried out and diversity indices (Simpson's and Shannon's) were calculated based on size and relative quantity of terminal fragments. Bacterial cell count of each water sample was determined using fluorescent microscopy. Cell count values of chlorinated and unchlorinated water samples were 10^4-10^5 cell/ml and 10^5-10^6 cell/ml respectively. Chlorinated and unchlorinated samples were also separated based on their T-RFLP profile. Unchlorinated water samples were characterised by very diverse communities with oligo-heterotrophs and chemolithotrophs. Communities of chlorinated pump stations were less diverse and dominated by members of genera *Methylocella* and *Mycobacterium*. Community structure of Újpalota pump station (chlorinated) was similar to unchlorinated samples due to dominancy of *Sphingomonas* spp. An other chlorinated pump station, Rákospalota showed as high diversity as unchlorinated samples.

PRELIMINARY RESULTS OF THE THIRD STUDY ON THE ANTIMICROBIAL SUSCEPTIBILITY OF *BACTEROIDES FRAGILIS* GROUP STRAINS IN EUROPE

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Bacteroides species are anaerobic bacteria that are predominant components of the bacterial flora of mucous membranes and are therefore a common cause of endogenous infections. *Bacteroides* infections can develop in all body sites, including the CNS, the head, the neck, the chest, the abdomen, the pelvis, the skin, and the soft tissues. Inadequate therapy against these anaerobic bacteria may lead to clinical failure. The *B. fragilis* group is more resistant to antibiotics than most other anaerobic bacteria, and it is well known, that the level of this antimicrobial resistance can differ from one geographic area to another or from one hospital from another. The aim of our study was to analyze the susceptibility trends to nine antibiotics of *Bacteroides fragilis* group isolates following of the previous two survey studies performed by the ESGARAB (ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria) in 1990 and 2000. *B. fragilis* group isolates were collected during the study period from 11 different European countries. The antimicrobial agents included were: ampicillin, amoxycillin-clavulanc acid, cefoxitin, piperacillin-tazobactam, imipenem, clindamycin,, moxifloxacin, tigecyclin and metronidazole. Minimal inhibitory concentrations (MICs) were determined according to the reference agar dilution method described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). 474 *B. fragilis* group isolates were included in the study. Abdominal infections and wounds were the most common sources of isolation and *B. fragilis* was the dominating species. 10.3% of the strains were resistant to amoxycillin-clavulanc acid, 19.8% to cefoxitin and 10.6% to moxifloxacin. Less than 1% was resistant to metronidazole, only 1.1% were resistant to imipenem, 3.1% to piperacillin-tazobactam.. No tigecyclin resistant isolate was found in this study. Antimicrobial resistance among the *B. fragilis* group is increasing. The variations observed in the susceptibility patterns of the *B. fragilis* group isolates emphasize the need to continue monitoring the emergence of resistance in order to guide the selection of the most appropriate antibiotic therapy scheme for anaerobic infections.

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COMPARATIVE CHARACTERISATION OF *BOTRYTIS CINEREA* POPULATIONS FROM DIFFERENT PLANT SPECIES

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The Ascomycete *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana*) is a necrotrophic fungus that causes grey mould disease on a very broad host range (more than 250 plant species) and inflicts serious crop losses worldwide. In viticulture, it is commonly known as botrytis bunch rot, in horticulture it is usually called grey mould. The fungus gives rise to two different kinds of infections on grapes. The first, grey rot, is the result of consistently wet or humid conditions, and typically results in the loss of the affected bunches. The second, noble rot, occurs when drier conditions follow wetter, and can result in distinctive sweet dessert wines, such as Aszú of Tokaj. Botrytis fruit rot (grey mould) caused by the fungus Botrytis cinerea is the most important disease of strawberry and raspberry worldwide. The disease causes severe preharvest and proharvest losses primarily due to infections of fruit and flowers, especially under humid conditions when daytime temperatures are moderate to warm. Information about the populations of plant pathogen fungi is essential for the

effective and economic protection. A plant pathogenic fungal population with high level of genetic variation is likely to adapt more rapidly to fungicides or resistant host plants than populations with little or no genetic variations, and information on the level of migration between populations and on the presence or absence of sexual reproduction within a population may indicate how rapidly will novel (fungicid resistant or more pathogenic) genotypes spread between populations. Application of the tools provided by recent advances in population genetics and biology are crucial in gathering those information. In the initial stage, 86 isolates of grapevine berry-growing *B. cinerea* from various locations of the Tokaj wine region, 70 isolates of strawberry and 39 isolates of raspberry from various locations of East and North East Hungary were collected. Individual strains were obtained by single-spore isolation. Characterization of their genotype was done by analyzing MSB1 minisatellite sequences, which is located in the intron of the ATP synthase. Its 37-bp repeat unit is AT-rich, and it is found at only one locus in the genome. In general, sequence analysis revealed a high degree of genetic diversity and the combination of alleles suggests the presence of sexual reproduction in the areas, while the disperse distribution of the genotype indicates high migration rate.

CULTIVATION-BASED APPROACHES TO CHARACTERIZATION OF TCE CONTAMINATED SITES

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Halogenated hydrocarbons are unfortunately common contaminants in soil and groundwater in Hungary. Conventional pump-and-treat technologies have limited effectiveness in remediating groundwater pollution. Stimulation of dechlorinating microorganisms is potentially the most promising and cost-effective technology for remediating contaminated sites. To use bacteria for bioremediation, it is important to obtain strains that are specific to particular contaminated sites, are culturable, and have high pollutant-degradation activity.Some of these pollutants are used as electron donor and carbon source under aerobic conditions. Chlorinated solvents are also degraded under anaerobic conditions, in which they are utilized as an electron donor and carbon source or can serve as an electron acceptor to support respiration (halorespiration). Groundwater samples were taken during gas injection and analyzed for changes in bacterial populations. Chemical analysis was performed in a standard laboratory, the following compunds were examined: pH, sulphate, soluble Fe, TOC and halogenated hydrocarbons. The aerobic populations were monitored in groundwater using the most probable number (MPN) technique. During the aerobic experiments, bacteria were cultivated on Nutrient Agar and R2A Medium plates from the groundwater. Anaerobe cultivation procedures also was done on PMB, Thioglycolate Medium and Ferrihydrite Medium.

The MPN count was initially high, under the treatment the total number of microorganisms was increased, but the microbial diversity was decreased. These bacterial data were compared to physical parameters (pH, dissolved oxygen, redox) and contaminant (TCE, cDCE, VC) concentrations. Phylogenetic analysis showed all bacterial groups in the groundwater samples: Actinobacteria, Firmicutes, α-, β- and γ-Proteobacteria, Bacteroidetes. Most high-density bands were classified into the phyla Proteobacteria. The isolates belong to *Acinetobacter* sp., *Arthrobacter* sp., *Pseudomonas* sp., *Sphingomonas* sp. may be involved in pollutant degradation. By specific detection of dehalorespiring bacteria four examined organisms were detected, which are *Dehalococcoides ethenogenes*, *Dehalobacter restrictus*, *Desulfomonile tiedjei* and *Desulfuromonas chloroethenica*. The presence of these organisms was monitored both on the contaminated sites. The specific

detection of sulfate-reducing bacteria also was done. Results were compared to chemical data, under the treatment the number of sulfate-reducing bacteria was increased.

THE EFFECT OF DIFFERENT LITTER INPUT ON SOIL RESPIRATION IN AN OAK FOREST

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We established a long-term field study in a sessile oak - turkey oak forest in Central Europe Hungary (Síkfőkút Experimental Forest (SIK) to address how detritus quality and quantity control soil organic matter (SOM) accumulation and soil respiration. The changing litter input affect the soil microbial community and this have an effect on soil CO_2 efflux too. Due to the climate change, the species composition of forest has been changing, and the total leaf litter production has been slightly decreasing. The recent, and relatively rapid, rise in atmospheric CO_2 has the potential to alter the cycling and storage of carbon in terrestrial ecosystems. The SIK DIRT (Detritus Input and Removal Treatments) plots consist of treatments that double leaf litter, double woody inputs, exclude litter inputs, or remove root inputs via trenching and no inputs at all, and the control plots. Macro and microclimatic changes and seasonality strongly influence temperature and moisture content of the soil, which affect on soil microbial processes proved. At the exclusionary treatments (No Litter, No Root, No Input) significant soil respiration decrease can be see only after 5 years. The soil respiration at Double Litter treatment in the first tree years slightly increased, but from the 4th year decreased. The seasonal changes of soil respiration show well correspond with the seasonal changes of soil temperature. With increasing soil temperature, the soil respiration is exponentially rising. Soil respiration response to soil temperature was strongly and it was significantly influenced by treatment, and treatment effects increased with time. According to our long-term litter manipulation field experiment, at the No Litter, No Root and No Input treatments the soil respiration decreased. It may be hypothesized that global warming will raise the efflux of CO_2 from the soil by the soil temperature raise, otherwise may be the absence of moisture will decrease the CO_2 efflux. It follows that both of these processes together will determinate the way of changes (change of litter production, microbial activity so the SOM accumulation too).

ABUNDANCE AND GENETIC DIVERSITY OF ADENOVIRUSES HOSTED BY GULLS

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A comprehensive screening of samples of Swedish wild seabirds for adenoviruses (AdVs) revealed that numerous novel si- and aviadenoviruses occur in gulls (Laridae). We analyzed around 350 field samples collected for avian influenza virus survey. Nucleic acid extracts of the samples were prepared on 96-well plates, then processed by robot techniques to perform consensus nested PCR. After the PCR, the products were studied by manual electrophoresis. PCR of samples, found positive, was repeated manually. The amplified DNA fragments were purified and sequenced to compare with other adenovirus sequences. Out of the 36 positive samples, 26 originated from different gull species. Namely, there were 22 from black-headed gull (*Larus ridibundus*), 2 from great black-backed gull

(*Larus marinus*), 1 from herring gull (*Larus argentatus*), and 1 from Caspian tern (*Hydroprogne caspia*). Interestingly, the nucleotide sequence of 10 Swedish samples was identical with that of the first gull AdV, formerly found in Hungary. This has been the only positive out of 11 Hungarian gull samples, and has clustered into the genus Aviadenovirus. The remaining 16 Swedish gull AdVs proved to represent distinct types belonging to the avi- or siadenoviruses. Based on the preliminary phylogenetic analyses, the putative gull AdVs form monophyletic groups in each genus, and can be classified into several virus species. These results imply that gull AdVs are also a good example for the hypothesized co-evolution of host animals and viruses. It is noteworthy that different avian hosts seem to generally be more permissive for AdVs than mammals are.

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NEW GROWING EXPERIMENTS OF A HUNGARIAN MUSHROOM SPECIES

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The *Agaricus macrosporoides* Bohus has been collected and described by Gabor Bohus. He collected this species from the Hortobágy in Hungary. More wild strains have been collected from specific habitats of the Hortobágy plain. The morphological and growing features can make this mushroom suitable for fresh market. A. macrosporoides Bohus strains show greater resistance against mycopathogens in contrast with the traditional cultivated white button mushroom (*Agaricus bisporus*) does not show these features. We tried to select such a strain, which is more economical to grow than other strains and the traditionally grow *Agaricus bisporus*. Besides we took into consideration the claims of the growers and consumers too. It is important that the selected strain has to have greater gastronomical value than the white button mushroom. We set growing experiments with 4 strains and examined the fruit body formation with and without casing soil. We tried more climatic effects during the growing period. More pesticides were tested against pathogens which can also appear in growing of the Agaricus bisporus. We compare the yields and the morphology of the fruit bodies all the strains. All things considered a new growing technology has been work out in this project.

EFFECT OF DIFFERENT SOIL MICROBES ON THE BIOACCESIBLE AMOUNTS OF DISTINCTIVE PESTICIDES

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Characterization of adsorption feature of widely used pesticides to soils has high environmental relevance. There are thousands species of soil-microorganisms with variable sensitivity and affinity to pesticides. Extra adsorbing surface for pesticides is provided by the presence of these organisms and their metabolites, moreover the effect of their enzymatic activity should also be taken into account.

Our work signifies major innovations in terms of applying different extraction solvents and involving pesticides which have not been studied so far for accessibility. Besides of making a comprehensive comparison between different experimental methods to model bioaccessibility of pesticides with

applying 5 extraction models our major goal was to estimate the influence of soil- microorganisms on the bioaccessible amounts of pesticides. The five pesticides examined were simazine, carbendazim, acetochlor, chlorpyrifos and diuron. For the examination sandy, brown forest and alluvial soils were applied. The extracted amounts were determined by GC/MS and HPLC/MS techniques. For reveal the effect of the microbial flora of the soils on the extent of adsorption of pesticides to soil, sterilized, air dried and microbiologically active soils were studied.
The estimation of microbial activity of soils were based on the hydrolysis of fluorescein diacetate [3',6'-diacetylfluorescein (FDA)] by several enzymes. The activity of the all soil types displayed marked differences. In all cases fewer pesticides were gained from microbiologically active soils than from the air-dried samples. According to the results of our studies focusing on estimation of the effect of soil microflora on the accessible amounts of different pesticides it could be stated that fewer amounts of the pesticides become accessible for the living organisms in case of soils having a normal microflora. Aqueous extraction solvents proved to be suitable for assessment of the accessible amounts of pesticides, as their effectivity was at least as high as that of organic solvents.
As a result of our studies a clarified picture was acquired to characterize the extent of pesticide-soil interactions and to model pesticides' fate in the natural environment.

THE MODE OF ACTION OF PRIMYCIN ANTIBIOTIC: MEMBRANE DYNAMICS EXAMINATIONS BY EPR

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The primycin is a non-polyene macrolide antibiotic. The mode of action of primycin was investigated using well characterised polyene-sensitive ergosterol-producing parental 33erg+ and ergosterol-less polyene-resistant mutants erg-2 of *Candida albicans*.The minimal inhibitory concentrations (MIC) to primycin of parental 33erg+ and its mutant erg-2 proved to be 12 μg/ml and 16 μg/ml, respectively. The MICs measured by microdilution test were 64 μg/ml for 33erg+ and 128 μg/ml for erg-2. These data suggested that the target of primycin is the plasma membrane and the membrane composition influences the mode of action of this antibiotic. Treatment of cells with 128μg/ml primycin resulted in significantly increased loss of metabolits absorbing at 260nm specially for the parental strain 33erg+. To determine the membrane dynamics induced by primycin, electron spin resonance spectroscopy (EPR) was used. For measurements of membrane processes spin- labelled 5-SAS was applied. Phase transition temperatures of untreated ergosterol- producing strain 33erg+ and its ergosterol-less mutant erg-2 were 12°C and 10°C reflecting the differences in membrane composition.
The phase transition temperature of treated strains was 16°C in both cases. In the saturation transfer measurement, the rotation correlation time was 30 ns by the control and 300 by the treated cells of 33erg+. In the case of erg-2, control cells exhibited 100 ns by the treated cells showed 1000 ns. In the fluorimetry measurements, the lifetime and anisotropy decay were determined. Primycin treated (64 μg/ml for 15 min.) cells of 33erg+ and erg-2 showed that primycin increased membrane rigidity and alteration on the membrane dynamics exhibited time and concentration dependence.

INTERFERON-GAMMA MEDIATED ALTERATIONS ON THE LIPID METABOLISM IN *CHLAMYDIA TRACHOMATIS* INFECTED MURINE EPITHELIAL CELLS

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Chlamydia trachomatis is an obligate intracellular bacterium that primarily targets columnar epithelial cells of the conjunctiva and the genitourinary tract. The bacterium is mainly eliminated by IFN-gamma produced by CD4+ and CD8+ T-lymphocytes. The combined impact of *Chlamydia trachomatis* infection and IFN-gamma on the transcriptome of murine epithelial cells is partially desribed and is mainly focused on the direct anti-chlamydial effector genes, such as the p47 GTPase family. We wanted to extend this screen to other cellular processes such as proinflammatory gene expression, cytokine-chemokine synthesis, antigen presentation and basic metabolic pathways of the host. The BM 12.4 murine primary oviduct epithelial cell line was infected with *Chlamydia trachomatis* L2 (MOI=1) in the presence or absence of IFN-gamma (20U/ml). Epithelial cells were harvested 24 h post infection, total cellular RNA was extracted, amplified and hybridized to the Affymetrix 430 A2 whole-genome mouse chip.
The addition of IFN-gamma has a multiplicative effect on the gene expression of the epithelial cells. The IFN-gamma alone or the chlamydia infection alone upregulated the expression of 80 and 179 genes respectively, while in combination 492 genes. The genes that showed a significantly higher expression when IFN-gamma were present included both proinflammatory genes and also host genes that are responsible for the activation of innate and adaptive immmune responses. The impact of IFN-gamma on the dowregulated genes was even more dramatic. The IFN-gamma alone or the chlamydia infection alone downregulated the expression of 5 and 27 genes respectively, while in combination 490 genes. Functional analysis of the downregulated geneset revealed that the most significantly impacted host metabolic pathways were the cellular cholesterol biosynthesis, and the ATP producing fatty acid beta-oxidation. These functional groups contained 27 genes including key enzymes involved in these metabolic processes. Interestingly, both the host cholesterol and the host ATP sources were shown before to be required for chlamydial growth, but they have not been implicated in the IFN-gamma mediated anti-chlamydial effector mechanisms.

EXAMINATION OF YEAST DIVERSITY IN „SZEKSZÁRDI KADARKA”

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Kadarka is an old red wine grape variety, most popular in Hungary, where it was introduced with the Turkish occupation. It is an important constituent of the Hungarian red cuvée Bull's Blood of Eger or Szekszárd. The aim of this work was to study wine yeasts isolated in spontaneous fermentation of Kadarka must in order to obtain more information about the indigenous flora.
To investigate the natural yeast microbiota, we took samples from Kadarka must of the vintage of 2006 and 2007. We sampled the musts 15 times during the fermentations to follow the changes of the

population dynamics. 100 mg/kg SO_2 was added to the must in 2006 and the first sample was taken on the third day of the fermentation. In 2007 no sulphur was added to the must before the sampling on the first day. We isolated 544 yeast strains in the two vintages, all of these isolates were subjected to conventional taxonomic tests. At the conventional tests we examined the morphology, sporulation, growth at various temperatures and the utilisation of carbon and nitrogen sources of our isolates. To verify the results of the taxonomic tests, representative strains of the species found were also subjected to molecular analysis including PCR-RFLP of the ITS1-5.8S-ITS2 region of the rDNA and sequencing of the D1/D2 domain of the rDNA gene. The nuclear gene MET2 digested with EcoRI and PstI and NTS2 region digested with BanI were applied for the delemination of the Saccharomyces sensu stricto group. For karyotyping chromosomal DNA was prepared from this strains and the chromosome-size DNA molecules were separated by CHEF-DRII and DRIII electrophoresis systems. According to these tests the isolates from 2006 probably belong to *Metschnikowia pulcherrima, Hanseniaspora uvarum, Candida zemplinina* and *Saccharomyces cerevisiae*. At the third day of the fermentation all four species were present. Three days later, we could not isolate *C. zemplinina* strains, after the third sampling only *Saccharomyces* strains were found. In 2007 we could isolate the same species and *Aureobasidium pullulans*, a yeast-like fungus which was present in the must for 3 days. *M. pulcherrima* was present for 10 days and *H. uvarum* for 14 days. At the end of the fermentation we could isolate *S. cerevisiae* and *C. zemplinina* strains. Without sulphur, the fermentation is a more complex microbiological process.

TAXONOMIC IDENTIFICATION OF YEASTS ISOLATED IN SPONTANEOUS FERMENTED CIRFANDLI

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The Cirfandli is a rare grape variety which is cultivated just in Pécs and it's countryside in Hungary. The aim of this work was to study wine yeasts isolated in spontaneous fermentation of Cirfandli must in order to obtain more information about the indigenous flora. To investigate the natural yeast microbiota, we took samples from Cirfandli must of the vintage of 2006. We sampled the must 15 times during the fermentations to follow the changes of the population dynamics. The first sample was taken on the third day of the fermentation.

The isolates were subjected to conventional taxonomic tests and a molecular analysis. At the conventional tests we examined the morphology, sporulation, growth at various temperatures and the utilisation of carbon and nitrogen sources of our isolates. To verify the results of the taxonomic tests, representative strains of the species found were also subjected to molecular analysis including PCR-RFLP of the ITS1-5.8S-ITS2 and NTS2 regions of the rDNA and sequencing of the D1/D2 domain of the rDNA gene. Other gene like MET2 was also analysed in the case of Saccharomyces sp. strains by PCR-RFLP. For karyotyping chromosomal DNA was prepared from these strains and the chromosome-size DNA molecules were separated by CHEF-DRII and DRIII electrophoresis systems. According to these tests the isolates probably belong to *Metschnikowia pulcherrima, Hanseniaspora uvarum, Torulaspora delbrueckii* and *Saccharomyces cerevisiae*. On the third day of the fermentation all four species were present. Three days later, we could not isolate *H. uvarum* and *T. delbrueckii* strains, after the fourth sampling only *Saccharomyces* strains were found.