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INTEGRATED MICROBIAL ACTIVITIES FOR MONITORING OF BIOREMEDIATION OF CADMIUM-CONTAMINATED SOIL CULTIVATED BY *MEDICAGO SATIVA*

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Microorganisms play key geoactive roles in the biosphere, particularly in the areas of element biotransformations and biogeochemical cycling, metal and mineral transformations, decomposition, bioweathering, and soil formation. All kinds of microorganisms, including prokaryotes and eukaryotes and their symbiotic associations with each other and 'higher organisms', can contribute actively to geological phenomena, and central to many geomicrobial processes. Microorganisms have a variety of properties that can effect changes in metal speciation, toxicity and mobility, as well as mineral formation or mineral dissolution or deterioration. Industrial and urban wastes, agricultural application and also mining activities resulted in an increased concentration of heavy metals in soils. How plants and soil microorganisms cope with this situation and the sophisticated techniques developed for survival in metal-contaminated soil. Soil contamination with cadmium is a serious global issue due to its mobility and toxicity to plants and soil organisms. Cadmium is considered particular toxic towards soil microorganisms, although severe adverse effects have generally only been detected for high Cd concentrations. Current problems of agricultural soil, which cause difficulties for human health, can be partially solved by the phytoremediation technologies application, designed to eliminate pollutants from the environment by the use of green plants. The application of Cd at different concentrations (0, 20, 40, 80, 160 and 320 mg/kg soil) was investigated. Alfalfa was grown in a Cd-contaminated clay loam brown forest soil. Control soil without plants was included in the experiment. Growth of alfalfa was stimulated in the lowest concentrations of Cd-amended soil. All measured parameters of soil microbial activities were affected by higher Cd concentrations. The decrease in CFU was most significant in the case of heterotrophic bacteria and spore-forming bacteria. Significant inhibition of microbial biomass-C occurred in soil highly contaminated by heavy metals. Generally, the values of enzymatic activities were highest in the soil above the source of contamination and they were decreased as approaching the source of contamination. Our results demonstrate that several parameters of microbial activities could be used as good indicators of increasing concentrations of Cd in soil.
The activities of soil dehydrogenase, phosphatase, β-glucosidase, urease, protease and cellulase were greatest in low Cd-amended soil and cultivated by alfalfa. In conclusion, the cultivation of alfalfa in Cd-amended clay loam brown forest soil reduce the bioavailable Cd pool seems a promising method to enhance productivity and plants health grown on Cd-contaminated soil.

THE USAGE OF REAL-TIME PCR TECHNIC TO MONITOR SPECIFIC EUBACTERIA IN BIOGAS PRODUCING SYSTEM

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Biogas has become one of the most important renewable energy carriers. It is produced in many countries at large scale, combining waste disposal with energy generation. There is still a substantial potential in improving the biotechnological aspects of the process. The microbiological events leading to biogas formation can be divided into three phases. The first step is the hydrolysis of large molecules utilizing bacterial exoenzymes. In the second phase the acetogenic microbes uses the smaller oligomeric substrates to generate volatile fatty acidst, mainly acetic acid. The third step is methanogenesis, which is carried out by archeabacteria.
One of the possibilities to enhance the biogas producing process is the addition of a bacterial culture that broadens one of the bottlenecks in the well organized microbial food chain. The availability of reducing power, e.g., in the form of hydrogen generated in situ, is a bottleneck recognized in our laboratory earlier. In laboratory batch fermentations and in a field experiment the hypothesis was corroborated and addition of good hydrogen producing bacteria led to faster decomposition of various substrates.
In this study systematic experiments were conducted in 5 litre CSTR (continuous stirred) fermentors, designed for biogas research at laboratory scale. These devices model the real-life, large scale biogas production plants much better than the routinely used batch systems. Thermophilic conditions were selected because the microbial diversity in the thermophilic natural consortia is lower, which requires a thorough inspection of the

microbiological profiles. Molecular biological methods were developed in order to detect the single species in the mixed consortia. We selected the Real-Time PCR technique, because this method can be used, to determine the amount of one selected bacterial DNA from the assortment of genomic DNA in the fermentation sample. Specific primers were designed, targeting selected and characteristic genes making sure that similar sequences could not be found in other microorganisms forming the consortium. Results have shown, that using higher organic total solid (oTS) values, the intensification process can be sustained during the whole process.

DIROFILARIA AND DIROFILARIOSES IN AUSTRIA

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Human dirofilarioses are mainly induced by two species, *Dirofilaria* (*Nochtiella*) *repens* RAILLIET & HENRY, 1911, which is the causing organism of (sub)cutaneous dirofilariosis, and *Dirofilaria* (D.) *immitis* (LEIDY, 1856), the causative organism of pulmonary dirofilariosis. Both *Dirofilaria* species are transmitted from natural final hosts (mainly dogs, cats or other carnivores) to humans by mosquitoes of the family Culicidae: *Anopheles*, *Aedes* and *Culex*. Until recently neither *Dirofilaria repens* nor *D. immitis* infestations of natural hosts as well as of humans were registered in Austria. However, the first autochthonous human case was observed in 2006, and in 2009 several dogs infected by *D. repens* have been found. In total 16 human cases (15 imported and 1 autochthonous case) of (sub-)cutaneous dirofilariosis have been registered (by histological-parasitological and/or molecular-biological methods) in Austria between 1981 and 2009. In addition, three human cases of suspected *D. immitis* infestations have been registered in recent years; the suspicion was based on clinical, anamnestic and parasitological-serological data.

MONITORING OF HIV PANDEMIC IN HUNGARY BY MOLECULAR VIROLOGICAL METHODS

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Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), displays a high degree of genetic diversity due to its rapid replication, high mutation rate and frequent recombinations. The genetic variability of HIV type 1 (HIV-1) is most pronounced in the env gene coding for the HIV surface glycoprotein (gp120). Various mutations in the HIV-1 pol gene confer high-level of antiretroviral drug resistance to reverse transcriptase (RTIs) and protease inhibitors (PIs). Positive selection and accumulation of drug resistant HIV variants is a major cause of treatment failure. Although available combinations of antiretroviral drugs have significantly decreased the mortality and morbidity related to HIV, their widespread use also caused the emergence of drug resistant strains. The aim of our study was to characterize the HIV-1 strains prevalent in therapy naive HIV-positive individuals in Hungary. HIV-1 subtypes were determined using nested PCR by amplifying the V1-V5 and V3 regions of the proviral env gene, and DNA sequencing. Antiretroviral drug resistance was predicted by sequencing the reverse transcriptase and protease coding regions amplified following RT PCR of viral RNA genomes. The sequences were analysed using the Stanford's HIVdb program to determine the drug resistance mutations. Classification of HIV-1 strains showed that subtype B is still the predominant HIV-1 clade in Hungary: all the 17 samples studied carried subtype B HIV variants. The prevalence of HIV-1 strains with transmitted drug resistance mutations in newly diagnosed individuals is 24% (4/17) in Hungary according to this survey. There was no major mutation associated with drug resistance in the protease gene. NRTI (nucleoside reverse transcriptase inhibitor) resistance mutations were observed in 2/17 (12%) cases. Both M41L and T215E mutations induce a low or intermediate level of drug resistance. NNRTI (non-nucleoside reverse transcriptase inhibitor) resistance mutations including K101Q, K103N or Y181C were detected in 2/17 (12%) patients associated with intermediate or high level of drug resistance. The data obtained may help in choosing the proper antiretroviral drug combination to treat HIV infected patients.

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IMPROVEMENT OF BIOGAS PRODUCTION VIA MICROBIOLOGY

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Due to the shortage of the fossil fuels and the environmental damages caused by their excessive use the utilization of biomass as a sustainable and renewable energy source gains importance worldwide. One of the means to exploit the chemical energy stored in biomass is biogas production. The anaerobic digestion process works with a wide range of substrates, including organic waste streams (e.g., municipal waste and waste water), byproducts of industrial activities and food processing, and plant biomass grown for energy production purpose. In order to make the process economically more attractive the efficiency of the biodegradation needs improvement.

Among the significant recent advances in understanding the ecology of anaerobic biodegradation of organic wastes is the recognition of the close syntropic relationship among the three distinct microbe populations and the importance of H_2 in process control. The regulatory roles of hydrogen levels and interspecies hydrogen transfer optimize the concerted action of the entire population.

During anaerobic biodegradation hydrogen concentration is reduced to a much lower level than that of acetate. In addition, the hydrogen partial pressure can change rapidly, varying by an order of magnitude or more within a few minutes. We have shown that under these circumstances addition of hydrogen producing bacteria to the system and thereby shifting the population balance brings about advantageous effects for the entire methanogenic cascade. The decomposition rate of the organic substrate increases and both the acetogenic and methanogenic activities are remarkably amplified.

The phenomenon manifests itself under both mesophilic and thermophilic conditions, albeit the microbiological composition of the respective natural microbial consortia would differ. The effect should also be observed when the microorganisms are grown on various substrates. From the numerous potential hydrogen producing candidate strains, *Enterobacter cloacae* was selected at mesophilic temperatures, and *Caldicellulosiruptor saccharolyticus* turned out to be an outstanding candidate at thermophilic temperatures. A thorough investigation was carried out to identify the parameters, which may be responsible for the increase in biogas formation. These led to the conclusion that the observed intensification effect took place only when the biogas generating consortium and the hydrogen producing bacteria were in close contact in the same space.

Molecular biological methods were used to follow the fate of the hydrogen producers in the anaerobic reactors. In the mesophilic system hypF-minus *E. coli* mutant strain provided direct evidence, linking the beneficial effect to the presence of an active hydrogen-producing enzyme.

GENETIC TOOLS FOR *THERMOPLASMA ACIDOPHILUM*: ACHIEVEMENTS AND APPLICATIONS

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Thermoplasma acidophilum is a thermo-acidophilic archeon, which grows optimally at 58 ^{o}C and pH 1,5- 1,8. *T. acidophilum* has become an important model organism of visual proteomics studies in the last decade due to its low genome complexity and its resemblance of several metabolic pathways to eukaryotic cells. As yet, genetic tools for genetic engineering and functional studies were missing which hindered thorough analysis and verification of hypotheses. Therefore, the main scope of our work was to establish basic techniques and tools for genetic manipulations of this archaeon. Firstly, gelrite and silica based solid media were developed for clonal selection. In parallel, shuttle vectors and chromosomal integration vectors were constructed in which the gyrB gene of the novobiocin resistant *T. acidophilum* strain was employed as resistance marker. For transformation, several methods like electroporation, lipofection, magnetofection and gene gun techniques were tested. None of the individual procedures worked effectively, but elements of these methods were combined leading to successful transformation of *T. acidophilum* cells. Novobiocin resistant *T. acidophilum* cell lines were easily generated with our shuttle vector however stable maintenance of the vector was parse. Instead, most of the cells had recombination events in which processes the resistance gene from the plasmid recombined into the chromosomally encoded wild type gyrase gene. To circumvent this phenomenon the less homologous

Thermoplasma volcanium gyrB gene was mutated by exchanging residues in the critical positions to turn it novobiocin resistant and used in further experiments.
Based on the *T. volcanium* novobiocin resistant gyrase gene three genetic manipulation systems are being developed. To express, capture and characterize tagged proteins/protein complexes of interest a., an *E. coli – T. acidophilum* shuttle vector is being constructed in which the expressed genes are under the control of a glucose inducible promoter and b., a chromosomal integration system is being tested by which a 6xHis-tag is introduced/fused to the C-terminus of the gene. In the latter case, the endogenous gene is replaced by a tagged version via homologous recombination, thus avoiding mixed population of tagged and untagged proteins in the cells. Paralelly, a knock-out mutagenesis system (c) has been developed and tested to deplete proteins involved in protein turnover (e.g. 20S proteasome, thermosome, tricorn protease). Basic elements of genetic manipulation have been developed for *T. acidophilum*. Application of these genetic tools will open avenues for deeper insights in cellular processes of T. acidophilum, and to capture and characterize proteins of interest.

THE EFFICIENCY OF DIFFERENT BIOLOGICAL SEWAGE TREATMENT TECHNOLOGIES IN THE REDUCTION OF THE NUMBER OF THE BACTERIA

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The efficient function of sewage treatment works is essential for the quality of surface waters. There are many factors which can modify the number of bacteria in cleaned water leaving sewage treatment works. Beside environmental conditions and chemical, biological and physical characteristics of untreated sewage, the cleaning technology applied also determine the removal efficiency.
We analyse untreated sewage, cleaned sewage and sewage sludge in three sewage treatment works using different biological cleaning methods. In the course of sewage treatment, a number of micro-organisms accumulate in sewage sludge. In the knowledge of chemical parameters (NH_4 - nitrogen (untreated: 35 – 75 mg/l, cleaned sewage: <1 – 40 mg/l), phosphorus (untreated: 6 – 15 mg/l, cleaned sewage: 0.2 – 2 mg/l), floating matter (untreated: 100 – 350 mg/l, cleaned sewage: 25 – 40 mg/l), and dry-matter content (untreated: 850 – 1450 mg/l, cleaned sewage: 570 – 980 mg/l). The change of the number of bacteria shows the efficiency of different methods in identical conditions. From micro-organisms we define *Escherichia coli* and *Enterococcus faecalis* bacteria. Both are indicator species, so their quantity refers to the quality of water.
The continuous examination of the microbiological parameters of cleaned sewages is essential in the reduction of environmental charge and the conservation of the quality of surface waters.

MICROBIOLOGICAL AND CHEMICAL ANALYSIS OF A DEEP WELL BASED DRINKING WATER DISTRIBUTION SYSTEM

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An urban drinking water distribution network and the connected deep wells were investigated by microbiological and chemical methods as part of an investment to improve the quality of the drinking water. The examined drinking water showed less stability than drinking waters in common. In the tap water samples, biological floccules have appeared after 3-5 days of standing depending on the temperature. The aim of this study was to reveal the reasons behind this decreased stability.
Samples were taken from the wells and at different points along the distribution system. The microbial communities were investigated with Terminal Fragment Length Polimorphism (T-RFLP) DNA fingerprinting method. Based on the T-RFLP results, a similarity tree was constructed and the results were compared with classical microbiological and chemical parameters using Principal Component and Correspondence Analysis. Three strongly differing representative samples were chosen to make a 16S rDNA composite clone library. The 192 clones of the library were grouped by ARDRA. 99 clones formed 17 groups (major clones), while the rest of the clones showed unique restriction patterns (minor clones). Sequence analysis was carried out on the major clones and most of the minor clones. The sequences were compared to the ARB and EZTAXON databases. Community DNA based results were supplemented with chemical and classical microbiological investigations

(both based on environmental health standards), with cell counts using epifluorescent microscopy, and with measurements of some special physical and chemical parameters.
Through our examinations we revealed that the water is anaerobic along the whole drinking water distribution system and a remarkable methane concentration is also detectable. The bacterial communities of the well waters and the tap waters are overlapping, but are showing a consequently different composition. We did not detect any pathogen microorganisms among the clones. A great part of the present microorganisms showed close relationship with obligate or facultative anaerobic bacteria. The number of microorganisms related to bacteria which utilize one-carbon or short carbon chain compounds were also dominant.
We conclude that beside the notable amount of methane, the presence of such microbes could stand in the background of the decreased stability of the drinking water.

DETECTION OF NEW ADENOVIRUSES IN BIRDS

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The family Adenoviridae consists of five accepted genera (*Mastadenovirus, Aviadenovirus, Siadenovirus, Atadenovirus and Ichtadenovirus*). Avian adenoviruses are present in 3 genera, namely in *Aviadenovirus*, *Siadenovirus*, and *Atadenovirus*. Our aim was to detect adenoviruses from poorly studied bird species and to confirm the hypothesis on the co-evolution between adenoviruses and their hosts. Altogether 306 samples of 50 different bird species were screened with a DNA-dependent DNA polymerase gene specific PCR method. A 300 bp part of this gene was amplified and used for diagnostic purposes and preliminary pylogenetic analysis.
Altogether 57 samples were found to be positive in AdV infection. Fourty-four of these viruses belong to the genus *Aviadenovirus*, 9 to *Siadenovirus* and 4 to *Atadenovirus*. This extremely high positivity (18.62%) can be explained by the large number of samples from dead birds. Adenoviruses are known to be secondary pathogens, that is to say they persist in the host, and manifest during another more serious disease that the host suffer from.
We found 5 aviadenoviruses, 4 siadenoviruses and 2 atadenoviruses, all of wich proved to be new types. In addition we found several adenoviruses that have been already detected earlier, or were very similar to earlier detected ones. In birds, most often aviadenovirus were found, wich are considered to have continuously co-evolved with the birds. Siadenoviruses, may have derived from amphibians while atadenoviruses have reptilian ancestry and they got into birds during host-switching. They occur in a smaller number of bird species and they are more pathogenic in birds than aviadenoviruses. We did a phylogenetic tree reconstruction by protein distance matrix analysis. The position of the novel adenoviruses in this reconstruction reaffirms the co-evolutionary hypothesis. The five distinct genera represent the major evolutionary lineages, and within the three genera there seem to be clear clusters for avian viruses found in related avian hosts.

IMPORTED VIRAL ZOONOSES IN HUNGARY

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As a result of the increasing tourism, the incidence of different imported infectious diseases in Hungary might increase in the future as well. This presentation demonstrates those imported viral infections which were diagnosed during 2010 by indirect immunfluorescent method in the National Reference Laboratory for Viral Zoonoses at the National Center for Epidemiology. Three Dengue cases were found, which were imported from three different regions of Asia. The cases presented in the form of typical Dengue fever with arthralgia, fever and exanthema, with full and spontaneous recovery. Diagnosing acute Dengue viral infection can be a challenge, because of the difficulties imposed by the presence of cross-reactions with other flaviviruses (for example yellow fever or tick-borne encephalitis vaccination, previous West Nile virus infection etc.) and also the eventual serological reaction for other viral agents, like parvovirus with similar clinical manifestations. In acute Dengue infection, determination of the causative serotype is time-consuming and labor-intensive. At the time of the abstract transmission, preliminary results show an acute Chikungunya infection imported from South-Africa, but serological examinations are still ongoing. The disease presented in milder form with fever, pain in the limbs and some maculopapular exanthema. The recovery was full and spontaneous. In this case, the serological (immunfluorescent) cross-reactions with other alphaviruses (for example Sindbis virus) showing similar symptoms and similar geographic locations, might be a problem and be worth considering. Both Dengue fever and Chikungunya infections are zoonotic diseases, the causative agents of both are arboviruses. The vectors of

the Dengue virus belonging to the flavivirus genus are Aedes mosquitoes. While the vectors of the Chikungunya virus belonging to the alphavirus genus are different Aedes and Culex spp mosquitoes. This presentation covers the clinical, epidemiological and microbiological aspects of these infections.

HUMAN STRONGYLOIDIASIS: A CASE REPORT

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Strongyloides stercoralis is a worldwide distributed nematode of the small intestine. This helminth is the only, which eliminates larvae through the feces. When the peristaltic movement of the bowel is decreased, the rhabditiform larvae transform into infectious, filariform larvae, which can penetrate the intestinal wall and complete the life cycle, causing hyper infestation. In many cases, the infection is asymptomatic or has mild symptoms. However, in immunocompromised patients, it can cause severe complications with a death rate of 60-85%. In this abstract we report the case of a male, 64 year old patient who underwent a serious gastrointestinal condition. During hospitalization he also suffered a surgical intervention, splenectomy. Following the intervention the patient had broncho-pneumonia, sepsis and heart failure. He dyed in hospital being suspected of disseminated strongyloidiasis affecting the central nervous system and invading other organs, too. The diagnosis was based on symptoms and gastric biopsy. The patient had chronic liver cirrhosis due to alcohol consumption, which was a major risk factor for disseminated strongyloidiasis.
The autopsy revealed invasive gastric strongyloidiasis associated with acute enteritis, the wall of the small bowel been autolysed. Although, repeated stool sample examinations by microscopy proved to be negative, duodenal mucosal scrapings showed a heavy load of Strongyloides stercoralis females, rhabditiform and filariform types of larvae, and eggs. The presence of filariform larvae was a clear evidence for the autoinfection leading to invasion. Biopsy specimens collected from the gastric wall confirmed the invasive character of the infection with the presence of the parasites in the mucosa. No other body site was affected by the parasite, the infection remained localized in the intestinal tube. In conclusion, examination of stool samples is not enough for a correct diagnosis in the case of strongyloidiasis, other techniques, like biopsy can be helpful. Although, the suspected death cause was hyperinfection with S. stercoralis, the autopsy infirmed that diagnosis, the lung, CNS and other organ biopsies denied the presence of *Strongyloides stercoralis.*

CHARACTERIZATION OF THE HEMICELLULOLYTIC SYSTEM OF THE PLANT WALL DEGRADER MODEL ORGANISM *THERMOBIFIDA FUSCA*

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The thermophilic actinomycete, *Thermobifida fusca*, is the major degrader of plant cell wall in compost piles due to its complex cellulolytic and hemicellulolytic systems. The multiple secreted *T. fusca* cellulases have already been investigated but little is known about its more complex hemicellulolytic enzyme system.
Based on genomic sequence analysis, four xylanases (Xyl10A, Xyl10B, Xyl11C, BXyl) and two mannanases (BMan, Man5C) were identified as part of the hemicellulolytic system of T. fusca strain TM51. The genes encoding the hemicellulases were overexpressed in *E. coli* host and the proteins were subjected to extensive biochemical characterization. These hydrolases are modular proteins with a conservative, TIM barrel type catalytic domain that belongs to Clan A of Glycosyl Hydrolase Families and act in a double displacement mechanism. The only exception is BXyl, whose catalytic modul has a unique five-bladed b-propeller fold. The mechanism, how BXyl cleaves the glycosidic bond, leads to the inversion of anomeric configuration supported by our NMR data. The extracellular hemicellulases (Xyl10A, Xyl10B, Xyl10C, Man5C) all randomly hydrolyse internal bonds in hemicellulose chain liberating oligosaccharides made of up to 12 sugar units. These hydrolases are hyperthermostable enzymes as maintaining their catalytic activity around 80°C. The intracellular BXyl and BMan attack xylo- and mannooligosaccharides from the non-reducing end and remove monosaccharide units with 50°C temperature optimum. A synergistic relationship was detected among the *T. fusca* endoxylanases (Xyl10A, Xyl10B) and b-xylosidase (BXyl), when they act in a concerted manner on a largely insoluble xylan. Utilizing this effect, we have developed an enzyme based kit comprising *T. fusca* xylanolytic enzymes for quantitative determination of xylan content in plant cell wall.

The stability of *T. fusca* hemicellulases is achieved partly by a number of ion pairs that form on the surface of the proteins and by oligomerization. Oligomerization is prevalent in the active form of the intracellular enzymes (BXyl, BMan). The endo acting *T. fusca* hemicellulases carry family 2 cellulose binding domain (CBM2), which specifically bind to crystalline hemicellulose. The active site topology of the intracellular hemicellulases (BMan and BXyl) was examined using two different approaches. In case of BMan, the active site nucleophile was identified by site-directed mutagenesis. To probe the active site of BXyl, a mechanism trap (N-brom-acetyl-xylopyranozyl-amine) was employed that is able to form covalent bond with exposed carboxyl side chains. In the ligand binding channel, four amino acids with carboxyl groups were modified, which suggests a unique arrangement of two Asp-Glu pairs.
This study was supported by the NKTH TECH_08-A3/2-2008-0385 (OM-00234/2008) MYCOSTOP grant.

ESTIMATING THE FREQUENCY OF SUPERINFECTION IN A LARGE EUROPEAN COLLABORATIVE HIV DATABASE

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Superinfection (infection of an HIV positive individual with another virus strain) can facilitate the evolution of HIV by allowing for the recombination of distinct viral lineages. In particular, it might enable the transmission of drug resistant viruses to individuals with drug-sensitive strains who have previously responded well to therapy. We have analyzed routinely collected genotyping data from a large European collaborative HIV database to estimate the frequency of superinfection. We used sequence data spanning the protease and partial reverse transcriptase regions from the Virolab and EuResist databases. 4656 patients (gender: male=3140, female=1363, unknown=153; riskgroup: heterosexual=1178, IDU=1073, MSM=951, other=158, unknown=1296) had at least two sequences in the database, with a total of 14196 distinct sequence entries (of which 89.0% belonged to subtype B). Superinfection was indicated when sequences of a patient failed to cluster together in maximum likelihood phylogenetic trees constructed from all available sequences. Putative cases of superinfection were then further investigated with Bayesian phylogenetic methods.
We identified ~300 patients with sequences clustering robustly into at least two distinct lineages (indicative of superinfection) in ML phylogenies; 37 cases represented infection with two distinct subtypes. Bayesian reconstruction of selected subsets of the phylogeny supported ~100 cases of superinfection with high posterior probabilities; support for the remaining cases proved weak to moderate.
Superinfection can occur relatively frequently, and might therefore play an important role in the transmission of drug resistance. We note, however, that our analysis was necessarily confined to patients with at least two available sequences, and might therefore have overestimated the prevalence of superinfection in the general HIV positive population. We have demonstrated that routinely obtained genotyping data can provide sufficient phylogenetic signal to infer superinfection, although further data might be needed to resolve some of the cases.

POSSIBLE RESISTANCE MECHANISM BEHIND ELEVATED TIGECYCLINE MIC'S IN *BACTEROIDES* STRAINS

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Tigecycline is a broad-spectrum antibiotic agent, that was developed in response to recent increase in antimicrobial resistance. Tigecycline is the first member of the glycylcycline family, a half-synthetic derivate of minocycline. The modification of the basic structure protects tigecycline from frequent resistance mechanism that inactivate tetracycline. Tigecycline shows good activity against clinically significant Gram-positive and Gram-negative bacteria, including the most frequent anaerobic pathogens, the *Bacteroides* strains.
During a recent Europe-wide surveillance of the antibiotic resistance of different *Bacteroides* strains a very good activity was found against tigecycline with a resistance rate of 1.7% according to the CLSI resistance breakpoint (MIC $\geq$16 μg/ml).

HEAT ADAPTATION OF *CRONOBACTER SAKAZAKII*

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Cronobacter sakazakii, formerly known as *Enterobacter sakazakii*, is a Gram-negative, facultatively-anaerobic, oxidase negative, catalase positive, rod-shaped bacteria of the family Enterobacteriaceae. *Cronobacter* was first proposed as a new genus in 2007 as a clarification of the taxonomic relationship of the biogroups found among strains of *Enterobacter sakazakii*. This proposal was validly published in 2008 with 5 species (*C. sakazakii, C. malonaticus, C. turicensis, C. muytjensii* and *C. dublinensis*).
Cronobacter are recognized as causative agents of neonatal bacteraemia, meningitis and necrotizing enterocolitis. Although rare, some cases have been linked to the consumption of contaminated reconstituted infant formula. The literature suggests that premature infants and those with underlying medical conditions may be at highest risk for developing E. sakazakii infection. Though infant infections are basically traced back to the consumption of powdered milk products, *Cronobacter* species are frequently isolated from the environment, plant material (wheat, rice, herbs and spices) and various other food products as well. Mild treatments and environmental effects often present sub lethal stresses to microorganisms that either lead to their death or cause stress reactions in the surviving cells increasing their resistances to similar or other kinds of stresses.
Mild preservation treatments desired by the consumer represent a danger to food safety because of the stress adaptation abilities of microorganisms. We have studied the effect of sub lethal heat treatment on the survival and the growth parameters of *Cronobacter sakazakii*. Heat destruction at various temperatures of cultures of Cronobaccter sakazakii type strain and isolate grown at 37°C in TSB (Tryptone Soy Broth) and TGY (Tryptone Glucose Yeast Broth) was examined with tradition plating and conductance measurement.
In our experiments the effect of preconditioning (keeping the bacteria at 20°C and 50°C respectively prior heat treatment) was examined on the heat resistance of the studied strains. According to the results it can be stated the efficiency of the heat treatment depends greatly on the pre life of the microbes.

IMPACTS OF WASTEWATER SLUDGE ON CARBON DYNAMICS AND BIOCHEMICAL ACTIVITIES IN MEADOW CHERNOZEM, "KOVÁRVÁNY" BROWN FOREST SOIL

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Soil is a dynamic system in which microorganisms perform important tasks in soil organic matter (SOM) transformations and nutrient cycles. The application of wastewater sludge (WS) increases SOM and improves soil structure. The WS is comparatively not clean, containing relatively low and high amounts of heavy metals (HMs), such as Cd, Pb, Cu, Hg, As, etc. A 90 days pot experiment was conducted to study the influence of soil amendment with WS of low (L) and high (H) HMs content at different mixing rates (soil/WS at 100/0, 80/20, 60/40, 40/60, 20/80 and 0/100% w/w) on some functioning microbial groups (total aerobic bacterial and fungal populations, cellulose decomposers and phosphate solubilizers) and six enzymatic activities (fluorescein diacetate, dehydrogenase, β-glucosidase, urease, phosphatase, aryl-sulphatase), microbial biomass carbon (MBC), total organic C (TOC), water and hot soluble C (WSC and HWSC), soil respiration (CO_2-release), total nitrogen content (TNC) and plant productivity in clay loam brown forest soil (Gödöllő, Hungary).
In comparison with unamended soil, WS addition increased C and N contents, enzyme activities, and enumeration of cellulose decomposers, phosphate solubilizers and plant dry weight. These indicating an increase in the microbial metabolism in the soil as a result of the mineralization of biodegradable C and N fractions contained in the amendments. However, the decreasing in the investigated parameters at higher amendment rates possibly being due to the higher HMs contents. When soil amended with LHMs, dehydrogenase activity was significantly correlated ($P < 0.05$) with TOC, WSC, HWSC, MBC and CO_2-release as well as other enzymatic activities. These results illustrated that dehydrogenase activity could be a good bioindicator of soil microbial activities. Also, the WS of HHM content significantly decreased soil bioproductivity, reduced it's the biochemical properties, and lowered the microbial contents in the soil in comparison with WS of LHM content. Total common bean dry matter yield and total N increased linearly with increasing WS application rates.
Soil samples amended with WS rates of 40 and 60 % exhibit higher in crop dry matter, microbial contents and enzymatic activities than the control soil and at higher mixing rates more than 60%. However, these parameters return quickly to background dosage, as soil microorganisms rapidly mineralize the added OM in the form of WS. These results indicated that application of WS of LHMs contents to soil stimulates the growth of soil

microorganisms, probably due to the OC and other nutrients than the control soil. Overall, our results demonstrated that soil amended with LHMs content can improve soil quality and soil biological and biochemical properties. Finally, the concentrations of HMs introduced into the soil must be regularly measured.

GENERAL REQUIREMENTS FOR THE ORGANISATION AND OPERATION OF PCR LABORATORIES FOR DIAGNOSTIC PURPOSES

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For ensuring the safety of consumers, the rapid detection of pathogens and other microbial contaminants in food is critical. One of the more and more widely used techniques is the PCR reaction. Beside all the advantages, this biochemical reaction has a strong cross contamination hazards. Therefore, out of the general microbiology laboratory requirements management of a PCR laboratory has to meet special requirements. The aim of these industrial laboratories is the detection (analysis of presence/absence) or quantification of pathogenic or spoiling microorganisms, and the possibility or occurrence of contamination could lead to false positive results, which have to be prevented and controlled efficiently.
As regards the organization of a PCR laboratory for diagnostic purposes the ISO standard 22174:2005 recommends the "forward flow" principle and the systematic containment of the methodological steps involved in the production of the results. The theory of "forward flow" helps the food examining laboratory to avoid the contamination of PCR reaction mixture by previously amplified target sequence. It is recommended that minimum four distinct areas (sample preparation, preparation of reaction mixture, addition of nucleic acid to the reaction mixture and detection and confirmation of PCR amplified nucleic acid) with their own working facilities should be separated. The application of PCR controls improves further the reliability of results derived from PCR amplification. For the detection of pathogenic microorganisms from food and animal feeding stuffs the ISO 22174:2005 standard gives recommendations. Special consideration is needed when the PCR amplified nucleic acid and materials used for the detection and confirmation should be disposed.
This work was supported by EU „PathogenCombat" FP6 007081 project.

BATS AS NEWLY DISCOVERED HOSTS FOR ADENOVIRUSES

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The PCR is a powerful tool for the specific and, nowadays even for the non-specific, detection of different viruses. As we have reported at the previous congress, the screening of randomly collected samples from different birds resulted in the recognition of an unexpectedly huge number of putative novel adenoviruses. Recently, several samples from bats have also been tested. To our surprise, the proportion of positive cases for adenovirus was almost as high as among the avian samples, significantly exceeding the number obtained when screening the samples of other mammalian hosts. The virus reservoir status of birds and bats can be explained partially by the similarities in their lifestyle. Just as birds, bats also live in large and dense colonies. The flocks of different species of birds or bats, respectively, often occupy the same or neighboring territories. Another characteristic feature shared by both animal groups is the ability to fly and regular migration, although this latter one usually means only a daily traffic between the sleeping and feeding sites in the case of bats. Long distance (over 1,000 km) migration of bats is very rare and is confined to less than 0.02% of all the species. Apparently, all these specific qualities of the way of living facilitate the easy spread and persistence of different microorganisms including a large selection of both RNA and DNA viruses.
In the early fifty's, bats were used as experimental animals in the examination of transmissibility, pathogenicty, and immunogenicity of a wide range of viruses known at that time. For a couple of decades, the virological researches connected to bats were focused on rabies virus. Since the recognition of the henipavirus and coronavirus infections in bats, these animals have been being again in the foreground of virological studies.
Almost simultaneously, bat adenoviruses have been detected and even isolated in Japan, Germany and China. Since the very first description in 2008 by Maeda et al., three isolates and more than 10 putative types have been published. The first full genome sequence was also published early this year by Li et al. It seems that the hypothesized co-evolution can be observed between the bats and their adenoviruses too. The *Chiroptera* is one of the richest orders of vertebrates with more than 1,100 species. It is divided into two large groups (corresponding to suborders) the *Microchiroptera* and *Megachiroptera*. Adenoviruses obtained from these two

different bat lineages appear in large distance from each other on the phylogenetic tree reconstructions. The striking similarity between the canine adenoviruses and some bat adenoviruses prompted us to speculate on their possible close common ancestry.

PROTEOMIC ANALYSIS OF EFFECT OF FACTOR C IN *STREPTOMYCES*

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Streptomycetes are Gram-positive filamentous soil bacteria. Their complex life cycle which finally results in sporulation is connected with production of secondary metabolites. Members of the genus *Streptomyces* produce over 60% of all known antibiotics and a wide range of industrial enzymes. The variation of onset of morphological differentiation and antibiotic production imply the presence of complex regulatory network consisting of multiple signal transfer systems acting independently or having complex interactions. Analysis of mutant strains showing different aberrations of differentiation are really important in identification of those genes that are involved in this pathway. Expression of the secreted regulatory protein Factor C from low copy plasmid in an A factor (a small microbial hormon-like molecule that induces both morphological and physiological differentiation) non producer bald mutant *S. griseus* NRRL B-2682 AFN (a strain that does not form aerial mycelium) restored aerial mycelium, mature spore and antibiotic formation on solid medium. In the wild type *S. griseus* NRRL B-2682 we failed to identify a facC homologe. Despite the absence of facC, response elements of its signal transduction pathway are present in *S. griseus* NRRL B-2682 and its bald mutant. During the proteomic analysis we compared the extracellular protein pattern of the above mentioned wild-type strain, its A factor non-producer bald mutant and the S. griseus NRRL B-2682 AFN/pSGF4 which harbours the facC gene in low-copy number. We tried to find out which genes are involved in aerial mycelium and antibiotic formation regulated by Factor C furthermore to identify the mechanisms applied by the bald mutant trying to help the bacterium to continue its blocked life cycle.

FIFTY-YEAR-STUDIES OF THE SOIL-PLANT-MICROBE-ENVIRONMENT INTERACTIONS AND IMPACT OF THE "MANIPULATION"

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Studies of the soil-plant-microbe-environment interactions have started 50 years ago in the RISSAC HAS, when the previous "Sopron School of Soil-biology" has moved to Budapest from the West Hungarian University (WHU) of Sopron. At that time the special research group was headed by Dániel FEHÉR (1890-1955), as one of the classics of the Hungarian soil-science, an internationally recognised botanist, plant-physiologist and microbiologist. From this team the main profiles in the Department of Soilbiology and Soilbiochemistry was developed by other classics, such as József SZEGI, István Mihály SZABÓ, Mária MARTON, Ilona BUTI, Ernő MANNINGER and also Mihály KECSKÉS, who was founder of the Section Agricultural and Food Microbiology at the Society of Hungarian Microbiologists. Main focus of the research from beginning up till now is the eco-physiology of microorganisms in various soils and their impact on the plant nutrition. Studies on: the chemical- and microbiological composition of soils (N, P, K and humus-content), the interrelations with soil-pH, electric conductivity and the soil-life; indirectly with the soil carbon-dioxide (CO_2) release. Focuses on the seasonal and spatial variability of soils, initiated mainly by the climatic factors, have started also by D. FEHÉR by searching the biological reasons and consequences of those ecological parameters. The Soil-biological Laboratory in Balatonfüred and the Research Centrum of Soil-life-sciences in Kisújszállás was also founded at that time in Hungary, highlighting the inevitable importance of the soil(micro)biological studies.
During those 50 years, research topics have remained generally the same, but starting from the "natural" forest ecosystems, special attention is given nowadays for the impact of "man-made" activities in the anthropogenic soils, influenced by the direct and indirect effects of the xenobiotics and/or the industrialisation. in the agriculture and the environment. Some of the methods in the classic microbiology can be still usable for the studies, however modern, up to date "functional" approach is also involved in assessing the quantitative and qualitative soil-micro-(rhizo)-biological parameters (with short- and long-term backgrounds of research). Beyond the monitoring points, the potentials of "man-ipulation" will be discussed through the applicability of

usual or alternative organic and inorganic amendments and beneficial microorganisms in the phyto(rhizo)technologies, as widely accepted methods of the soil-plant-(microbe)-animal-human food chain.
Supports of the EU-Fp7 Soil-CAM project, the Hungarian Research Fund (OTKA K68992), the TÁMOP (4.2.2-08/1-0016), the bilateral agreements (RAS-HAS, CSIC-HAS) and the relevant COST Actions (8.31, 8.59, 8.70, FA0950) are highly acknowledged.

THE LIFE CYCLE OF STREPTOMYCETES, THE WONDER BUG THAT MAKES A LOT OF DRUGS

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Streptomycetes are filamentous, spore forming Gram positive bacteria that constitute a major part of the soil bacterial communities. The success of the streptomycetes is likely due to their mycelial growth habit, their capacity to consume otherwise refractory organic materials by producing extracellular hydrolases and their ability to produce and secrete small molecules that modulate the growth of competing microorganisms.
These small molecules, the so-called secondary metabolites make them especially good sources of medically important compounds: antibiotics, enzymes, enzyme inhibitors, modulators of the immune system, chemotherapeutic agents, effectors of plant growth, etc. Their life cycle on solid medium initiates with spore germination, leading to growth of vegetative multi-genomic filamentous cells, called substrate hyphae. These grow into the medium, thereby sampling nutrients across a variety of microenvironments. These cells are non-motile; therefore if local conditions are unfavorable, the organism depends on sporulation for dispersal.
Spore formation involves the development of aerial hyphae that grow out of the soil and extend vertically into the air. During aerial mycelium formation many cells in substrate mycelium die and lyses which is considered as a programmed cell death and their lysis supply the growing aerial hyphae with nutrients. At this stage of development enzymes, that degrade the biopolymers of the decaying hyphae are presumably required.
Cell division is rare in the substrate hyphae, explaining the multigenomic nature of those cells. In the contrary in aerial hyphae concerted septation takes place resulting in pre-spores that finally results in mature rounded pigmented spores that are resistant to many environmental challenges. Their eventual release enables the initiation of the growth cycle elsewhere. The onset of development is temporally relates to the production of antibiotics and other secondary metabolites. Autoregulatory molecules play a key role in controlling both the onset of cellular differentiation and secondary metabolism. The best studied autoregulator is A-factor (2-isocapryloyl-3Rhydroxymethyl-γ-butyrolactone), a small microbial hormone like molecule (243 Da) that induces both morphological and physiological differentiation in *Streptomyces griseus*. The γ-butyrolactone regulatory system is widespread in streptomycetes. Another interesting autoregulator of cell-cell communication and cytodifferentiation is the secreted signaling protein Factor C (molecular mass 34.555 kDa) originally isolated from the culture fluid of "S. griseus 45H", which was recently shown to be identical to a laboratory strain known as *Streptomyces flavofungini*, itself a member of the *Streptomyces albidoflavus* species group. The Factor C producer strain like *S. griseus* readily sporulates in submerged culture.
The isolation and identification of Factor C, the distribution of it in the microbial word and the surprising connection between these two highly divergent types of signaling molecules (A factor and Factor C) as well as the possible interplay between their regulatory networks will be the focus of this lecture. I will also highlight a possible practical outcome (a sensitive aspergillosis test) of the Factor C research.

THE COMPLEX EXTRACELLULAR BIOLOGY OF *STREPTOMYCES*

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Members of the genus *Streptomyces* are the most abundant bacteria in soil. Their success could probably be explained by their mycelial growth habit sampling the soil for nutrients, the huge capacity to produce extracellular hydrolases for the degradation of otherwise refractory organic materials like chitin and cellulose, their use of non-catalytic substrate binding proteins for the uptake of nutrients, and the production of a large number of small molecules, the so called secondary metabolites that modulate the growth of competing microorganisms. When local conditions for growth deteriorate, they form spores that are highly resistant to unfavorable conditions and are responsible for dispersal. Evidence suggests that spore formation and the production of secondary metabolites are linked and have a common regulation. The involvement of two highly

divergent extracellular signaling molecules, the small (243 Da) microbial hormone-like lipid soluble γ-butyrolactone A-factor that induces both morphological and physiological differentiation in *Streptomyces griseus* and the protein factor C (molecular mass 34500 Da) that also plays a key role in cellular communication and cytodifferentiation are studied in detail in the regulation of sporulation and antibiotic production. Their regulatory pathways and intimate interactions will be used to show the importance of extracellular events. The unusual distribution of factor C-like genes in microorganisms and a practical asset of it will also be discussed.

DETECTION AND CHARACTERIZATION OF HUMAN PARECHOVIRUSES IN ARCHIVED CELL CULTURES, IN HUNGARY

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Human parechoviruses (HPeV) belonging to the family Picornaviridae are widespread enteric pathogens and associated with various clinical symptoms in humans. There is no report for detection of the circulating parechoviruses in Central Europe. The aim of this retrospective study was to detect and characterize human parechoviruses in cell cultures with "enterovirus"-like cytophatic effect (CPE) archived between 1990 and 2000, in Hungary. Fecal samples from children with symptoms of gastroenteritis under age of 10 were cultured as a previous routine diagnostic laboratory protocol for "enterovirus" on GMK and "293" cell cultures from years 1990 to 2000, in Baranya county, Hungary. Minimum of 2 cell cultures indicating CPE were archived and deeply chilled (-80°C) in each year. Specimens were tested retrospectively, in 2009, for HPeV by reverse transcription–PCR (RT-PCR) method using 5'UTR conserved primers. Specific primer pairs were designed to determine the complete nucleotide sequence of the structural region (VP0-VP3-VP1) of HPeV.
Nine (9.1%) of 66 archived samples found to be HPeV-positive. Six (67%) of 9 HPeV-positive samples were HPeV1, 2 (22%) were HPeV4 and 1 could not be determined. Three HPeV1 clusters were identified according to the isolation date originated from years 1990/1991, 1992/1995 and 1998.
This is the first detection of human parechoviruses in Central Europe. Detection and genetic characterization of HPeV in available historical samples infected with previously unidentifiable agents with "enterovirus-like" cytopathogenic effect help to understand the genetic diversity and evolution of these viruses.

MONITORING OF WINE FERMENTATION INOCULATED WITH INDIGENOUS STARTERS OF *SACCHAROMYCES CEREVISIAE*

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The role of *Saccharomyces cerevisiae* in wine production has been pointed out by several authors, demonstrating that this yeast dominates the fermentation process due to its efficient fermentative metabolism. Its activity may occur by two approaches: a) spontaneous fermentation, which is performed by the yeast populations present in grape must and permits the participation of several strains, presumed to be autochthonous *Saccharomyces* strains, better adapted to the environmental conditions and potentially able to imprint a locally distinctive style to the wine; b) inoculated fermentation, which takes place by the use of selected S. cerevisiae starters, in the form of active dry yeasts, to ensure the reproducibility of fermentations and the final product quality. Today, the majority of wine production is based on the use of commercial strains, which have been selected for their superior properties for winemaking, but the same strains are used to ferment grape musts of different origin and variety. On the other hand, there is increasing interest in the selection of new indigenous *S. cerevisiae* strains from the vineyards and wine fermentations, which are better adapted to local fermentation conditions and specific grape must composition. These wild *S. cerevisiae* strains can also reflect the biodiversity of a given region. In addition in inoculated fermentation it must be underlined that indigenous yeasts can still participate in fermentation and there is no guarantee that the inoculated yeasts will predominate during fermentation. It means that if the starter culture and the inoculation techniques are not perfectly controlled, the selected yeast will not predominate, and wild yeasts can manage the process due to a better adaptation to the wine cellar environment. Therefore, rapid and simple methods for yeast strain identification in fermentations would be useful to ensure the guidance of the fermentation by the inoculated yeast. These methods should clearly differentiate between the inoculated S. cerevisiae strain and the wild *S. cerevisiae* strains present in grape must or in the cellar.
Here we report the results of a study regarding the evaluation of the effective dominance of the inoculated strain

during the fermentation process. In particular, two *S. cerevisiae* selected strains, isolated from Aglianico del Vulture grapes, were tested during inoculated fermentations at pilot scale in three wine cellars, producing Aglianico del Vulture wine in the Basilicata region (South Italy). Yeast colonies, sampled during the processes, were identified and *S. cerevisiae* strains were differentiated by RFLP-mtDNA. By this technique *S. cerevisiae* isolates showed a different dominance of inoculated strains in function of the wine cellar. In two cellars, all the isolates showed the same restriction profile, identical to the starter pattern, whereas in the third cellar a significant percentage of *S. cerevisiae* isolates exhibited mtDNA-RFLP patterns different from the starter profile, demonstrating a low dominance of the starter in the fermentation of the third cellar. Although the inoculated strains were found with high frequency, other yeasts (*S. cerevisiae* and non-*Saccharomyces*) developed, contributing to the fermentative process and to the organoleptic quality of the final wine.

THE MYCORRHIZAL COMMUNITY UNDER PHOSPHORUS STRESS IN THE SOIL WITH DIFFERENT GENETICAL TYPES

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In the experiment series were examined the relation between different stress effects and the mycorrhizal colonization in the Agricultural Office of County Baranya Plant Protection and Soil Conversation Directorate Soil Biology Laboratory. Our aim was: how the mycorrhiza colonization shapes the soil when the content of the ortophosphate changes. The experimental soil types were Ramann's brown forest soil (BAR4), calcareous meadow soil (BAR6) and calcareous chernozem (BAR4). The experimental material was *Triticum aestivum lupus*. *Glomus intraradices* (AM-27) was used as inoculum in the experimt, 5 g inoculum was added to a pot. Phosphate threatment was done, when the experimental plants were 5 weeks old. Two dose (D2= 50ppm, and D3= 100ppm P_2O_5 per opportunuty) and naturally controll (D1) were used. After four weeks the plants were harvested. Measured parameters were: plant root mass, green-mass, chemical parameters, soil chemical and microbiological parameters: CFU og microfungi, total number of microorganisms and the intensity (M%) of the mycorrhizal infection. During the phosphorus stress experiment the observed parameters, consequently the mycorrhizal colonization depended on the type of the test soil. The experiment has hypothesis, that the plant mycorrhiza connection was destored by enhancing quantity of the ortophosphate depending on the type of soil.

AFLATOXIN B1 DEGRADATION ACTIVITY OF SOIL BACTERIA ISOLATED FROM OIL POLLUTED SITES

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AflatoxinB1 is toxic and carcinogenic metabolite produced by species of *Aspergillus flavus* and *A. parasiticus*. Beside its cytotoxic effects it is one of the most powerful natural mutagenic pollutants. Therefore it is not surprising that among mycotoxins aflatoxin has far the lowest regulatory limit – as low as 4 µg/kg (EFSA, 2006) – in food and feedstuffs. The total annual worldwide loss due to aflatoxin in food and feed industry is up to billion Euro range. In our lab during the last decade oil degrader bacterial strains were isolated by the use of special selection methods from oil contaminated sites of Hungary. Our microbial strain collection contains about 300 different strains that are able to degrade different hydrocarbon compounds presented in oil polluted sites.
In a preliminary experiment 20 strains were selected according their excellent aromatic hydrocarbon (PAH) degrading activity. As PAHs have similar structure to aflatoxinB1 (AFB1), these aromatic decomposer strains were chosen for mycotoxin biodegradation experiments. Our aim was to monitor the biodegradation of AFB1 and to measure the resulting genotoxic level of the degradation residuals by bacterial biotests. The AFB1 degrading activity of 20 selected strains, which were representatives of the genera *Pseudomonas, Streptomyces, Microbacterium, Arthrobacter Pseudoxanthomonas, Gordonia, Chryseobacterium, Paracoccus, Ochrobactrum* and *Sphingopyxis* were measured in contaminated liquid cultures. AFB1 concentration was 4 µg/ml in the starting media. The residual mycotoxin levels were detected by ELISA and chemical analytical tests, respectively. From the investigated 20 strains 18 could degrade AFB1 significantly. The best AFB1 degrader was *Pseudomonas pseudoalcaligenes* with 98,5 % degrading activity. The genotoxic effects of the degraded

mycotoxin containing samples were analysed by SOS-Chromotest. We found that the genotoxic effect of AFB1 is still detectable at 0,078 µg/ml concentration. SOS-Chromotest was successfully used to select microorganisms that have the best AFB1 degrading potential and can degrade AFB1 without genotoxic by-products.
This study was supported by the NKTH TECH_08-A3/2-2008-0385 (OM-00234/2008) MYCOSTOP grant and KMOP 1.1.1.-07/1-2008-0002 project.

PREVALENCE OF WU AND KI POLYOMAVIRUSES AMONG RENAL TRANSPLANTED PATIENTS

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It is suggested that the two recently discovered human polyomaviruses KI (KIPyV) and WU (WUPyV) are widespread viruses. However, little is known about their transmission and these viruses have not yet been linked to a specific primary disease. There is no clear ethiological link between respiratory diseases and WUPyV and KIPyV, but these viruses were frequently detected in respiratory specimens suggesting transmission via this rute. At the same time, frequency of the viruses in respiratory specimens did not show differences between asymptomatic individuals and patients with respiratory symptoms, but in connection with KIPyV significantly higher prevalence was observed among immunocompromised, stem cell transplanted patients. High reactivation frequency of WUPyV and KIPyV (similar to that of BKV) was found among AIDS-patients. Our aims were the followings: to determine the prevalence of KIPyV and WUPyV in blood, urine and respiratory specimen of renal transplanted patients and to reveal the possible connection of these viruses to any clinical symptoms. For these purposes nested PCR, sequencing of the amplicons (all PCR positive) and qunatitative real-time PCR were used. Up to the present 450 plasma, 106 urine and 137 respiratory specimens were examined. 3 plasma samples were positive for KI and 8 for WU virus, the prevalence of these viruses was 2.4 % (11/450). The copy numbers of the viruses were below the detectable levels At the same time higher prevelane of WU and KI was revealed in urine samples: 13.2 % (14/106). 4 samples were positive for KIPyV and 10 carried WUPyV DNA. In most of the samples the copy numbers of the viruses were below the detectable levels, or were low (1-2.2 X 10^1 Geq / ml). 12 (8.7 %) out of the 137 examined respiratory samples were positive for viral DNA: 8 were positive for KI and 4 for WU viruses. Collection and statistical analyses of the clinical data are planned.

EXAMINATION OF YEAST DIVERSITY IN „BADACSONYI KÉKNYELŰ"

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Kéknyelű is a traditional Hungarian grape variety which is grown only in the Badacsony wine-growing region. The aim of this work was to study the indigenous yeast flora of Kéknyelű wine. We took samples from fermenting Kéknyelű musts in vintages 2006 and 2007. To monitor population dynamics, we sampled the musts several times during fermentation. We isolated 534 yeast strains in the two vintages. All isolates were subjected to conventional taxonomic tests, in which their morphology, sporulation, growth at various temperatures and the utilisation of carbon and nitrogen sources were examined. On the basis of the results the isolates were grouped and then representative strains of the groups were also subjected to molecular analysis including PCR-RFLP of the ITS1-5.8S-ITS2 parts of the chromosomal rDNA region and sequencing of the D1/D2 domain of the large subunit rRNA gene. The nuclear gene MET2 digested with EcoRI and PstI and the NTS2 region digested with BanI were applied to the differentiation of species in the *Saccharomyces* sensu stricto group. For karyotyping, chromosomal DNA was prepared from the strains and the chromosome-size DNA molecules were separated by CHEF-DRII and DRIII electrophoresis systems. According to the results, the isolates from year 2006 belonged to *Metschnikowia pulcherrima, Hanseniaspora uvarum, Candida zemplinina, Torulaspora delbrueckii, Saccharomyces cerevisiae* and *Saccharomyces uvarum*. After one week of fermentation, only *Saccharomyces* strains were present in the must. In 2007 we also detected *Saccharomyces bayanus* and *Aureobasidium pullulans*, a yeast-like fungus which was present in the must for 3 days. The fermentation in 2007 was slower allowing *M. pulcherrima* and *H. uvarum* strains to be present for almost two weeks. At the end of this fermentation, *Saccharomyces* and *C. zemplinina* strains were found in the wine.

TAXONOMIC IDENTIFICATION OF YEASTS ISOLATED FROM SPONTANEOUSLY FERMENTING "EZERJÓ"

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The aim of this work was to study wine yeasts isolated from spontaneously fermenting Ezerjó must in order to obtain more information about the indigenous flora. To investigate the natural yeast microbiota, we took samples in the vintage seasons 2006 and 2007. We sampled the musts 10 times during the fermentation process to monitor population dynamics. The isolates were subjected to conventional taxonomic tests and molecular analysis. In the conventional tests, we examined the morphology, sporulation, growth at various temperatures and the utilisation of carbon and nitrogen sources by the isolates and grouped them on the basis of the results. Then representatives of the groups of isolates with identical properties were also subjected to molecular analysis including PCR-RFLP of the ITS1-5.8S-ITS2 and NTS2 parts of the chromosomal rDNA region and sequencing of the D1/D2 domain of the large subunit rRNA gene. The gene MET2 was also analysed in the case of *Saccharomyces* sp. strains by PCR-RFLP. For karyotyping, chromosomal DNA was prepared from the isolates and the chromosome-size DNA molecules were separated by CHEF-DRII and DRIII electrophoresis systems.
On the basis of the results of the tests, the isolates from 2006 could be classified to *Metschnikowia pulcherrima, Hanseniaspora uvarum, Rhodotorula glutinis, Torulaspora delbrueckii, Saccharomyces cerevisiae* and *Saccharomyces uvarum*. At the beginning of the fermentation *Aureobasidium pullulans* strains were also present. In 2007, we detected *Metschnikowia pulcherrima, Torulaspora delbrueckii, Saccharomyces cerevisiae* and *Candida zemplinina* strains.

DIAGNOSTIC RESULTS OF PANDEMIC INFLUENZA INFECTIONS IN 2009 AND 2010 AT THE DEPARTMENT OF CLINICAL MICROBIOLOGY

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The previous influenza pandemic occurred more than 40 years ago. The majority of those physicians who participated in the treatment of patients of a similar occurrence had subsequently retired. Virologists had been thinking seriously of the next pandemic because of the increased number of susceptible patients. As the recent pandemic started in the Southern-Hemisphere, in Europe we had sufficient time to prepare against influenza infections. The researchers involved in the influenza pandemic in the Southern-Hemisphere used their experience to make proposals for the countries in the Northern-Hemisphere, which proved absolutely correct. Nevertheless not unexpectedly, influenza-like infections began very rapidly in Szeged.
Throat and nasopharyngeal swabs were taken. Automated nucleic acid isolation was used. Real-time RT-PCR was performed for the influenza diagnosis. Immediate diagnoses were given to the clinicians from 8.00 in the morning until midnight. 510 clinical samples were sent to our laboratory from November 27 to December 31 in 2009, and 52.6% of the samples proved positive. From 1 January to the end of May in 2010, 193 samples arrived: the positive rate was 19.2%.
The proposals of the medical virologists from the Southern-Hemisphere were absolutely correct, but the primary prevention in Szeged was low. The applied influenza antivirals were effective. It is our conviction, that the usual influenza screening was not sufficient for an influenza pandemic situation. Under such circumstances, diagnostic laboratory examinations too are very important.

VACCINE COVERAGE OF CONJUGATE PNEUMOCOCCAL VACCINES AGAINST CLINICAL AND CARRIED ISOLATES IN HUNGARY

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There are 2 types of vaccines against pneumococcal infections: the 23-valent polysaccharide vaccine (Pneumovax) and the conjugate vaccines (Prevenar, Synflorix). Only the conjugate vaccines can be used for children < 2 years old, due to the improper antibody response against polysaccharides at such young age.

Prevenar (PCV-7) was included in the national vaccination scheme in Hungary as a suggested vaccine in April 2009, and since then the vaccination rate has reached a very high level. Furthermore, Prevenar was substituted by the newer Prevenar-13 in June 2010. Due to these important changes, we would like to give an overview of the theoretical vaccine coverage based on our research results.
We performed an epidemiological analysis of 150 pneumococcal strains isolated from severe infections, as well as 184 strains carried in the nasal flora of healthy children attending day-care centres. The species identity of all strains was confirmed by the presence of the lytA gene. Their antibiotic sensitivity was determined by E-test. Serotyping was done by the combination of conventional agglutination and a PCR-based method. Resistance to penicillin was only 1.4% in the clinical group and zero among the carried isolates, however, macrolide resistance was a bigger problem. We could identify the serotypes of 136 clinical and 125 carried strains. The most frequent types in the clinical group were 6 (29.4%), 14 (19.1%) and 19 (8.8%), followed by types 23, 3 and 9; and similarly, types 6 (32.0%), 14 (17.6%) and 19 (15.2%), followed by types 15, 11 and 23 in the carried group.
Based on our results, and taking certain cross-protections into account, Prevenar (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) would provide protection against 68% of invasive infections and against 76% of pneumonia cases. Synflorix contains 3 additional serotypes (1, 5, 7F), which are rare in Hungary, therefore is only slightly better than Prevenar. On the other hand, Prevenar-13 has 3 further serotypes (3, 6A and 19A), which are all important in Hungary, therefore the vaccine coverage increases to 87.5% in this case. Regarding the carried isolates, coverage is a bit lower (75.2% for Prevenar and 77.6% for Prevenar-13), due to the several non-vaccine types found in this group. In summary we can conclude that, due to the high coverage rate, introduction of Prevenar-13 as an obligatory vaccine would be welcomed in Hungary.

OCCURRENCE OF AFLATOXIN-PRODUCING *ASPERGILLUS* SPECIES ON MAIZE IN HUNGARY

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CHARACTERIZATION OF VANCOMYCIN RESISTANT ENTEROCOCCI ISOLATED DURING A SIX-YEAR SURVEY IN DEBRECEN

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In developed countries vancomycin resistant enterococci (VRE) have become widespread nosocomial pathogens in the last decades. However, until recently they have been rarely encountered in Hungary. Here we report on a six-year survey conducted at the Hospitals of the University of Debrecen, Medical and Health Science Center.
The Bacteriological Diagnostic Laboratory of the Department of Medical Microbiology has been screening VRE on 6 mg/l vancomycin containing BHI agar plates since 2004. During the period of investigation, between January 2004 and December 2009, altogether 7271 clinical samples were found to contain various species of enterococci. Among these strains, 17 were moderately resistant to vancomycin by E-test according to the EUCAST breakpoints. Bacterial species were initially identified by routine laboratory methods, and the results were confirmed by species specific PCR of the D-alanin-D-alanin ligase (ddl) and the superoxid dismutase (sodA) genes. We found, that in agreement with data from other European countries *E. gallinarum* was the most common pathogen (9 samples). The incidence of infections caused by this species alarmingly increased in the last 2 years of our study. We used multiplex polymerase chain reaction (PCR) to identify the vancomycin resistance genes vanA, vanB, vanC1/C2, vanD, vanE and vanG. All of the 17 strains proved to contain vanC1 or vanC2. The latter were differentiated by RFLP, which proved that 4 bacteria carried the vanC2 gene and the rest were vanC1 positive. According to the literature vanC genes are usually found in E. gallinarum and E. casseliflavus. Interestingly, in our study we also found 3 vanC1 and 3 vanC2 carrying E. faecalis species.

The genetic diversity of the 6 *E. faecalis* species was determined by MLST of 7 housekeeping genes (gdh, gyd, pstS, gki, aroE, xpt, yiqL) as described at the www.mlst.net website. Our data proved that the 6 isolates belonged to different sequence types. By sequencing both strands of the acetyl-Coa acetyltransferase (yiqL) gene of strain 6130/II, we found a new allele, with a single nucleotide replacement of C to T at position 187 (as compared to allele yiqL-8). The curator of the website assigned allele 64 (yqiL-64) to the new yqiL sequence, and ST-336 to the new allelic profile. Genetic relationships between the enterococcus strains were analyzed by PFGE fingerprinting, and antibiotic resistance profiling by E-test. According to the PFGE dendrogram, two pairs of *E. gallinarum* were closely related to each other, however, the members of the pairs exhibited different resistance against daptomycin or gentamicin, respectively. Gentamycin resistance was confirmed by PCR of the bifunctional aminoglycosid resistance gene aacA-aphD.
In summary, our results show that the incidence of vanC related vancomycin resistance in enterococci is still low in our hospitals (0.2% of all enterococcus infections). The isolates had no epidemiologic connection to each other, thus we concluded, that the infections occurred sporadically.

EFFECT OF HERBAL EXTRACTS ON THE GROWTH OF PATHOGENIC BACTERIA

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Dilutions of fourteen commercially available herbal extracts were tested on the growth of different human pathogenic bacteria. From peppermint-, lemon-, balm-, eucaliptus-, fennel-, cinnamon-, spearmint-, grapefruit-, indian balm-, lavender-, clary-, sage-, rosemary-, clove-oil, the extracts of cinnamon and clove prooved to be the most effective in the case of the investigated strains of the following human pathogenic bacteria: *Acinetobacter baumannii, Enterococcus faecalis, Enterococcus faecium, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella Typhimurium, Shigella flexneri, Staphylococcus aureus, Streptococcus faecium, Yersinia enterocolitica, Campylobacter jejuni*. Rosemary prooved to be ineffective in all cases, while suppressing effect of the other extracts depended on the oil concentration. Among the investigated bacteria *K. pneumoniae* and *P. aeruginosa, S. Typhymurium* prooved to be the most resistant since most of the extracts that were effective in hindering the proliferation of other bacteria, could not inhibit the growth of these three isolates. Basing on the results further investigations are planned to clear up what major components are responsible for the antimicrobial effects of a certain herbal extract and what mechanism(s) lead to the inhibition of proliferation.

GENOME ANALYSIS OF FISH ADENOVIRUS, THE ONLY MEMBER OF THE FIFTH GENUS OF *ADENOVIRIDAE*

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Adenovirus-like particles have been observed in different fish species including cod, dab and sea bream, but the only isolate available to date is from the white sturgeon (*Acipenser transmontanus*). This virus strain (WSAdV-1) was propagated on an epithelial cell line (WSS-2), prepared from the spleen of white sturgeon. After purification and concentration of the virions, the viral DNA was extracted and randomly cloned using PstI and HindIII restriction enzymes. More than 100 plasmid clones were screened and sequenced partially, but only a small proportion of them was found to contain inserts of adenoviral origin unequivocally. With degenerate consensus primers, fragments from the DNA polymerase and the IVa2 genes were amplified by PCR. The central genome region spanning from the gene of the IVa2 to that of the 33K protein was also acquired by "joining" PCRs. To obtain the end regions of the genome, a series of unidirectional PCRs were performed with specific, outward directed primers. The construction of the genetic map and the design of the PCRs were hampered by the unusual location (close to the left-hand end of the genome) of the fiber genes. The sequence of ITRs was determined with the use of a RACE kit. The size of the full genome is expected to consist of more than 50 kilo base pairs (kb) with a fairly equilibrated G+C content of 43%. Until now, the frog adenovirus (FrAdV-1), with the smallest known genome size of hardly exceeding 26 kb, was considered as the most ancient AdV. The fish AdV was expected to have even shorter and simpler genome. To the contrary, the genome of WSAdV-1 is significantly longer than those of the aviadenoviruses. Phylogenetic calculations with a variety of partial or full sequences (from the DNA polymerase, terminal protein precursor, penton base and hexon genes) showed that WSAdV-1 does not belong to any of the existing genera but represents a distinct lineage within the

family Adenoviridae. A new, fifth genus with the name Ichtadenovirus had been proposed, and officially approved. This was further supported by the unusual genome organization that does not comply with the scheme established as a conserved core in every former genus of this virus family. In all previously sequenced AdVs, the conserved genome part stretches between the genes of the IVa2 and the fiber, and contains 17 genes plus the U exon. In the genome of WSAdV-1 leftward from the IVa2, four putative ORFs were found, three of which are on the r strand and seemingly encode fiber-like proteins. The predicted protein product of the fourth ORF (closest to the IVa2 gene, also on the l strand) shows homology to bacterial and bacteriophage proteins. At the right hand end of the genome, downstream from the gene of the 33K, no homologues of the pVIII or the U exon could be identified. Instead, numerous tentative ORFs of yet unknown function were found. One seems to be a member of the Ig superfamily. Another one contains a sulfotransferase domain. Two novel ORFs show homology to the NS protein of parvoviruses. Similar gene homologues exist also in aviadenoviruses.
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SIMPLE MONITORING OF SOIL BIOLOGICAL ACTIVITY AS INFLUENCED BY APPLICATION OF COMPOST OF DIFFERENT INCUBATION TIME

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Exogenous organic matter is frequently used as a source of soil organic matter. The knowledge of short-term effects of amendments becomes essential when considering, e.g., the development of soil-conservation strategies. However, the simple monitoring of soil organic matter (SOC) content which changes very slowly means that many years are required to measure significant changes in soil quality.
In recent years, soil biochemical parameters such as soil respiration and enzyme activities have been seen to be early and sensitive indicators of soil changes and so can be used to predict long-term trends in the quality of soil.
The following study was carried out to assess the effects of different rates (0, 2.5, 5 and 10%) of fresh and 6 month old compost applications on physical, chemical and biological properties of clay loam brown forest soil.
The results showed an increase in soil respiration. Enzyme activities in soil were found to be increased after addition of compost material. Also, soil pH and dissolved organic carbon (DOC) were affected at modest levels over the 6 month incubation period by any of the four rates of the amendments. An increase in application rates of compost greatly increased the biological parameters studied. The application of 6 month incubated compost material improved soil metabolic activity as measured by increasing microbial activities of cellulose decomposing and solubilizing phosphate ability, dehydrogenase, urease and basal respiration. Compost of 6 month-old seems to be more suitable than fresh one to be used in soil remediation and as a source of immobilized enzymes. It was found that microbial biomass C was significantly correlated with soil pH, soil organic C, K_2SO_2-dextractable C and especially with the basal respiration rate. In contrast, application of 6 month old compost had a significant ($P \leq 0.05$) positive effect on the hot water carbon (HWC) levels amended soil. HWC was positively correlated with soil microbial biomass-C and total carbohydrates. The HWC was positively correlated with WSC and total organic C. Most of these measurements have been actively promoted as key indicators of soil quality. More research would be required under field conditions in agro-ecosystems to determine the dynamics following changes in soil use and management practices.

"BACTERIAL INTERFERENCE" - A NEW APPROACH TO TREAT RECURRENT URINARY TRACT INFECTIONS

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Recurrent urinary tract infections (recurrent UTI) affect a major portion of the population worldwide, and present a significant health challenge in industrialized countries. Acute antibiotic therapy and permanent maintenance dose antibiotic prophylaxis are traditional ways of intervention. With emergence of multiresistant pathogens also in community acquired infections these approaches do not promise an overall remedy to the issue. Therefore there is an urgent need for new approaches to struggle with this query. It is questionable if vaccination provides a reasonable alternative to the "classic" methods as it is not likely that a vaccine with protective potential covering the full range of the involved serogroups could be developed. A recent

approximation is based on the idea that a microbe resident on mucosal membranes offers a colonisation resistance against new invaders. In the recent years this approach called "bacterial interference" is the focus of studies on urinary tract infections. Escherichia coli strains isolated from asymptomatic bacteraemia (ABU) cases seem to be good candidates for eliciting this beneficial interference with real urinary pathogens. It has been shown by Zdziarski et al. (Infect. Immun., 2008) that ABU strains harbour virulence genes with mutations preventing the phenotypic appearance of these traits. Furthermore in human studies Sundén et al. (Int. J. Antib. Agents, 2006) have accounted for the preventive effect of ABU strains to a subsequent infection by a virulent strain. As safety is a pivotal requirement for bacterial interference we have performed various virulence assays in mice to evaluate the innocuous nature of the standard ABU strain E. coli 83792. In respiratory, intravenous and ascendant urinary tract models the strain proved to be entirely avirulent. These results point to the harmless nature of the stain at least in the immunocompetent host. The question remains if an incidental translocation of the ABU strain from the bladder to the tissues/circulation may develop a disease in the immunocompromised organism. Studies to elucidate the possibility of this hazard are in progress.
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CHARACTERIZATION OF BACTERIUM-KILLING MECHANISM AND EFFICIENCY OF *MEDICAGO* NCR PEPTIDES

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ISOLATION AND EXAMINATION OF MICROORGANISMS DETECTED IN INDUSTRIAL WASTEWATER SAMPLES CONTAINING HYDROCARBONS

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The worldwide usage of hydrocarbon originated products increases the importance of refinery industry and raises the necessity of the strict regulation and control of the applied processes. Refinery stations must minimize the emission of hazardous substances to ensure the protection of the environmental elements. However, effluent industrial wastewaters may contain traces of hydrocarbon compounds that can emerge in the recipient surface water. The raising issue of hydrocarbons in natural waters may be solved by the microbial community of industrial wastewater. To verify this possibility our aim was to examine hydrocarbon containing wastewater samples of three different Hungarian refinery plants with respect to the following aspects: the number of cultivatable microorganisms, the species level identification of the isolated strains and finally, the oil degradation ability of these isolates. During our work the appearance of opportunistic pathogen microorganisms was followed with particular attention. Sampling and experimental period was 2008-2009.
Colony Forming Units were determined with a simple cultivation process in solid nutrient medium to make following isolation possible. Species level identification of the isolated strains was carried out by the sequence analysis of the variable region of 16S rDNA. Hydrocarbon degrading ability of the isolated and identified strains was determined with a semi-quantitative gravimetric procedure.
Based on our results, several microbial species and genus were identified in industrial wastewater samples. Our experiments verified that some of these species can contribute to the safety elimination of hydrocarbon components due to their established capacity for degradation, such as *Acinetobacter venetianus, Microbacterium oxydans, Ochrobactrum tritici, Pseudomonas putida* or *Leucobacter iarius*. In the case of species Ochrobactrum tritici and Leucobacter iarius our investigations are the first verification of hydrocarbon utilization ability that means novel result in the field of bioremediation. Several pathogenic microorganisms such as *Pseudomonas aeruginosa* were detectable in wastewater samples with verified virulence-related features that call the attention to biological safety problems of industrial wastewaters. Based on the results, the isolated and identified, non-pathogenic strains with up to 79.3% oil degrader ability can be the basis of bioremediation technologies for the treatment of industrial wastewaters.
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TTV INFECTION OF PREGNANT WOMEN IN HUNGARY

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Although the pathological roles of TTV have not yet been clarified, high prevalence rates of TTV DNA were found in patients with haemophilia (67–75%), intravenous drug users (40%) and haemodialysis patients (46%). Recently, several reports suggested that the prevalence of TTV infection is more prevalent in patients infected HIV. The mode of transmission of TTV is not known, but the possible sources includes i.) zoonotic origin as the TTV genome has been detected in several domestic animals, including pigs, cattle, sheep, cats and dogs; ii.) contaminated shuman vaccines; iii.) enteric transmission as TT virus genes in faeces and influent as well as effluent water samples from wastewater treatment plants were detected.
To obtain a preliminary picture about the presence of TTV in Hungarian women and the viral ability to spread vertically blood samples of seven pregnant women (A-G) were examined for TTV together with the cord blood (A1-G1) of the newborns and colostrum (A2-G2). Samples were collected at Clinic of Gyneology Semmelweis University of Medicine. Blood and colostrum collection were approved by the Ethical Commitee of Semmelweis University and acceptance claims were signed by the women. Furthermore, hereby all samples are indicated by capital letters to ensure protection of privacy. DNA was extracted by standard Trizol method followed by PCR. PCR conditions and primers applied were reported by Kekarainen et al (2000). All colostrum samples proved to be negative for TTV. TTV were revealed in six blood samples of pregnant women (B-G) and cord blood samples (B2-G2). These observations may be of clinical relevance in perinatal counseling emphasising the human TTV infection is present in Hungarian population and colostrum does not play a role in vertical transmission.

GENETIC DIVERSITY OF *MONILINIA FRUCTIGENA* POPULATIONS FROM HUNGARY

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Monilinia fructigena is a troublesome fungal pathogen of stone and pome fruits in Eurasia, that produce brown rotting symptoms on infected rosaceous tissues. Morphological variances among the in vitro culture of *M. fructigena* is well known [1]. For this reason, the goal of our study was to investigate the suitability of ISSR markers for detecting genetic diversity in *M. fructigena* populations.
30 isolates of M. fructigena were gathered from various regions of Hungary (4, 4, 10, 3, 4, and 5 from Hajdú-Bihar, Békés, Szabolcs-Szatmár-Bereg counties, Mátra, North Transdanubian and Balaton regions, respectively) on pomaceous trees. Each isolate was prepared from rotted fruit and cultured on potato dextrose agar (PDA) incubated at 22°C. All isolates were identified as *M. fructigena* based on morphological characteristics and molecular features developed by fragment size of multiplex PCR products [2].
12 primers were tested previously applied to *M. fructicola* [3]. Among them, the primer $(GACA)_4$ was definitely suitable for establishing genetic diversity of *M. fructigena*. Polymorphic bands amplified by primer $(GACA)_4$ were used to genotype all isolates. The sizes and the numbers of amplified fragments ranged from 250 to 2500 base pairs and from 9 to 12, respectively. Fragments between 300 and 450 as well as at 1000 base pair showed differences among isolates. Based on 300-450bp-sized fragments, two genetically different groups were determined and within this additional subgroups could be formed with the presence or absence of about 1000bp-sized fragments. Our results confirmed that there are notable genetic diversity among *M. fructigena* population similary to *M. fructicola* [3].
The study was supported by a grant of OTKA T 78399 and a János Bolyai Fellowship awarded to IJ Holb.

INVESIGATION OF THE SUBTYPE DISTRIBUTION OF GENOGROUP 1 TORQUE TENO VIRUS IN MUCOSAL SAMPLES COLLECTED FROM THE UTERINE CERVIX AND THE HEAD AND NECK REGION

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Torque teno virus (TTV) lineages may play different roles in different diseases. Present study aims at comparing diversity of genogroup 1 TTVs in a Hungarian population at two mucosal sites, head and neck region and the uterine cervix in individuals with normal mucosa, in patients with potentially malignant (oral lichen planus, oral leukoplakia and cervical atypia) and malignant lesions (oral squamous cell, laryngeal and cervical cancers).
The N22 segment commonly used for phylogeny was directly sequenced for all genogroup 1 TTV positive samples. All previously described Hungarian TTV sequences found in the GeneBank were included for comparison. Sequences were classified into genotypes and subtypes using well-defined reference sequences.
The commonest subtypes were 2c followed by 2b, 1a and 1b. Subtypes 2b and 2c were evenly distributed among cervical samples; subtype 1a was more frequent in patients with cervical atypia or cancer. Subtypes 2c was more frequent than 2b in samples from the head and neck region. Other Hungarian sequences were also dominated by subtype 2c. Genetic diversity within subtypes was relatively low (<0.04) in case of genotype 1, but much higher in subtypes 2b (0.08) and 2c (0.15). Comparing subtype distribution to available data suggested a geographically or racially determined pattern; Far Eastern studies report dominance of subtypes 1a and 1b, while subtype 2c seems to be common in Central European populations.
In conclusion, subtype distribution may be more important than genotype distribution in association with diseases, and subtypes show variations in frequency in different geographical regions or different tissues.

DEGRADATION OF TRICHLOROETHYLENE BY *PSEUDOMONAS PUTIDA* F1

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Trichloroethylene (TCE) is a suspected carcinogen and is one of the most commonly detected volatile organic contaminants in groundwater. Considering this, research that has focused on both anaerobic reductive dechlorination and aerobic degradation via cometabolism has been undertaken in our laboratory. A number of studies have demonstrated that aliphatic and aromatic hydrocarbon-degrading bacteria, such as those that degrade phenol and/or toluene are capable of aerobic cometabolic transformation of TCE to readily degradable oxygenated compounds. Under anaerobic conditions, PCE (tetrachloroethylene) and TCE have been reductively dechlorinated by mixed cultures to less-chlorinated ethenes and, under certain conditions, to the nontoxic products ethene and ethane. Under aerobic conditions, PCE is considered nonbiodegradable, while TCE can be broken down to mainly nontoxic products by certain nonspecific oxygenases, such as methane monooxygenase or toluene dioxygenase.
The primary aim of the present study was to investigate the toluene dioxygenase system of the *Pseudomonas putida* F1 bacterial strain responsible for the high TCE-degrading activity in the chemostat enrichment. Additionally, we have examined the ability of bacterial cells grown in the presence of different concentrations of toluene in fluid medium.
The research was supported by the EU and co-financed by the European Social Fund through the Social Renewal Operational Programme under the project CHEMIKUT (TÁMOP-4.2.2-08/1-2008-0012). The financial support of TEVA Hungary Ltd. is also highly appreciated.

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

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Lactose is intracellularly hydrolysed by *Aspergillus nidulans*. Classical mutation mapping data and the physical characteristics of the previously purified glycosyl hydrolase enabled the in silico identification of clustered, divergently transcribed intracellular beta-galactosidase (bglD) and putative lactose permease (lapA) genes. Phylogenic studies refuted the widespread misbelief that only lactose-utilising yeasts (a.o. *Kluyveromyces lactis*) are sources of beta-galactosidase with a neutral pH-optimum.
bglD and lapA were perfectly co-expressed in response to D-galactose, lactose or (at lower levels) to L-arabinose. However, no transcript was detectable in the co-presence of glucose and either of the above inducers. Interestingly, transcript analyses in CreA mutants showed derepression of both genes to a basal level under all non-inducing growth conditions, including on glucose, while full induction only occurred on lactose and galactose in the absence of glucose, indicating a prominent role for inducer exclusion in the system's regulation.
To confirm lactose permease function, the lapA gene was deleted. Unexpectedly, disruptants were still able to grow on liquid lactose medium, albeit at a much lower rate than wild type controls, and behaved normally on galactose. The effect of lapA deletion was exacerbated at lower lactose concentrations, suggesting the existence of a second uptake component of lower affinity.

CONIDIOSPORES OF *ASPERGILLUS NIGER* ARE UNABLE TO GERMINATE ON D-GALACTOSE

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The majority of Black Aspergilli (*Aspergillus Section Nigri*), including *Aspergillus niger*, as well as many other Ascomycetes fail to grow on D-galactose as a sole carbon source. The phenomenon was proposed to be the consequence of a missing D-galactose phosphorylating activity. In this study we provide evidence that contrary to this suggestion, *A. niger* does contain a galactokinase homologue which is expressed on D-galactose, and cell-free extracts of *A. niger* are able to phosphorylate this sugar. We furthermore show that although *A. niger* mycelia transport and subsequently grow on D-galactose, conidiospores are unable to germinate on it. In agreement with these observations, we demonstrate the presence of an intact Leloir-pathway that is responsible for channelling D-galactose into the glycolytic pathway in *A. niger*, and show that these genes are expressed on D-galactose (and also on lactose, D-xylose and L-arabinose) both in the mycelial and the conidiosporal stage. We conclude that the inability of *A. niger* to grow on D-galactose is restricted to the conidiospores, and that this phenomenon is not due to the impairment of the Leloir-pathway.
The project was supported by the Hungarian Scientific Research Fund (OTKA K67667) and the National Office for Research and Technology (NKTH A2-2006-0017). EF is supported by a Bólyai János Research Scholarship (BO/00519/09/8).

HEAT-LABILE ENTEROTOXIN OF ENTEROTOXIGENIC *ESCHERICHIA COLI* INCREASES THE ADHESION ABILITY TO INTESTINAL EPITHELIAL CELLS

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Enterotoxigenic *Escherichia coli* (ETEC) is an important diarrhogenic pathogen of both the farm animals and humans. The heat-labile (LT) enterotoxin produced by ETEC not only stimulates host diarrhea, but has been shown to promote the colonization of small-intestine by adhesin producing ETEC strains. To acquire quantitative data on how the LT toxin increases the adhesion to intestinal epithelial cells we used the derivatives of the K88ac adhesin producing E. coli strain 1836-2: 8035 contains the intact LT operon cloned into pBR322;

8221 contains the LT operon with an R192G mutation in the A subunit also in pBR322; and 8589 contains only the cloned LTb subunit in pBR322. Strain 8017 served as toxin negative control. Adhesion assays were performed on cells of the IPEC-J2 intestinal epithelial cell line. We further assessed whether host protein synthesis or other biological activities were essential to LT-enhanced bacterial adherence by cyclohemide treatment of IPEC-J2 cells prior to performing adhesion assays. We found that the strains expressing the intact toxin, a non-toxigenic mutant of LT or the toxin's B subunit (8035, 8221, 8589) each were able to induce significantly higher adhesion when comparing to non-toxigenic adhesin expressing or non-expressing strains (8017, G58-1). We did not find significant differences in adhesion to metabolically active as opposed to cells whose protein synthesis was blocked by 25ug/ml cycloheximide. This suggests that LT enhancement of adhesion is dependent on the LTb subunit and is not appreciably affected by host cell activity.

CHARACTERIZATION OF MICROSCOPIC EUKARYOTE ASSEMBLAGES WITH GROUP-SPECIFIC INVESTIGATIONS IN LAKE FERTŐ (NEUSIEDLER SEE, AUSTRIA/HUNGARY)

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Lake Fertő is the westernmost and largest steppe lake in Eurasia. Significant part of this shallow, alkaline, meso-eutrophic lake is covered by reed, but there are numerous reedless inner ponds of variable size within the reed belt, which is enmeshed with canals connecting the inner ponds with the open water areas.
In this study, samples were taken from three different water types: from a turbid open-water area, from a brown-water inner pond and from a small pond near the lake. We applied epifluorescence microscopy for the detection and enumeration of planktonic eukaryotic picoalgae. Taxon-specific PCR-DGGEs (denaturing gradient gel electrophoresis) were performed for the characterization of the groups *Chlorophyta, Cercozoa* and *Ciliophora* based on the 18S ribosomal RNA gene. Microscopic investigations showed that there are significant differences among the samples, e.g. the lack of eukaryotic picoalgae in the open water area. Molecular fingerprints have also revealed the temporal and spatial clusters of samples. Sequence analysis of excised DGGE bands confirmed the presence of various picoeukaryotic algal genera (*Choricystis*, *Chloroparva*) and several members of *Ciliata* and *Cercozoa* in Lake Fertő.

FUNCTIONAL ANALSIS OF HPV 31 LCR (LONG CONTROL REGION) SEQUENCE VARIATION

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Cervical cancer, caused by infection with high-risk human papillomavirus (HPV) type 16, 18 and 31, is a major public health problem in Hungary. It is well known that the genomes of HPV types differ geographycally. We studied the functional consequences of genomic sequences variation within the LCR (long control region) of HPV type 31. HPV 31 LCR contains a promoter which contains binding sites for cellular factors (such as SP1 and TFIID) as well as binding sites for viral factors (E1, E2). This region also contains elements for the replication and episomal maintenance of the viral genome. We worked with clinical samples from women who had colposcopical and or cytological atypia in the cervix. First we amplified a part of the HPV 31 LCR (nt 7381-7814) with PCR from 43 clinical samples. To study the point mutations in the LCR fragments, we used SSCP (single strand conformational polymorphism) analysis. According to the SSCP patterns of the LCR fragments, seven different variants were selected for further analysis. The whole LCR of HPV type 31, was amplified from these samples and cloned into pGL2 basic luciferase reporter vector for sequence analysis and functional studies. The DNA sequence of our variants were compared to variants published in the literature. In order to study the transcriptional activity of the different HPV 31 LCR variants, the luciferase constructs are transfected into HeLa cells and luciferase tests are performed.

EVALUATION OF E-TEST METHOD FOR DETERMINING MICAFUNGIN MICS FOR 360 CLINICAL ISOLATES OF 10 *CANDIDA* SPECIES

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Micafungin (MICA) is an echinocandin with potent activity against yeasts. However, the utility of MICA Etest has not yet been determined against a larger number of *Candida* clinical isolates. MICA was tested in vitro against 100 *Candida albicans*, 66 *C. glabrata*, 40 *C. tropicalis*, 42 *C. krusei*, 54 *C. parapsilosis*, 15 *C. inconspicua*, 15 *C. lusitaniae* 11 *C. kefyr*, 7 *C. guilliermondii* and 10 *C. dubliniensis* isolates using CLSI broth microdilution method (BMD). As some *Candida* species frequently shows paradoxical growth (PG; growth of *Candida* species in the presence of low MICA concentrations, no growth at intermediate concentrations, and growth again at high MICA concentrations) with MICA; the occurrence of PG was also evaluated. The Etest results correlated well with reference MICs. After 24 h, agreement within ± two dilutions was 96-100% for all *Candida* species, with the exceptions of *C. kefyr* (81%) and *C. lusitaniae* (87%). After 48 h, agreement increased to 98-100 % for all species. In the BMD method PG was noted in 17% (17/100) and 63% (25/40) of *C. albicans* and *C. tropicalis* strains, respectively; but not in any other tested species. Using Etest, the PG detected by BMD was seen in case of seven out of the 17 C. albicans and in case of all 25 *C. tropicalis* isolates.
Etest proved to be a useful method not only for MIC testing, but to detect paradoxical growth for MICA against *Candida* clinical isolates.
This study was supported by Richter Gedeon Nyrt.

HIGH-LEVEL RESISTANCE TO CIPROFLOXACINE COMPROMISES VIABILITY AND POSSIBLY ALSO CAPACITY OF DISSEMINATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

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Though clonal shifts of MRSA have been common in recent years accross Europe the background of strain replacement remains obscure. Data collected from a Hungarian hospital suggest that resistance of MRSA to non-beta-lactam antibiotics could impact the dissemination of the isolates. The effect of fluoroquinolone resistance on the viability of individual MRSA isolates has been investigated. Community-acquired MRSA strains susceptible to ciprofloxacine (MIC kisebb egyenlő 0.16 mg/L) were turned resistant by exposure to ciprofloxacine then changes in growth rates have been determined spectrofphotometrically. MRSA isolates with high-level resistance to ciprofloxacine (MIC nagyobb egyenlő 32 mg/L) showed a significant slowdown in growth rates that could compromise their ability to disseminate by colonizing patients. The identity of the ciprofloxacine susceptible and resistant isolates was confirmed by pulsed-field gel electrophoresis (PFGE). PFGE also detected mutations in the resistant isolates the determination of which is in progress. Authors thank the Department of Bacteriology, National Center for Epidemiology for kindly providing some of the isolates.

HOST-PATHOGEN INTERACTION: VIRULENCE AND PATHOGENESIS OF THE EMERGING HUMAN FUNGAL PATHOGEN *CANDIDA PARAPSILOSIS*

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LACK OF GENETIC OR EPIGENETIC ALTERATIONS IN THE BACKGROUND OF INITIAL REPONSE TO CIDOFOVIR AND LATER RELAPSE IN A SEVERE JUVENILE RECURRENT RESPIRATORY PAPILLOMATOSIS CAUSED BY HPV11

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A patient with severe juvenile recurrent respiratory papillomatosis characterized by palatal spread and high relapse rates (31 surgical interventions within nine years) was treated with intralesional cidofovir instillation multiple times. Clinical and virological status was monitored for five years prior to cidofovir treatment, throughout the therapy and for one year after cessation of cidofovir administration. Three HPV11 isolates, one detected prior to, one during the initial good response to and one after the failure of the cidofovir regimen were sequenced entirely using direct sequencing of both strands by the ABI BigDye terminator v3.1 Cycle kit in an ABI 3100 Genetic Analyzer. CpG methylation of the long control region (LCR) was examined by sequencing the whole LCR after bisulfite modification.
All three whole genomes were identical; no single nucleotide polymorphisms, insertions or deletions were detected. The genomes were closest to Slovenian HPV11s sequenced from laryngeal papillomatosis samples (Gene Bank accession numbers FN907975 and FN907964), but carried unique polymorphisms/mutations in the E5 and L1 ORFs, however, these did not result in amino acid replacements. In the LCR two unique nucleotide changes were identified at positions 7480 and 7902. The latter is immediately next to the E2 binding site of the LCR. All CpG islands were non-methylated in all three HPV11 genomes. Based on these data, the late therapeutic failure with cidofovir was not caused by genetic or epigenetic alterations in the virus. The unique nucleotides found in the LCR may play a role in the extreme aggressivity of this HPV11 strain.

FOODBORNE HUMAN PARASITOSES IN HUNGARY: DIAGNOSTIC TECHNIQUES AT THE DEPARTMENT OF PARASITOLOGY, RESULTS FROM THE LAST DECADE

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Foodborne human parasitoses came to the front in the last decade as, in consequence of the effects of globalization (the number of travelers and importation of exotic foods is increasing and sanitation practices vary worldwide) pathogens of foodborne illness less common in the particular geographical area are beginning to be seen. This is the reason why public health concerns must include events happening all around the world. The aim of our study was to examine whether the Department of Parasitology of the National Center for Epidemiology (NCE) has the diagnostic capacity to detect all the foodborne human parasitoses caused by the pathogens listed among the most important emerging foodborne parasites (Dorny et al, 2009) or the foodborne zoonotic pathogens grouped in the "group of significant importance" (Cardoen et al, 2009). A further aim of our study was to examine whether the pathogens in point did occur in Hungary in the last decade (2000-2009) or not. For this reason, the results by the laboratories of the National Public Health and the Medical Officer's Service (NPHMOS) were reviewed and are presented here. The international scientific literature was reviewed by searching the "PubMed" electronic bio-medical database for important foodborne parasites. The relevant Hungarian results from the last decade were collected from the annual reports by the laboratories of the NPHMOS. The Department of Parasitology (NCE) has the diagnostic capacity to detect all the foodborne human parasitoses caused by the pathogens listed among the most important emerging foodborne parasites or the foodborn zoonotic pathogens grouped in the "group of significant importance". Eight of the 26 pathogens in point were detected in Hungary in the study period. As it was documented in the annuals, there was one human case of diphyllobothriosis, dozens of cases caused by *Cryptosporidium parvum*, the *Taenia*, and the *Trichinella* species, although the *Trichinella* species were not grouped in the "group of significant importance" by Cardoen et al. There were more hundred cases of echinococcosis detected, and more thousands of laboratory confirmed cases caused by *Giardia lamblia* or *Toxoplasma gondii*. All of these infections are known as waterborne or are of zoonotic origin. *A. lumbricoides* was not listed among the most important emerging foodborne parasites by Dorny et al, although the classical route of transmission for ascariosis is the ingestion of contaminated vegetables or fruits. More hundred human cases of ascariosis were detected in Hungary in the last decade.
As the source of foodborne parasitic infections are usually the unwashed or improperly washed vegetables or

fruits, raw or improperly processed meat or meat products, foodborne parasitoses can be prevented by avoidance of the use of raw or improperly composted manure or irrigation water containing untreated sewage, controlling rules by processing foods, and education of people.

SCREENING FOR NEW TAXA FROM ACTINOMYCETE STRAIN COLLECTION BUILT IN THE EARLY SEVENTIES

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Actinomycetes are one of the most significant prokaryote groups from a biotechnology aspect, since they are used as industrial sources of enzymes (hydrolases, transferases, esterases etc.). Moreover, they represent an important group for the pharmaceutical industry, as they produce a wide spectrum of antibiotics, as well as antifungal and antiviral substances. Thus, isolation and identification of new actinomycete strains, and their screening for considerable industrial properties have a great importance nowadays. Our aim was the molecular taxonomic classification of the actinomycete strain collection of Agruniver Holding Ltd. formed till the beginning of 1970. We had limited information about the original strain collection as well as the freeze dried microbe strains. Out of the strain collection that makes up circa 400 items, we have already recultured and identified hundred strains by molecular methods on the base of their 16S rDNA sequences analyses. In the PCR reaction 27f and 1492r universal primers were used. Sequencing was performed with an ABI Prism 310 device, and leBIBI software parallel with Blast analysis were used for sequence based identification. On the base of our findings, the major part (80%) of the identified strains belongs to the Streptomyces genus. Besides actinomycetes, one *Ureibacillus* and two *Thermoactinomyces* species were also identified. In this study, we report the description of a new species candidate *Streptomyces* strain. Strain K23 shared up to 97% similarity with sequences of its closest relative type strains of *S. griseochromogenes* and *S. canarius* (97% homology). Interestingly, strain K23 shared higher than 99% homology with *S. viridochromogenes* JCM 4437. Based on detailed electron microscopic observations of spore morphology and the results of molecular taxonomic investigations (cell wall-, quinone analysis, API 50CH, API ZYM), in which *S. viridochromogenes* DSMZ40110 type strain, JCM 4437 and strain K23 were compared, we discovered that *S. viridochromogenes* JCM 4437 strain was originally misidentified by phenotypic methods in the 1960s. DNA–DNA homology values between strain K23 and JCM 4437 was higher than 80 %, while relatedness between strain K23 and *S. viridochromogenes* type strain (DSMZ) was only 65%. Our results warranted the proposal of strain K23 as the new species *Streptomyces küsteri* sp. nov, parallel reclassification of *S. viridochromogenes* JCM 4437, which also would be placed in this new taxon.

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CONVERSION OF CELLULOSE CONTAINING PLANT BIOMASS TO HYDROGEN BY *CALDICELLULOSIRUPTOR SACCHAROLYTICUS*

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Our developed civilization has enormous energy demand; the fossil fuels are used much faster than their formation rate, consequently, their depletion is expected within couple of decades. With decrease of the crude oil and the natural gas resources, attention increasingly turns to renewable energy sources and alternative energy carriers. Hydrogen is a promising, clean, alternative energy carrier. During its combustion, the greenhouse gas CO_2 is not released. Biohydrogen can be generated in light-dependent and in light-independent – dark fermentative - processes. Many studies have described approaches, technologies for fermentative hydrogen production from enzymatically pretreated lignocellulosic biomass, but only few papers discussed the possibility of hydrogen evolution from untreated cellulosic waste. The main objective of this work is the development of a single step procedure for bioconversion of unpretreated filter paper to hydrogen.. For this pupose, a strain having cellulose degrading and hydrogen producing capacity is necessary. The Gram positive, extremely thermophilic, anaerobic *Caldicellulosiruptor saccharolyticus*. growing at 60-70^{o}C, is capable to use various monomeric and

polymeric sugars, such as cellulose. Furthermore, during its fermentative processes it produces acetate, lactate, CO_2 and - most importantly - hydrogen, therefore it seemed to be a good choice for our purposes.
In this study, we tested whether *C. saccharolitycus* can efficiently convert untreated papers to biomas and – if so - how this process can be further improved. We used various sugars as stimulators of the bioconversion. As expected, *C. saccharolitycus* consumed the monomeric sugars within the first few days of growth, then the bacterium started to utilize the untreated filter paper. From the sugar specificity, it is likely that some of these monomeric sugars could stimulate the cellulolytic activity of the cells, thus paper decomposition can be promoted by sugars. In parallel, we monitored the hydrogen evolution from sugars and filter paper alone and in various combinations and it has been clearly demonstrated that *C. saccharolyticus* was able to produce significant amount of hydrogen from untreated cellulolosic biomass, namely paper.

ASSOCIATION OF TYPE III LATENCY OF EPSTEIN-BARR VIRUS WITH APICAL PERIODONTITIS LESIONS

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Apical periodontitis is a polymicrobial inflammation possibly caused by a co-infection of opportunistic endodontic bacteria and herpesviruses, particularly Epstein-Barr virus (EBV). The aims of this study were to determine the presence of EBV in apical periodontitis in an eastern Hungarian population and to analyze the association with clinical symptoms and patient history. Fourty samples with apical periodontitis and fourty controls were included in the study. EBV DNA was detected by nested polymerase chain reaction (PCR) and EBNA2 mRNA type III latent infection was detected by reverse transcription PCR. EBV DNA and EBNA2 mRNA were found in patients with apical periodontitis at significantly higher levels compared to controls (72.5% vs 2.5, $p<0.0001$ and 50% vs 2.5%, $p<0.0001$, respectively). Symptomatic cases had a higher incidence of EBV DNA (82%) and EBNA2 mRNA (71%) versus asymptomatic cases (65% and 35%, respectively). In specimens with radiographic bone destruction more or equal to 5 mm, a higher incidence of EBV DNA (91%) and EBNA2 mRNA (76%) were found compared to cases with bone destruction less than 5 mm (53% and 21%, respectively). These results suggest that EBV should be considered as a relevant factor in the pathogenesis of apical periodontitis and is mostly associated with symptomatic and large sized periapical lesions.

STRESS RESPONSE AND SECONDARY METABOLITE PRODUCTION IN FUNGI

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Fungi continuously adjust their cellular physiology to adapt to abiotic stress factors, such as extreme temperature and pH, oxidative and osmotic disturbances, nutrient depletion, ultraviolet irradiation or sub-lethal concentrations of xenobiotics in the ecologically diverse habitat, where they live, thrive and propagate. At molecular level, fungi sense and transduce the stresses by (i) the HOG-(**h**igh **o**smolarity **g**lycerol) type MAPK pathway, (ii) the Slt2-type MAPK dependent and MAPK independent CWIS (**c**ell **w**all **i**ntegrity **s**ignaling) pathway and the (iii) adenylyl cyclase – protein kinase A (cAMP-PKA) pathway. After activation of these signal transduction pathways by diverse stress factors, the phosphorylation cascade phosphorylate a number of substrates including transcription factors that in turn cause changes in gene expression patterns and subsequent protein synthesis. Besides well studied examples of stress adaptation, lesser known side effects of the stress response can be alterations in secondary metabolite profiles and increased drug tolerance. Research at the Mycology Group of HAS–SZIU has shown that disruption of selected elements of the signal transduction pathways mentioned above may lead to drastic changes in secondary metabolite production of toxigenic species of *Fusarium*. Deletion of the HOG1 orthologue in *F. proliferatum* resulted in increased sensitivity towards a range of abiotic stressors. The ΔFphog1 mutants showed enhanced sensitivity to N-starvation stress paralleled with increases in FUM (fumonisin) gene expression and fumonisin B1 production. ΔFpacy1 mutants of the same fungus species obtained by targeted gene disruption of the adenylyl cyclase gene produced high amounts of bikaverin, another polyketide derivative, whereas only traces of this metabolite was detected in the wild type. The response of such mutants to stress factors created by an artificial environment that prevails during industrial fermentation has practical implications.
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CELL LENGTH GROWTH PATTERNS IN THE FISSION YEAST CELL CYCLE

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In the first half of the 20th century yeasts have become model organisms in cell cycle studies. The cylindrically shaped fission yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. Length growth patterns may therefore indicate connections between volume changes and cell cycle events. The classical method to study the growth of individual cells is time-lapse microphotography; cells are growing on the surface of an agar pad in a thermostated photomicroscope, and later on the growth pattern of cell length can be simply studied by a projector. A size control acts in every cell cycle to keep the size of the cells constant. With further analyses of growth patterns we are able to estimate the position of this size control.
In different cell types, there is considerable controversy concerning the exact growth profile of size parameters during the cell cycle. Linear, exponential and bilinear (i.e., two linear segments with a rate change point (RCP)) models are commonly considered, and the same model may not apply for all species. Selection of the most adequate model to describe a given data-set requires the use of quantitative model selection criteria, like Akaike Information Criterion (AIC), which are suitable for comparing differently parameterised models. A linearised biexponential model (called bilinear) was applied, which makes possible a smooth, continuously differentiable transition between two linear segments. Essentially all the quantitative selection criteria considered here indicated that the bilinear model was somewhat more adequate than the exponential one for fitting the fission yeast cell data. We have analysed the length growth pattern of 60 fission yeast cells from each strain (wild type, wee1Δ mutant, cdc2-3w cdc25Δ double mutant and cdc2-3w cdc25Δ pyp3Δ triple mutant). The above mentioned model selection criteria were used for discriminating among linear, exponential and bilinear models and selecting the most adequate one in the case of all these cells' length growth patterns. Although relatively small differences were found in several cases, essentially all the quantitative selection criteria considered here indicated that the bilinear model was generally more adequate than either the exponential or the linear ones. In the case of wild type and wee1Δ mutant cultures, more than 2/3 of the cells showed a bilinear pattern, while in the case of cdc2-3w cdc25Δ double and cdc2-3w cdc25Δ pyp3Δ triple mutants, the ratio of bilinear cells was slightly above 50%. “Average cells” were also constructed from all the individual cells' data for all four strains, whose patterns were definitely found to be bilinear by any criterion used. In wild type and wee1Δ mutant cells, size control seems to act before the RCP, while in cdc2-3w cdc25Δ double mutant and cdc2-3w cdc25Δ pyp3Δ triple mutant, size control seems to act after the RCP. In the case of bilinear patterns, the slopes of the two linear segments were also compared with t-test (Statistica® software, Homogeneity of slopes function).
This project is supported by the Hungarian Scientific Research Fund (OTKA K-76229).

PATULIN-INDUCED PLASMA MEMBRANE FLUIDIZATION AND OXIDATIVE STRESS INDUCTION IN *SCHIZOSACCHAROMYCES POMBE*

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Patulin is the toxic secondary metabolite of the different species of *Penicillium, Byssochlamys* and *Aspergillus*. Previously, we demonstrated that patulin fluidized the membrane of *S. pombe* cells, and the altered membrane structure increased the efflux of 260 nm absorbing substances on a concentration dependent manner [Horváth et al., Food and Chem. Tox., 2010 48; 1898-1904].
The oxidative stress generating effect of patulin was also investigated in *S. pombe* cells. In case of the examination of reactive oxigen species, a 60 minute 500 and 1000 μM patulin treatment induced significant increment of intracellular superoxide and peroxide concentration. 60 min 500 μM and 1000 μM patulin treatment caused a 2.4 fold and 6.3 fold increase in intracellular peroxide concentration, respectively, while the superoxide concentration in case of 500 and 1000 μM patulin increased 1.98 fold and 2.38 fold, respectively. 500 μM patulin treatment for 60 min caused significant increment in the specific enzyme activity of Cu/Zn SOD, CAT, GST and decrement in the intracellular concentration of GSH. The oxidative stress induction effect of patulin was also tested on MAPK signal-transduction mutants of *S. pombe*. Sty1 and pap1 mutants of *S. pombe* strains turned out to be sensitive against patulin. mRNA expressions of pap1 and sty1 were measured by quantitative real time PCR and oxidative stress response was studied in RNA expression level.

DEVELOPING OF SCFV LIBRARY TO PURIFY PROTEIN COMPLEXES FROM *THERMOPLASMA ACIDOPHILUM*

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We are developing an antibody library based protein purification method for structural and functional analysis of protein complexes of the thermoacidophilic archaeon *Thermoplasma acidophilum*. This method is based on affinity selection of antibodies produced by the phage display technology (PDT), and our target antibodies specifically recognize native protein complexes. In the PDT the single chain fragment variable (scFv) of the IgG is fused to the phage minor coat protein and expressed by the pCANTAB 5E phagemid vector in *E. coli* TG1 strain. In the presence of M13K07 helper phage the scFv can be incorporated into the capsid particle during phage assembly. Phages displaying specific scFv antibodies against T.acidophilum proteins were selected by biopanning and used to infect TG1 cells to create monoclonal *E. coli* cell lines. The specificity of the selected scFv clones were tested in monoclonal ELISA and Western-blot assays against high molecular weight proteins of T. acidophilum. In the next step selected phages were used to capture protein complexes, however our phage based pull down experiments didn't result sufficient amount of target proteins, because the ratio of the displayed scFvs is proportional to major coat proteins which influenced the binding capacity of the system. To overcome of this hurdle target scFvs were re-cloned and expressed in a modified pET28 expression vector to obtain larger amount soluble antibodies in E.coli cells. We established an elegant purification method to capture native protein complexes by mixing and incubating *E. coli* cell extract containing His-tagged scFvs with *T. acidophilum* cell extract followed by NiNTA chromatography. With an additional separation step on Superose 6 column ~98 % protein purity could be obtained, which was suitable for electron-microscopic single particle analysis in cases of proteasome and thermosome complexes. The pET28 based scFv expression system worked well, however there were examples when His-tagged scFvs couldn't be expressed. To solve this problem we have developed a new pCANTAB vector, in which we designed a His-tag and a TEV-protease cleavage site to be able to cut off the scFv from the phage particle and purify it by affinity chromatography. To further fine tune the system we introduced a library clean-up step to reduce the over-representation of phages/antibodies against known and abundant protein complexes. Applying thermosome and proteasome specific biopanning and ELISA assays over 90 % of the clones were excluded from the library enriching phages against other targets. We demonstrated the viability of our purification technology on the example of two well-studied complexes, and in the future we will use phages/antibodies of the enriched PDT library to capture and characterize less abundant and unknown protein complexes.

CONFORMATIONAL ANALYSIS OF ANTIMICROBIAL PEPTIDE PALINDROMES CONTAINING PROLINE RESIDUES

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Several antimicrobial peptides (AMPs) possess a characteristic feature with regard to their sequences, namely they contain palindromic segments with various lengths. In the case of these AMPs, the palindromes can play an important role not only in the determination of their three-dimensional (3D) structure but also in their biological effects. Among these AMPs, indolicidin, tritrpticin and metalnikowins are the representatives of palindromic peptides containing proline amino acids. Indolicidin (H-ILPWKWPWWPWRR-NH_2) was first purified from the cytoplasmic granules of bovine neutrophils, while tritrpticin (H-VRRFPWWWPFLRR-OH) was identified in the porcine bone marrow, and finally metalnikowin IIA (H-VDKPDYRPRPWPRPN-OH) and metalnikowin III (H-VDKPDYRPRPWPRPNM-OH) were isolated from the hemipteran insect *Palomena prasina*. In the case of the palindromic segments of these AMPs, a detailed conformational analysis was performed applying the simulated annealing (SA) method, in order to characterize their structural and conformational features. Since the above-mentioned palindromes contain Pro amino acids, different stereoisomeric forms could be distinguished, according to the cis-trans isomerism of Xaa-Pro peptide bonds. Thus, the four stereoisomers of the palindromic segments of indolicidin and tritrpticin, as well as the eight stereoisomers of the palindromic segments of metalnikowins IIA and III were modeled, respectively. For the conformers obtained from the SA simulations, the presence of different secondary structural elements was investigated, and various types of β-turn structures (i.e.

types I, III, VIa1, VIa2 and VIb β-turns) were determined in certain tetrapeptide units of the stereoisomers. Furthermore, short helical regions were also detected in the case of peptide sequences containing trans Xaa-Pro peptide bonds, such as 3_{10}-helices for the palindrome of indolicidin, and poly-proline II helices for the palindromes of all peptides. Nevertheless, various intramolecular interactions (i.e. H-bonds, aromatic-aromatic and proline-aromatic interplays) were identified, which played a relevant role in the structural stabilization of different conformations. Among them, the i←i+3 H-bonds formed between the backbone atoms contributed to the structural stability of types I and III β-turn structures, while the proline-aromatic interactions played an important stabilizing role in the case of type VI β-turns and cis Xaa-Pro peptide bonds.
On the whole, our results pointed out that the stereoisomeric forms of the palindromes of indolicidin, tritrpticin and metalnikowins showed characteristic structural and conformational properties, regarding their secondary structures and intramolecular interactions.
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EXAMINATION THE EFFECT OF PREBIOTIC COMPOUNDS ON PROBIOTIC BACTERIA REPRODUCTION

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Today is flourishing probiotic products and their effects on the body but the details are still unclear. Although many tests confirmed that some gastrointestinal diseases exert a beneficial effect of probiotic preparations in a recent series of experiments conducted on the therapeutic use of probiotic bacteria has highlighted the risk.
The direct application of probiotic bacteria while increasing attention is given to the compounds so-called prebiotics, which help keep the intestinal flora in balance. The role of the prebiotics 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 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.
One of the most thoroughly studied compounds in the prebiotic effect is inulin. The probiotic bacteria of inulin and its derivatives (in particular, and to a lesser extent, the *Bifidobacterium* genus of *Lactobacillus* species) effect in stimulating bacterial growth is manifested. The aim of our experiments were to determine whether different, presumably prebiotic compounds what kind of effects have on probiotic bacteria. Stimulate or reduce the reproductive rate, or indifferent to the effect of the examined strains. In our research we examine the effect of prebiotic compounds, such as inulin, resistant starch. *Bifidobacterium* and *Lactobacillus* bacteria strains were used for pure and mixed cultures. The results suggested that the addition of inulin stimulated the growth of bacteria, which resulted in an improved viability of these organisms.
The research was financed by the NKTH, NFÜ research programme.

IDENTIFICATION OF *LACTOBACILLUS* AND *BIFIDOBACTERIUM* SPECIES FROM HUMAN ORIGIN BY HIGH RESOLUTION MELT CURVE ANALYSIS

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Lactobacillus and bifidobacterial cultures are increasingly used as probiotics in pharmaceuticals and in foods. *Lactobacillus* and *Bifidobacterium* are shown to be of benefit in maintaining good health. Regular consumption of live cultures improve the gut microflora and reduces the number of bowel infections by reducing unwanted bacteria. 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 *Lactobacillus* and *Bifidobacterium* species, isolated from human fecal and breast milk samples, by the use of the elongation factor gene F-tot/R-tot primers. After the Real-time PCR the products were submitted to HRM analysis and the melting curves were compared.
DNA was extracted from the human samples and the reference

GENE EXPRESSION ANALYSIS OF DIFFERENT *SALMONELLA* SEROVARS INSIDE AVIAN MACROPHAGES

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Salmonella enterica usually infects animals and humans by the oral route. To induce gastroenteritis, the pathogen must reach the distal ileum, penetrate the mucosal epithelium and survive in the phagocytic cells of the host. Survival and replication inside phagosomes is crucial for successful systemic colonisation. Adaptation to this harsh environment requires complex and coordinated changes in bacterial gene expression that was extensively studied in the last decades. To explore the possible differences four representatives of the most frequently isolated invasive and non-invasive food poisoning serovars have been tested by microarray.
Bacterial gene transcription of *Salmonella Typhimurium*, *S. Enteritidis*, *S. Infantis* and *S. Hadar* strains at the genome level has been studied using DNA microarray. As an infection model for antigen-presenting cells avian macrophage cells have been used. Infected macrophages were differentially lysed. Bacterial RNA was extracted, amplified, labelled and hybridised to the whole genome gene expression microarray slides carrying serovar specific DNA probes. Extensive changes in gene expression profile of Salmonella Enteritidis isolated from HD11 macrophages indicate broad scale metabolic adaptation to the micro-environmental conditions to which the bacteria are exposed in vivo. The most characteristic metabolic changes enabled the remarkable overexpression of magnesium and phosphate uptake systems, indicating the limiting role of these elements. There were no signs of carbon-, nitrogen- or energy starvation, suggesting these factors are relatively easily available intracellularly. Overexpression of non-specific efflux systems and SOS response regulators could be observed, due to the reactive nitrogen and oxygen species produced by the host macrophages. Unlike the above functional groups, the vast majority of the surface antigens – including certain OMPs, chemotaxis proteins and flagellar compounds – were strongly repressed.

METAL BIOAVAILABILITY, CHANGES IN ENZYMATIC ACTIVITIES AND MICROBIAL PROPERTIES AFTER BIOREMEDIATION OF A HEAVY METAL-CONTAMINATED SOIL

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The bioavailability and toxicity of metal are very difficult to predict and there is no agreement on a single procedure to document such properties for metal. The total metal concentration in soil is generally acknowledged as the best approach whereby to understand the degree to which a soil may be contaminated or pose a health risk to humans. This approach requires comparing metal concentrations at a site in question to similar non-contaminated soils and will vary by soil type. Bioremediation technique of heavy metal-contaminated soil is based on the extraction or the stabilization of the contaminants. Soils can naturally reduce mobility and bioavailability of heavy metals as they are retained in soil by sorption, precipitation and complexation reactions. This bioremediation process can be accelerated by the addition of amendments. Nevertheless, microorganisms respond quickly to changes and can rapidly adapt to environmental conditions. Changes in microbial populations or their activities can precede detectable changes in soil physical and chemical properties, providing an early sign of soil improvement or an early warning of soil degradation. Microbial and biochemical parameters such as microbial biomass carbon, heterotrophic bacterial count, dehydrogenase, β-glucosidase, aryl-sulphatase, and urease activities, together with several chemical properties such as pH, $CaCl_2$ soluble heavy metals concentrations, total organic carbon and water soluble carbon were measured to evaluate changes in soil quality, after remediation of a heavy metal-contaminated clay loam brown forest soil.

The experiment was carried out using pots, filled with heavy metals (Cu, Ni and Zn) contaminated soil by 0, 20, 40, 80, 160 and 320 mg/kg soil in the presence of one month early wetted uncomposted barley plant residue mixed with soil at 20% (w/w). Unamended soil was used as control. Barley (*Hordeum vulgare* L.) was sown in the pots of 45% moisture content. The soil was sampled twice: one month and six months after amendment application. Results indicated that the higher amendments significantly affected the soil chemical properties: soil pH, total organic carbon and water soluble carbon decreased in the amended soils, while soluble heavy metal concentrations diminished. At the same time, higher MBC, enzymatic activities were found in the organically amended soils. Barley plant cover was important in restoring the soil chemical and microbial properties in all the soil samples, but mainly in those that were not amended organically.
Bioremediation measures improved soil quality in the contaminated soils. The bioremediation technique used improved soil chemical and biological conditions, amendments contributed to reduce heavy metal bioavailability, increase soil pH, and restore soil microbiological properties. Although this low cost bioremediation technique seem very suitable for restoration of extensive areas contaminated by heavy metals, further monitoring is necessary to prevent reverse effects of the amendments used.

PROTECTION OF QUAILS AGAINST HIGHLY PATHOGENIC H5 AVIAN INFLUENZA VIRUS WITH WHOLE, INACTIVATED NIBRG-15 VIRUS STRAIN CONTAINING, ALUMINIUM ADJUVATED VACCINE

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SAN quails were housed in a temperature-controlled environment with 12-h light:12-h dark cycles; food and water were delivered ad libitum. Vaccine and adjuvant: The vaccine contain whole formaldehide inactivated NIBRG-14 vírus. Active ingredient concentration was 7,5 mikrogram HA/ 0,1 ml with aluminium phosphate adjuvant The first group of quails (16 animals) were immunized one times with aluminium phosphte adjuvated vaccine subcutaneously. Every animals obtained 0,1 ml inoculum.
The second group of quails (16 animals) were immunized two times with aluminium phosphte adjuvated vaccine subcutaneously. Every animals obtained 0,1 ml inoculum. The first immunization took place same time as first group, and the second one was 2 weeks later. Highly pathogene Influenza A /Swan/Nagybaracska/01/05(H1N1) strain pathogenicity index is 2,6. LD50% is estimated formerly. Two or 5 weeks, as indicated, after the last immunization the quails were challenged. One hundred microliters of a one time , ten time and one hundred time LD50% of HPIA Swan/Nagybaracska/01/05(H1N1) (clade 2.1) virus was administered subcutaneously to anilmals, respertively. Mortality was monitored on a daily basis for 10 days.
Following assays is planed: HI, NI and MN . Challenge study . One times imminizing quails was protected against 100xLD50% of Influenza A/swan/Nagybaracska/01/05(H5N1).

FUNCTIONAL DAIRY PRODUCTS, BASED ON LACTOSE-HYDROLYSED MILK, SUPPLEMENTED WITH GREEN-TEA POWDER AND FOREST HONEY

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Probiotics are very important for the balance of colonic microbiota. Special fermented milks, based on lactose-hydrolysed milk may be succesful in the diet of the lactose-intolerant people. Supplementations with green-tea powder and honey give a good possibilities to produce functional foods, probiotic yogurts, which are rich antioxidant- and prebiotic compounds.These supplements are able modify gastrointestinal functions and colonic microbiota positively. Our fermentations were run in duplicate:200-200cm^3 of lactose-hydrolysed milk was fermented by *Bifidobacterium breve* and *Lactobacillus paracasei*, at 45°C.(The inoculation of probiotic bacteria was 3-3%.) The acidity (SH°) and the viable cell counts (Cfu) were examined in details during the fermentations. After the complette coagulation the probiotic fermented milks were supplemented and mixed with 0,2% of green-tea powder, and 10% of forest honey+ 0,2% of green-tea powder . After it the fermented and supplemented milk-samples were stored at 10°C for 2 weeks. At the end of storage the acidity and colony forming units were controlled, too.(The acidity was suitable: 31-35°SH and the viable cell count was more, than

10^7 Cfu/g yogurt.) The sensoric properties of the supplemented probiotic yogurts were compared with each-other by Kramer method.The probiotic yogurt-samples, which were supplemented with 10% of forest honey + 0,2% of green-tea powder, seemed to be significantly better, than thouse probiotic yogurts, which were completted only 0,2% green-tea powder.

INVESTIGATION OF FUNCTIONAL YOGHURTS IN A SIMPLIFIED ARTIFICIAL DIGESTION SYSTEM

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Traditional yoghurts, containing probiotic bacteria, are well known dairy products. Prebiotic carbohydrates are non-digestible ingredients that may improve the health and well being of consumers through the support of colonic probiotic bacteria. We envisaged functional yoghurts that contain resistant starch and inulin as prebiotic ingredients and additional minerals (as organic and inorganic salts), as well. In the current investigation we examined the effects of yoghurts supplemented by prebiotics and minerals on the colonic microbiota in a simplified in vitro digestion model.
Preparation of yoghurts: the yoghurt samples originated from a dairy factory in Eger, Hungary. The milk was inoculated by the standard yoghurt starter culture, filled into tubs, and then delivered to the laboratory. The prebiotic ingredients (two different resistant starches and inulin) and minerals were mixed with the yoghurt in different combinations. After the ingredients were added, the samples were incubated at 44 °C for 4 hours. At the end of the incubation, the yoghurt samples had the same consistency and appearance as the non-supplemented control samples and were stored at 4 °C until further use.
Artificial digestion model: the in vitro digestion model simulates a three-step digestion procedure in the mouth, stomach and small intestine. We modelled the intestinal reabsorption with the dialyzation of the chyme, then it was inoculated with one species of the most frequent probiotic bacteria (*Bifidobacterium* and a *Lactobacillus* sp.), and two common enteropathogens (*Bacteroides* and *Clostridium* sp.). The artificial chyme was cultured at 37 °C, under anaerobic atmospheric conditions for 48 hours. Samples were taken for microbiological aim right after inoculation, 24 and 48 hours after. Standard yoghurts were used as control. Prebiotic index was calculated and used to compare the prebiotic effect of the various supplemented yoghurts.
Our results showed that yoghurts containing one kind of resistant starch and inulin were fermented by the gut microflora, so the prebiotics resisted the break down effects of the simulated gastrointestinal tract and exerted prebiotic effect on the bifidobacteria and lactobacilli. However, this effect is most evident when resistant starch and inulin were combined: 1:1 ratio of resistant starch and inulin had the strongest proliferation stimulating effect on the model bacteria. Organic and inorganic salts did not have similar significant effects.
These in vitro results indicate that various combinations of resistant starches and inulin added to functional yoghurt can stimulate the proliferation of probiotic bacteria in the intestinal tract, hence improve the balance of the intestinal microflora.

IN VITRO ANTIMICROBIAL ACTIVITY OF PROPOLIS AGAINST PROBIOTIC BACTERIA

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Propolis is a resinous substance produced by honeybees and as a folk medicine it has been used worldwide to cure various diseases. Propolis exhibit bacteriostatic activity against different bacterial genera, especially Gram-positive species, and has a weaker effect on Gram-negatives. Propolis has a complex chemical composition that differs greatly between samples of diverse geographical and botanical origin. Its biological activities depend mainly on the presence of flavonoids, benzoic acids and their esters. Propolis can cure several diseases caused by microorganisms and it can inhibit the growth of the gastric pathogen Helicobacter pylori; however, there is limited literature on the susceptibility of beneficial probiotic bacteria. The aim of this study was therefore to investigate the antimicrobial effect of ethanolic extract of four propolis samples, originating from different regions in Hungary, on probiotic bifidobacteria and lactobacillus species.

Crude propolis was ground into powder and dissolved in 80 % ethanol to a final concentration of 0.25 g/ml at 37 °C for 48 hours. After dissolution, ethanolic extract of propolis (EEP) was filtered through 0.22 μm sterile filter. The antimicrobial activity of EEP against probiotic bacteria was tested using the disk diffusion method in Müller-Hinton agar and 80 % ethanol was used as a negative control. The antimicrobial effect of EEP samples was investigated after a three-step in vitro digestion procedure too, that simulated the digestive process in mouth, stomach and small intestine. The diameter of inhibition zones around each hole was measured and recorded. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined after incubation of probiotic species with different dilutions of EEP samples in MRS-cysHCl medium. Viable counts were determined after 24 hours on appropriate selective agar plates.
All the four bifidobacteria and five lactobacilli species tested were susceptible to EEP and digested EEP, too. The probiotics were resistant to the 80 % ethanol, but two lactobacilli strains, *Lb. acidophilus* DSM 20079 and *Lb. casei* DSM 20011 showed susceptibility against digested ethanol. However, in both cases the inhibitory zone around the propolis samples was higher than around the control. *Lb. acidophilus* DSM 20079 had the lowest MIC and MBC values, while *Lb. rhamnosus* DSM 20021 had the highest ones. There was an irrelevant difference between the antimicrobial activities of the four investigated propolis samples.
Because of its antimicrobial properties, propolis is widely used as medicine. However these results intimate that some components of propolis could have a negative effect of the beneficial species of the intestinal microbiota.

THYOLATED PYRIMIDINE COMPOUNDS AS HIV ENTRY INHIBITORS

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The initial molecular events of HIV infection, determined by gp120 and gp41 surface glycoproteins,as well as cellular receptors are with primary significance for HIV replication. Replication levels of recombinant HIV constructs (pseudovirions) that carried the glycoproteins of wild-type HIV isolates on a generic viral background correlated with replication of their corresponding natural isolates. The aim of this study was the analysis of coreceptor binding properties of different HIV isolates, and evaluating the anti-HIV effect *in vitro* of a chemically modified thyolated pyrimidine nucleotide UD31.
HIV-1 glycoprotein genes were amplified and cloned into the eukaryotic expression vector pCI resulting in a set of *env* expression plasmids. The other proviral plasmids carry HIV *gag-pol* genes and firefly luciferase or eGFP as reporter genes. Pseudovirions were produced by transfecting both pEnv pGag plasmids (with reporter genes) into HEK293T cells. In transfected cells phenotypical mixtures of constructs (pseudoviruses) form. To examine transfection efficiency at the time of pseudovirion harvest, expression of the reporter genes was observed upon luciferase activity or detecting eGFP expression. Human and animal cell lines, expressing CD4 and CCR5 or CXCR4 selectively or both, have been used for the determination of pseudovirion tropism. UD31 compound in concentrations 2.5-40 microgram/ml were added prior and at the time of infection of cells with HIV pseudovirions (moi: 2). HIV p24 antigen expression was quantitatively determined by Vidas Elisa. Antiviral effect of UD31 were also tested in a syntitium assay on MT-2 lymphoid cell lines infected with HIV-1 IIIB, treated with various concentration of UD31.
Preliminary results with UD31 showed a prominent antiviral effect in the concentration of 5 ug/ml. As UDs inhibits the glyceraldehydes-3-phosphate dehydrogenase (GAPDH), results suggest that this thiolated nucleotide may interfere with the function of the essential –SH groups of CD4 molecule (the primary receptor of HIV), and may function as an entry inhibitor for HIV.

NEXT GENERATION SEQUENCING OF HEAT STRESSED AND STRESS-FREE TRANSCRIPTOMES OF WILD TYPE AND HMGB-A/HMGB-B DOUBLE DELETED MUTANT STRAINS OF *ASPERGILLUS NIDULANS*

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Introduction of Next Generation Sequencing (NGS) method into molecular biological researches had a revolution-like impact on genome-scale and transcriptome researches. It made many investigations available meanwhile providing the most accurate and robust throughput data over conventionally used methods.

We had constructed hmgB-A and hmgB-B deleted mutants of Aspergillus nidulans and found that lack of either of chromatin associated high mobility group proteins caused deterioration of asexual reproduction process through conidia and stress tolerance of mycelia. Growth of single deleted mutants and wild type strain did not show significant differences. In contrast, double deleted mutant lacking both HmgB-A and HmgB-B proteins showed cell death when incubation temperature was shifted from 37°C to 42°C. To understand physiological processes leaded toward cell death in double mutant we had carried out NGS transcriptome analysis. First we analysed heat stress responses at transcriptome level in the wild type strain and found that out of 9410 genes 1086 and 1264 genes became significantly (more than 2 fold) up- or down regulated, respectively. In the double mutant the 1086 upregulated genes from wild type split up to three groups. 376 genes were counter-regulated, 280 genes showed significant lower level of upregulation than the wild type genes and 43 genes showed significant increase in upregulation. Out of the 1264 downregulated genes of wild type 274 genes were regulated on the contrary and 77 of the them were highly upregulated (more than four fold), while 55 genes showed stronger downregulation (more than two fold) than those of wild type. Physiological role of great majority of the differently transcribed genes is unknown or putative. However, the results of analysis definitely point toward those cellular processes that have the greatest impact on heat stress defense. Additionally, our data indicate the role of the high mobility group proteins HmgB-A and HmgB-B in the defense processes. Moreover, the analysis provides added value to the recently available annotated Aspergillus data bank since several unknown genes are experimentally proved to be clearly responsive to heat stress.
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CONSTRUCTION OF A BIDIRECTIONAL *E. COLI/ASPERGILLUS NIDULANS* AUTONOMOUS REPLICATIVE EXPRESSION VECTOR

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Molecular biology has been introduced into microbiological researches several decades ago, and since then continuous development of novel plasmid tools to support newly risen demands was needed.
Here we report the construction of a novel bidirectional *E. coli*/*Aspergillus* nidulans autonomous replicative expression vector that contains pantoB *selection* marker gene and NdeI, NotI cloning sites between Pgpd constitutive promoter and trpC termination sequences.
Development of the novel vector was based on the adaptation of pAnGFP, an *E. coli* based *Aspergillus nidulans* integrative GFP fusion expression vector through four steps of modifications. We eliminated the intrinsic single NdeI restriction site by cloning the PCR product of pantoB marker gene into the NdeI site. The cDNA fragment encoding GFP situated between the Pgpd constitutive promoter and the trpC termination sequences was also eliminated. In addition to the pantoB marker gene (for selection purpose) we cloned the amaI sequence (autonome replication sequence of *A. nidulans*) and established two unique cloning sites NdeI and NotI into the new expression vector. Size of the resulted cloning-and-expression vector became 13.2 kb.

PHENOTYPIC CHARACTERIZATION OF A CHROMATIN ASSOCIATED HIGH MOBILITY GROUP PROTEIN NULL MUTANT (HMGB-B DELETED MUTANT) *ASPERGILLUS NIDULANS*

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High Mobility Group B (HmgB) proteins are ubiquitous nuclear proteins, highly conserved across several species including mammals, plants, yeast, *C. elegans, D. melanogaster* and *P. falciparum*. Previous studies showed that they regulate and facilitate various DNA-related activities such as transcription, replication, recombination, repair and chromatin assembly. We have identified two HMGB type proteins (HmgB-A: AN2885.2 and HmgB-B: AN1267.2) in *Aspergillus nidulans* genome by in silico analysis and subjected them to functional analysis. Here we report the phenotypic characterization of the hmgB-B deleted mutant strain that provides a rough insight into the biological role of the HmgB-B chromatin associated protein.
Briefly, the deleted mutant produced inviable conidia at frequency of 99.4%; germination of the viable conidia (0.6% of the total conidia) showed a 24 hours delay compared to that of the wild type; the mutant colony failed to develop wild type like hyphal branches under 1M NaCl osmotic stress; the mutant was more sensitive against 1.8 mM diamide oxidative stress agent than the wild type.

Altogether the phenotypic characteristics of the deleted mutant indicates that the chromatin associated HmgB-B protein strongly modulates stress-linked processes either or both through influencing stress signal production, stress signal transduction pathways and stress responses.
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MOLECULAR EPIDEMIOLOGY OF *PSEUDOMONAS AERUGINOSA* IN A FIVE-BED INTENSIVE CARE UNIT WITH A PULMONOLOGY PROFILE

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Molecular epidemiology analysis of *Pseudomonas aeruginosa* was carried out in 2009 and 2010 in an intensive care unit treating mostly patients with severe chronic obstructive pulmonary disease requiring mechanical ventilation. For comparison a few isolates from 2008 were also included, as well as isolates from other non-ICU wards (pulmonology, oncology and outpatient wards) of the same clinic. In the study period, the patient turnover was slow in the ICU; the average number of hospital days per patient was 16 days (8 to 33 days). Antibiotic usage was frequent; the most popular drugs were piperacillin+tazobactam and amikacin.
Sampling of the patients in the ICU were always performed when the physician suspected an ongoing infection, patients with active infections were sampled almost daily in some cases. Isolates were derived mostly from bronchial washing or sputum samples; they were identified with traditional microbiological methods. Susceptibility testing was carried out by the CLSI disk diffusion method, antibiotics tested were imipenem, meropenem, piperacillin+tazobactam, ceftazidime, cefepime, polymyxin B, ciprofloxacin, amikacin, gentamicin and tobramycin. SpeI-macrorestriction patterns of 110 isolates (94 from the ICU and 16 from other wards) were determined by pulsed-field gel electrophoresis (PFGE).
Twenty unique patterns were found, out of which ten isolates were from non-ICU patients. Three and three isolates were unique for two different patients. The other 84 isolates formed clusters of 2-46 isolates, with five major clusters (A-E). Cluster A included eight isolates of five (four ICU and one pulmonology ward) patients isolated between March and December 2009. In cluster B, 14 isolates of six ICU patients were included isolated between December 2009 and February 2010. Cluster C was the largest cluster with 46 isolates of twelve ICU patients; these isolates were recovered between December 2008 and February 2010, representing an endemic clone in the ICU. One isolate was from a sample of a former ICU patient taken at the rehabilitation department. Cluster D was a small cluster of three isolates from two pulmonology ward patients, out of which one was admitted to the ICU later. Curiously, while pulmonology ward isolates were pansusceptible in this cluster, both ICU isolates showed resistance to ceftazidime, cefepime and ciprofloxacin. Cluster E was comprised of four ICU isolates of two patients recovered between January and February 2010. This cluster was characterized by a uniform resistance to ciprofloxacin and all tested aminoglycosides.
Most ICU isolates were resistant to ciprofloxacin and to all tested aminoglycosides, but the resistance pattern to beta-lactam agents was extremely variable even within some clusters. Isolates in cluster C varied from susceptibility to all antipseudomonas beta-lactam agents to non-susceptibility to any of them. The background of this variability remains to be determined.
PFGE revealed a hidden endemic presence of a major P. aeruginosa clone in the ICU, which caused clustering of cases repeatedly over the study period. Besides this major clone at least two important clones were present and caused case clustering, and several hidden epidemiological connections with other wards were identified.
This study was supported by the MEC8/2008 grant of the University of Debrecen.

PREVALENCE OF ASYMPTOMATIC CARRIERS OF ESBL PRODUCERS IN THE COMMUNITY IN NORTHEASTERN HUNGARY

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The aim of the study was to measure the prevalence of asymptomatic carriage of ESBL producer enterobacteria in the community for the first time in Hungary.
Stool samples of 807 asymptomatic individuals sent for Salmonella screening were investigated between April and September in 2009; 571 individuals (116 males, 455 females, mean age 32.9 years) were applying for

employment eligibility screening (workers in kindergartens, in food industry or catering and personnel in related training) and 236 (93 males, 143 females, mean age 66.3 years) needed the screening prior to admittance to long-term care facilities. Samples were directly plated onto eozin-methylene blue agar plates supplemented with 2 mg/l cefotaxime, recovered isolates were identified using standard microbiological methods and disk diffusion susceptibility testing was performed. Suspect isolates were tested for the ESBL phenotype by means of double disk synergy test. Isolates showing the ESBL phenotype were tested by PCR for the presence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes. Five aminoglycoside resistance genes commonly demonstrated in enterobacteria, aac(6')-Ib, aac(3')-IIa, ant(2")-Ia, aph(3')-Ia, ant(3")-Ia, were also sought for by PCR.
Out of the 807 patients 33 (4.1%) harboured ESBL producers, nine, 18, one and three was colonized with *Klebsiella pneumoniae*, *E. coli*, *Citrobacter* spp. and with two different ESBL producers (*E. coli* + *K. pneumoniae*) simultaneously, respectively. The prevalence among individuals screened for employment purposes and among long-term care facility applicants was 2.5% (13/571) and 8.1% (19/236).
bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were present in 16, 14 and 24 isolates, respectively. The proportion of isolates with bla_{CTX-M} gene was 57.1% and 63.2% in individuals screened for employment purposes and among long-term care facility applicants, respectively. Co-resistance to other antibiotic classes was frequently seen; almost all isolates (33/35) were resistant to doxycycline, 55.6% of isolates were resistant to ciprofloxacin and more than half of the isolates were resistant to gentamicin and/or tobramycin.
Twenty-three isolates harboured at least one tested gene, the most frequently found aminoglycoside resistance genes were aac(6')-Ib and aac(3')-IIa. In nine isolates, the phenotypically detected resistance could not be explained by the genes found; other, not tested genes are probably responsible for these resistances.
While the prevalence in individuals applying for long-term care facility admission is not surprising and can be explained by the probable previous multiple hospitalizations of these persons, the 2.5% prevalence is alarming, as in case of these individuals previous hospitalization is unlikely. This rate represents the carriage rate in the community most probably and is likely to be the result of transmissions outside the hospital environment.

DEMONSTRATION OF THE HYPERVIRULENT ST17 CLONE OF *STREPTOCOCCUS AGALACTIAE* IN HUNGARY

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Streptococcus agalactiae (Group B streptococcus, GBS) is well established as a major human pathogen causing serious infections primarily in neonates but affecting also adults. The screening of pregnant women for GBS prior to delivery is performed in many European countries but has not been introduced in Hungary. In addition, no data on the clonal distribution and virulence of GBS has been reported in Hungary to date. 100 strains of GBS, isolated from a variety of specimens at the Central Bacteriology Laboratory of Semmelweis University, between 2009-2010, were genetically characterized. The isolates derived mostly from cervical or vaginal exudates of pregnant women, from the ears or throat of newborns, and rarely from other adult specimens, e.g. urine. The strains were identified by routine biochemical tests and by detection of the GBS specific dltR gene by PCR. Of the nine serotypes of GBS currently recognised types I, II and III are the most prevalent, especially in neonatal infections and in asymptomatic carriers. The serotypes of 58 isolates were determined with the Pastorex latex agglutination test which distinguishes types I, II and III. The vast majority of the strains (36/58) belonged to type III, 7 isolates proved type I and 5 isolates showed type II. The remaining 10 strains were not typable by the Pastorex test. Interestingly, the type I strains were isolated mostly from the ears of newborns.
The ST17 clone of GBS identified by multi locus sequence typing (MLST) is recognised as a hypervirulent international clone associated mainly with invasive neonatal infections. ST17 comprised 25,9% of all strains among the Hungarian GBS isolates tested, which figure is in line with those in international reports. Most but not all of the ST17 strains belonged to serotype III. Further genetic characterization of these and additional Hungarian GBS isolates is in progress.

VACCINATION STRATEGIES AGAINST TRACHOMA – EXPERIMENTS IN *CYNOMOLGUS* MONKEYS

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Chlamydia trachomatis is the etiological agent of trachoma the world's leading cause of preventable blindness. Research during the past decades primarily focused on developing subunit vaccines, most of them focusing on MOMP, the major outer membrane protein of *C. trachomatis*. We have recently tested the most promising one in the nonhuman primate model. This subunit vaccine was native-MOMP, extract from infected cells in a way that preserved the native trimeric structure of the protein. Although we observed a significant reduction in bacterial shedding post challenge this partial protection was not reflected in any reduction of ocular pathology.
In order to identify other antigens as potential vaccine targets we analyzed the humoral immune response of nonhuman primates to chlamydial infection. Three cynomolgus monkeys were infected ocularly with *C. trachomatis*. Infection and ocular clinical disease persisted for 4-6 months and then resolved spontaneously. Monkeys exhibited significant protective immunity to re-infection characterized by rapid clearance of organisms and less severe ocular pathology. Sera were analyzed by radioimmunoprecipitation (RIP) with intrinsically radiolabeled, non-denatured chlamydial proteins. RIP was performed on heated and unheated radiolabeled lysates to identify conformation dependent antigen recognition. We observed a simple antibody recognition pattern using non-denatured antigens. Antibody recognition was restricted to the 40 kDa MOMP and several higher and lower molecular weight (mw) polypeptides. MOMP was recognized early and the antibody response was sustained until the spontaneous clearance of the primary infection. Antibody recognition of the higher and lower mw proteins occurred later during primary infection and peaked at the time of spontaneous resolution. A marked increase in recognition of these proteins was observed following ocular re-infection. The high mw proteins were identified by immunoblotting as HSP60, polymorphic membrane protein D (PmpD) and its proteolytically processed fragments. Antibody recognition of PmpD and most lower mw proteins were destroyed by heat treatment suggesting that the infection mediated antibody response is largely directed at conformational determinants. These findings suggest that conformational antigenic determinants of PmpD and other, yet to be identified antigens, are important antibody targets of protective immunity and represent novel trachoma vaccine targets. Developing a subunit vaccine based on delivering conformational antigenic determinants, however, might be challenging. One of the best and most commonly used approaches is the use of attenuated strains. We generated a *C. trachomatis* attenuated trachoma strain by curing the plasmid from a virulent clinical isolate. Ocular immunization of primates with the attenuated strain was highly immunogenic. Furthermore, infectious challenge demonstrated that 50% of the immunized animals had sterilizing immunity while the other 50% of the animals exhibited a significant partial protection. Our results could have an important impact on the future control of blinding trachoma.

BIOFILM ELIMINATION FROM MIXED BED ION-EXCHANGE RESIN OF A WATER PURIFICATION SYSTEM

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Ultra pure waters are critical requirements in many industrial environments, often suffering from microbial contamination. In such habitats (characterised by extremely low salt and nutrient concentrations) a common survival strategy is to form biofilms. In the supply water system of a Hungarian power plant biofouling and biocorrosion were observed. These phenomena cause reduced lifetime, effectiveness and yield as well as increased operational costs. During the last year microbial communities of the water purification system of a Hungarian power plant were examined and the most contaminated "critical points" of the system were determined. On two points (gravel filtration system and mixed bed ion-exchange resin) of the water purification system serious microbial contamination was observed. To reveal the possibilities of the elimination of biofilm bacteria from ion-exchange resins, biocide Kathon WT was used against the bacteria covering the resins. This biocide is effective against biofilms at low concentrations, it is biodegradable, water soluble, its toxicity is low and it is easy to deactivate. Based on previous results, in the present work the microbial contamination of a mixed bed ion-exchange resin was eliminated by using Kathon WT in the water purification system of the plant.

The aim of the present work was to eliminate the biofilms from the surfaces with the biocide, preserving the total ion exchange capacity of resins. A 25 ppm concentration of the active ingredients (5-chloro-2 methyl-4-isothiazolin-3-one, 2-methyl-4-isothiazolin-3-one) of Kathon WT was applied for 4 hrs with continuous mixing by air. After the chemical treatment the mixed bed ion-exchange resin was washed several times with refined saltless water until the active ingredients attenuated to the allowable concentration for being discharged into living water (0.0018 ppm). The effectiveness of the treatment was followed up by Scanning Electron Microscopy examinations of the resins. According to these results the biocide killed most of the bacteria. Additionally the ruined cells were removed by the washing steps. The analytical studies of the resins showed that the ion-exchange capacity increased in a small compass. Since these ion-exchange surfaces were covered by microbes before, this increase is caused by creating free places on the resins' surface by the treatment and the washing. Henceforth three further mixed bed ion-exchange resin columns will be treated by biocide Kathon WT in the water purification system of the power plant.

CONGENITAL AND PERINATAL CYTOMEGALOVIRUS (CMV) INFECTION

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Cytomegalovirus infection may be acquired prenatally or perinatally and is the most common congenital viral infection. Signs at birth, if present, are hepatosplenomegaly, microcephaly, jaundice, petechiae, periventricular calcifications, chorioretinitis, pneumonitis, prematurity and intrauterine growth retardation. Average incidence of congenital CMV infection is 1% of birth in Hungary per year. 1000 newborn acquire CMV infection with or without symptoms. If acquired later in infancy, signs may include pneumonia, hepatosplenomegaly, hepatitis, thrombocytopenia, and atypical lymphocytosis. Diagnosis of neonatal infection is performed by virus isolation on cell culture or by the amplification of the virus DNA.
Urine, cerebrospinal fluid (CSF) and blood samples were sent from the Neonatal Intensive Care Unit and from other Paediatric wards to the laboratory for CMV screening tests. Urine and EDTA-blood samples were used for the quantitative detection of CMV DNA. Samples were prepared by using MagNa Pure Compact Nucleic Acid isolation kit (Roche). Quantitative PCRs were carried out by using CMV LC PCR kit (Artus, Qiagen). Serum samples were used to determine CMV specific IgM and IgG antibodies.
Between 01. January 2007 and 2010 May 18 congenital/perinatal CMV infections were detected (male/female ratio was 10/8). All of the children had a positive CMV IgM titer, and positive urine and/or blood CMV PCR results. Majority of the newborn-babies had symptoms at birth, such as microcephaly, jaundice, splenomegaly or prematurity, while some of them had feeding difficulties, fever of unknown origin one-two month after birth.
Symptomatic neonates have a mortality rate of up to 30%, and 70 to 90% of survivors have some neurologic impairment, including hearing loss, intellectual disability and visual disturbances. Among asymptomatic neonates, 10% eventually develop neurologic sequelae. Ganciclovir treatment decreases viral shedding in neonates with congenital CMV and may prevent hearing deterioration at 6 months. When therapy stops, the virus is again shed; therefore, its role in treatment remains controversial. Non-immune pregnant women should attempt to limit exposure to the virus. Transfusion-associated perinatal CMV disease can be avoided by giving preterm neonates blood products from CMV-seronegative donors or leukoreduced products. Vaccines to prevent congenital CMV are being developed. Using CMV hyper immune globulin in pregnant women with primary CMV infection to prevent or treat congenital infection is also under investigation.

HUMAN UROPATHOGENIC *ESCHERICHIA COLI* STRAINS MAY CARRY HLYA PLASMID

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Cytolysin A (ClyA, HlyE, or SheA) and alpha-haemolysin (HlyA) are cytolytic pore-forming proteins. Cytolysin A can be found in *Escherichia coli, Shigella* species, and *Salmonella enterica* strains. Alpha-hemolysin or its ortologues are produced by Escherichia coli, Enterobacter cloacae, different Proteus species, and several other taxa. Both of these cytolysins are virulence factors. ClyA is encoded in the chromosome while hlyA genes may be found either on a pathogenicity island integrated into chromosome or on plasmids of different sizes. In our previous study we suggested a possible incompatibility between hlyA and sheA (clyA, hlyE) in the chromosome of E. coli.

Recently we found both virulence traits in some extraintestinal *E. coli* (ExPEC) strains from human or dog extraintestinal infections, and in some *E. coli* strains isolated from the pig intestine. In this study we investigated these *E. coli* strains for the location of the hlyA gene. As already known from other studies, the dog and pig isolates possessed with plasmid mediated alpha-haemolysin. Surprisingly, opposite to the existing assumption that human ExPEC harbours hlyA exclusively on the chromosome some of these strains presented also with plasmid located hlyA. As human and dog ExPEC may share identical somatic and flagellar antigens the zoonotic nature of these isolates is assumed. Thorough clonal analysis of the strains is in progress to evaluate this idea.

PREVALENCE OF HUMAN PATHOGEN VIRUSES IN HUNGARIAN SURFACE WATERS: THE FIRST RESULTS

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Surface water is a potential source of water-borne viral infections through recreational use or surface derived drinking water. Although a wide range of human viruses was identified in surface waters, it is still not part of the water quality monitoring in most countries. In Hungary, previous data on the viral prevalence was scarce. Our aim was to investigate the presence of human adenoviruses and caliciviruses, two of the most relevant groups of water-borne human pathogen viruses in Hungarian surface waters. Since summer 2006, in total 70 surface water samples were collected from 14 sites. The samples (10 L) were concentrated by membrane or glass wool filtration. The viral particles were eluted by beef extract and flocculated. Nucleic acid was extracted using magnetic silica bead extraction method. The presence of viruses was analyzed by group-specific PCR and confirmed by sequence analysis. The fecal indicator counts were also determined. Seasonal variability and the effect of sewage input were investigated by multiple sampling of the river Danube (n=24).
More than half of the examined samples (n=47) were positive for adenovirus, mostly type 40 and 41. The highest rate of adenovirus presence was observed in Danube. Calicivirus was present in 30% of all samples, mostly in Danube samples. The ratio of positive samples was higher in more polluted waters, however, direct correlation between the viral presence and the fecal indicator counts was not observed.
Adenoviruses were detected in practically all Danube samples. Based on the calicivirus data, it was observed that the viral presence was most pronounced in spring, probably due to the winter-spring seasonality of virus infection and the high precipitation. The effect of sewage input was difficult to assess because of the high ratio of positive samples, thus quantitative analysis will be necessary for further clarification.

IDENTIFICATION OF HOST ADAPTATION MARKERS OF *BORDETELLA BRONCHISEPTICA* WITH PCR-RFLP ANALYSIS

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Bordetella bronchiseptica is a widespread Gram negative pathogenic bacterium causing respiratory diseases in different mammalian species. It is known to play a role in the aetiology of infectious atrophic rhinitis of swine, in canine kennel cough, respiratory syndromes of cats, rabbits and guinea pigs, and sporadic human cases have also been reported. Motility is important in approaching a susceptible host and flagellin, the monomeric component of flagella, is a potent pro-inflammatory factor of *B. bronchiseptica*. Adenylate cyclase toxin induces cAMP accumulation in the target cells, consequently inhibits invasivity, moreover plays role in haemolysis. Study of the flagellin [flaA] and adenylate cyclase [cyaA] genes provides an alternative approach for genotyping of *B. bronchiseptica* and the results may be useful for finding incidental markers of host-adaptation.
Eighty three strains from different geographical regions and from different hosts were studied phenotypically with biochemical tests and haemolysis and genotypical characterisation with PCR-RFLP. The 2151 bp length PCR products of cyaA were analysed with SalI and NarI, the 1165 bp length PCR products of flaA were digested with HincII, BglI and MspI. Concerning biochemical reactions, the strains were different from each other only in nitrate-reduction. Fifty-five percent of the isolates produced haemolysis on blood agar. The cyaA gene was detected in 74 strains. On the basis of PCR-RFLP analysis, strains were grouped to 4 types, and the major type consisted of the 81 percent of the bacteria. All strains possessed flaA PCR product, and RFLP analysis resulted eight types (designated A to H). Most of the strains belonged to three major types (A, B, and C). Homogeneity of the Hungarian isolates originated from dogs (type A) and guinea pigs (type C) was established, and only one

strain from pig showed different fragments from the other isolates of porcine origin. Diversity and uniqueness of human isolates (type F) and turkey originated strains (type E and G) was also noticed. Signs of host adaptation were observed in distinct geographical regions. Prevalent types were established by PCR-RFLP analysis among foreign and Hungarian strains originated from pigs and dogs. Strains isolated from pigs had the same RE-type (B) at a rate of 89% while strains isolated from dogs had the same RE-type (A) at a rate of 71%. Isolates from rabbits showed RE-types A and B in equal portions.

GENETIC AND EPIGENETIC ALTERATIONS IN THE INK4A/ARF TUMOR SUPPRESSOR LOCUS IN HEAD AND NECK CANCER

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The INK4A/ARF locus on chromosome 9p21 encodes two distinct tumor suppressor proteins, p16INK4A and p14ARF, which share an exon using different reading frames. Both proteins play an important role in the Rb- and p53-mediated cellular growth control pathways. The p16INK4A regulates proliferation indirectly by suppressing hyperphosphorylation of pRb. p14ARF interacts with MDM-2 and stabilizes p53 in the nucleus by blocking its cytoplasmic transport and MDM-2-mediated degradation.
In human tumors chromosome 9p21 is a major site of chromosomal abnormalities. In several tumors, including head and neck cancers, deletion of exon1 and/or exon2 of the p16INK4A and p14ARF genes as well as methylation of their promoters have been implicated in the pathogenesis. We examined the alterations of p16INK4A and p14ARF genes using PCR-SSCP and the methylation status of their promoters by means of methylation-specific PCR in 37 oral (OSCC) and 28 laryngeal (LSCC) squamous cell cancers.
Lack of amplification suggesting exon deletion were found in ten, 19, nine and two cases for the p14ARF, p16 exon1 and for the common exon2 and exon3, respectively, in case of LSCC patients. In contrast, only one p16 exon1 deletion was suggested by PCRs in case of OSCC. SSCP pattern alterations suggesting mutations were found in 14/28 (50.0%) and 13/37 (35.1%) LSCC and OSCC patients in at least one of the examined exons. This means that the rates of all suggested genetic alterations (deletion or mutations) altogether were 13, 22, 14 and 12 in the p14ARF, p16 exon1 and for the common exon2 and exon3, respectively, in case of LSCC, while two, eight, four and six, respectively, in case of OSCC. In case of LSCC, one totally and one partially methylated p14 promoter was found, while p16 promoter was partially methylated in five case (total methylation was never found). In OSCC patients, p14 promoter was totally or partially methylated in one case each; p16 promoter was methylated totally in three and partially in six cases.
These data show that genetic alterations of the p14ARF and p16INK1A tumor suppressor genes are more common in LSCC than in OSCC, while epigenetic alterations seem to be more frequent in OSCC. Disruption or non-expression of the two examined tumor suppressor genes may play a role in the pathogenesis and/or progression of head and neck cancer, but their importance seems to be different in the two anatomical sites.

BIOFILM BACTERIAL COMMUNITIES ASSOCIATED WITH CAVE WALLS IN THE BUDA THERMALKARST SYSTEM

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Budapest has a special hydrogeological situation due to the even now active Buda Thermalkarst System, where the mixing processes of thermal- and meteoric waters and the probably microbiologically influenced cave formation can be well studied. During these studies bacterial communities of cm thick, reddish-brown biofilms associated with walls of Rudas-Török bath (RTB) and Molnár János caves (MJB) were investigated. SEM and light microscopic photos were made and clone libraries were constructed to analyze the bacterial diversities. Communities from the distinct sampling sites differed morphologically according to the microscopic examinations. Clone libraries included nearly 200 clones, respectively. ARDRA grouping of clones from both libraries resulted in wide phylogenetic diversities. The sequence analysis of 16S rRNS genes of the representative clones showed the highest similarities with uncultured clone sequences from different environmental (e.g. soil, cave, thermal spring) samples. According to the comparison with already described bacterial species, RTB and MJB clone sequences showed the highest correspondence with members of 17 phylogenetic groups: Aquificae, Thermodesulfobacteria, Chloroflexi, Nitrospirae, Cyanobacteria, Chlorobi,

Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Firmicutes, Actinobacteria, Planctomycetes, Acidobacteria, Bacteroidetes, Verrucomicrobia, Gemmatimonadetes. RTB and MJB clone libraries shared 12 common phyla, although the distribution of clones among the phyla was considerably dissimilar in the samples. Majority of RTB clones belonged to Deltaproteobacteria (44%), whereas dominant MJB clones affiliated mostly with members of Firmicutes (40%). Considering the metabolism of bacteria closely related to our clones, in both biofilms bacterial communities may participate mainly in the local sulfur and iron cycles.

COMPARATIVE STUDY OF MICROBIAL COMMUNITIES IN SOILS FROM TWO HUNGARIAN KARSTIC AREAS

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From two characteristic karstic areas of Hungary - Aggtelek National Park and Tapolca-basin – 17 different soil samples were taken in autumn 2009. The physical and chemical characteristics of the soils were determined by grain size analyses (by laser-diffraction method) and measuring humidity-, pH values, acidity and organic matter content. Microbial biomass C and N were determined by chloroform fumigation extraction method. The activity of microorganisms in soils were followed up by measuring basal- and substrate induced respiration (RESP and SIR) using gas chromatography. The phylogenetic diversity of bacterial communities was investigated by 16S rDNA based Denaturing Gradient Gel Electrophoresis (DGGE). In each soil samples multiple microbial biomass C- than N- values were detected. The lowest MBC/MBN rates could be calculated from the samples of the shallowest soil layers. Higher biomass as well as RESP and SIR values were characteristic to the surface and near-surface soil samples. The highest RESP, SIR, biomass C and N values were measured in the black rendzina soils from Aggtelek. The soil type influence on bacterial community structure was confirmed by the DGGE results as phylogenetic diversity of black rendzina soils was the most different. On the basis of each investigation, other soil samples separated mainly according to the sample depths and types, irrespectively of the sampling sites.

HETEROTRIMERIC G PROTEIN SIGNALING IS INVOLVED IN PAF TOXICITY IN THE RELATIVE *ASPERGILLUS NIDULANS* AND *ASPERGILLUS FUMIGATUS* SPECIES

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Heterotrimeric G proteins are conserved in all eukaryotes, playing a pivotal role in sensing the external cues and transducing the signal into the cells to elicit appropriate cellular responses. The system consists of a membrane bound G-protein coupled receptor (GPCR), heterotrimeric G protein α, β and γ subunits and a diverse group of effectors. Upon binding of ligands, GPCRs undergo conformational changes causing the GDP-GTP exchange of the G α subunit, which results in the dissocation of GTP-G α from the Gβγ heterotrimer. In fungi, G-proteins play integral roles in germination, vegetative growth, cell cycle control, mating, cell-cell fusion, morphogenesis, chemotaxis, pathogenecity and secondary metabolism (Yu, 2006).
It was shown in *Saccharomyces cerevisiae* and *Aspergillus nidulans* that antifungal proteins interfere with G-protein signaling. Using a transgenic *A. nidulans* strain that carries mutation in the fadA ($fadA^{G203R}$) gene, the G-protein coupled activity of PAF (*Penicillium chrysogenum* antifungal protein) was confirmed. The fadA gene encodes the heterotrimeric G-protein α -subunit FadA. In the dominant interfering $fadA^{G203R}$ mutant the dissociation of G α from Gβγ is inhibited. Growth assays unraveled reduced sensitivity of the A. nidulans $fadA^{G203R}$ mutant strain to PAF (Leiter et al., 2005).
Further investigations of *A. nidulans* and *A. fumigatus* mutants in G-protein subunits confirmed that PAF may induce detrimental effects through heterotrimeric G protein coupled signal transduction pathways. Similarly, dominant interfering mutation in the G α (GpgA) subunit ($gpaA^{G203R}$) of *A. fumigatus* showed resistance to PAF. Deletion of the Gα (αfadA) and Gβ(βgpgA) subunits increased PAF-tolerance both in *A. nidulans* and *A.*

fumigatus strains, but loss of the Gγ subunit (SfaD) not affected PAF sensitivity.
In Aspergilli the FadA(GpaA)-SfaD-GpgA heterotrimeric G protein plays a crucial role in proper control of vegetative growth, spore germination, asexual development and production of certain metabolites. Heterotrimeric G protein signaling is well known to regulate ion channels, which could directly account for the PAF-dependent K^+ efflux and plasma membrane hyperpolarisation and for the apoptosis inducing effect.
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BIOGAS PRODUCTION FROM PROTEIN RICH SUBSTRATES

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Biogas is a renewable energy carrier and the production of biogas is associated with double benefits: elimination of environmental pollution problems is coupled with the generation of useful energy. The utilization of the digestion effluent as fertilizer for agricultural application facilitates nutrient recovery and eliminates the need for artificial fertilizers, the production of which is a highly energy demanding process. Anaerobic digestion of slaughterhouse waste presents a specific task being rich proteins. For example blood has a very low C/N ratio therefore it is not a favorable substrate for biogas production. Several earlier attempts corroborated the inhibitory effects of elevated $NH_3 – NH_4^+$ concentrations on anaerobic digestion.
Anaerobic digestion of animal waste was investigated in batch reactor experiments at both 37°C and 55°C. In all experiments efficient degradation of blood containing samples were observed using a specially adapted microbiological consortium. Contrary to the findings published earlier ammonia did not inhibit the biogas process at concentrations lower than10 g N/dm^3.
At higher temperature and pH the free ammonia concentration increases significantly. The experiments were designed to compare the protein hydrolysis potential of substrates that were acclimated and non-acclimated to protein rich media at two different temperatures.
Proteinase activities of the consortia were monitored regularly. The changes in acetate and ammonium-nitrogen concentrations were followed during the fermentations. Volatile fatty acid compositions (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic acids) were determined using HPLC to monitor the microbiological activity in the reactors. Total carbon and total organic carbon contents have been examined to determine the C/N ratio of the biomass. Volumetric biogas yields gave information about the efficacy of the anaerobic digestion process. The composition of the evolved gas was determined by gas chromatography.

ADHESION POTENTIAL OF HUMAN ISOLATE *CAMPYLOBACTER JEJUNI* STRAINS TO THE EXTRACELLULAR MATRIX PROTEINS TYPE IV COLLAGEN, FIBRONECTIN AND LAMININ

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192 individual *Campylobacter jejuni* isolates were collected in the year 2006 from the South Hungarian region. Strains were collected from hospitalized patients. Although exact mechanism of the pathogenic process of *C. jejuni* is not revealed in detail yet, one of the first important step is colonisation. In this process extracellular matrix proteins (ECMPs) may play an important role. Aim of this study was to determine how different ECMPs can help this stage of the pathogenic process. Therefore we carried out solid- phase binding assays with three ECMPs such as type IV collagen, fibronectin and laminin. Efficiency of the tests were proved by using different concentrations of proteins, different colony forming units of bacteria and different incubation times. Based on these preliminary results, experiments were carried out with the most ideal combinations. Preferences in the binding abilities of individual strains to different ECMPs were revealed. On the other hand differences in binding

abilities to ECMPs could be also revealed among the members of the *C. jejuni* straincollection. All these results were analyzed in correlation with the available data of hospiatlisation. Based on our results we plan to carry out further investigations to clear which of the bacterial proteins are responsible for the ECMP binding ability and what additional role they could have in the pathogenic process.

INDUCTION OF DEOXY-GLUCOSE RESISTANT *DEBARYOMYCES OCCIDENTALIS* STRAINS FOR THE ENHANCEMENT OF AMYLOLYTIC ENZYME SYSTEM AND ETHANOL PRODUCTION ABILITY

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Starch containing raw materials or agricultural by-products are suitable substrates for bioethanol production by Saccharomyces cerevisiae after enzymatic pretreatment. Fermentation processes could be made more economical using strains which harbour amylolytic enzyme system, thus simultaneous saccharification and ethanol fermentation could be achieved. Several species of the *Debaryomyces* genus, like *D. occidentalis* are well known from their good starch utilizing and fermenting properties. Enzymatic activities of these strains, however, could be hampered by the sugar catabolite repression caused by high starch concentration.
Our aim was to induce catabolite derepressed *Debaryomyces occidentalis* mutants that have therefore an enhanced α-amylase and glucoamylase production ability. For isolation of such derepressed mutants the MIC value of deoxy-glucose was determined and an adequate method for random mutagenesis was worked out for *D. occidentalis* strains. Several mutant strains were isolated using this method and according to their glucose and starch utilizing properties two of them were selected for starch fermentation tests.
According to our results both of the investigated mutant strains had better starch utilization ability then the parental strain. This was attributable to the higher and faster α-amylase activity of the strains at elevated starch concentration which resulted in earlier glucoamylase and ethanol production. However, the produced ethanol did not exceed that of the parental strain but the usage of such derepressed mutant strains for ethanol production could shorten the time needed to perform starch fermentation processes.
This work was supported by Hungarian Government under the project GOP-1.1.1.-08/1-2008-0046.

HUMORAL IMMUNE RESPONSE AGAINST FLU WITH VIRUS NEUTRALIZATION TEST

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The virus neutralization test is a sensitive and specific assay applicable to the identification of virus specific antibody in human sera. Virus and serum are mixed under appropriate condition and then inoculated into cell culture like MDCK. The presence of unneutralized virus may be detected by reactions such as CPE(cytopathic effect), haemagglutination or/and neuraminidase inhibition.The CPE is allowed to develop over a period of days (depending on the virus and cell type). The virus endpoint titre is the reciprocal of the highest dilution of virus that infects 50% of the host system 50% of cell cultures develop CPE. This endpoint dilution contains one 50% tissue culture infecting dose ($TCID_{50}$) or one 50% lethal dose (LD_{50}) of virus per unit volume. The concentration of virus generally used in the neutralization test is 100 $TCID_{50}$ or 100 LD_{50} per unit volume. In the National Center for Epidemiology we detected the neuraminidase antibodies by neuraminidase inhibition test. This assay is based upon enzymatic removal by NA of the sialic acid residual of the fetuin. Fetuin is adsorbed on a plate and peroxidase conjugated peanut agglutinin recognizes and binds the free galactose: the amount of agglutinin, which is bound is proportional to the concentration of sialic acid that has been removed and, therefore, also proportional to the amount of NA which is present.

ENTOMOPHYL SOIL BACTERIA ISOLATED FROM WESTERN CORN ROOTWORM

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The currently known entomopathogenic bacteria derive from the *Bacillus, Pseudomonas, Xenorhabdus, Photorhabdus, Serratia, Streptococcus* and *Micrococcus* genera. Among them, *Bacillus* spp. are the most widely used in biological pest control in both agriculture and horticulture. This group of bacteria usually causes septicaemia in the insect host. Since the 1960's, over 90 *Bacillus* species were isolated from insects, plants and soil that have an entomopathogenic effect. It was observed that the number of insects decreased in the environment of soils inoculated with *Bacillus licheniformis, sphaericus, thuringiensis* and *cereus* strains.
Originating from America and making its first European appearance in Yugoslavia, the western corn rootworm (*D. virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae) has become a major limiting factor of corn production in Hungary, too. Following its first appearance in 1995, it has quickly spread to every county of Hungary, and has recently crossed the northern borders infecting neighbouring countries.
In our study we have collected root damaging insect larvae from western corn rootworm infected lands throughout the country and in order to isolate entomophyl bacterial strains.
The bacteria were isolated from whole segments and prepared intestinal tracts of living and deceased larvae, using the dilution-plating method, directly, or after enrichment in liquid broth. A great number of isolates were distinguished based on morphological and phase contrast microscopy examinations of single colonies.
In the course of presumptive microbiological identification, the isolates went under a Japanese Gram test followed by parallel culturing in carbon source supplemented nutrient agar, lauryl sulphate agar, MYP (*B. cereus* polymixin agar), XLD, MacConkey and CT selective/differentiating culture media. The isolates were then characterized in view of colony morphology. MYP agar is used with egg yolk enrichment and polymyxin B for isolation of *Bacillus cereus* from food. XLD agar is a selective growth medium for the isolation of *Salmonella* and *Shigella* species. MacConkey medium is used to differentiate strains of enteric pathogens and the coliform group. Caprylate-thallous (CT) medium is applied for selective isolation of *Serratia* and effectively discriminates against most non-*Serratia* strains, likely to be in the same habitats. Lauryl-sulfate is a selective medium used in the presumptive coliforms detection. Our laboratory has obtained about 1000 larval isolates of which 26 strains belonging to the *Bacillus* genus, 35 strains belonging to the *Serratia* genus and 18 belonging to the genera *Photorhabdus* or *Xenorhabdus* have been distinguished.
This work is supported by KMOP-1.1.1-08/1-2008-0042 grant of the National Development Agency, Hungary.

BACTERIOPHAGE-THERAPY AGAINST FIRE-BLIGHT OF APPLE TREES - ISOLATION AND CHARACTERIZATION OF PHAGES AGAINST *ERWINIA AMYLOVORA*, PENETRATION AND APPLICATION RESULTS

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Erwinia amylovora, a member of Enterobacteriaceae, is the causative agent of fire blight, which is a serious disease of some Rosaceae plants, including apple, pear, cotoneaster, pyracantha and hawthorn. The disease causes severe economic losses in Europe, North America, the Mediterranean region and New-Zealand.
Fire blight used to control with streptomycin, however, the extensive use of this aminoglycoside antibiotic has caused the increased prevalence of streptomycin-resistant *E. amylovora* strains resulting a reduced efficiency of treatments. One alternative way for treatment of fire blight could be the application of bacteriophages.

Phages against *Erwinia amylovora* were isolated from aerial tissues of healthy apple trees as well as from soil beneath apple trees displaying fire blight symptoms. 24 phage strains were classified as Caudovirales, mostly Myoviridae but two of them Siphoviridae, according to electron microscopy investigation. Their host specificity range against 6 E. amylovora, *Erwinia mallotivora* and *Erwinia raphontici* strains was determined. Three phage strains were selected for further experiments, based on their broad-range host specificity. All the three virus strains reduced the living cell number of *E. amylovora* in liquid culture in a significant manner, however, changes in titer and MOI varied during the investigated 48 hours. Preliminary penetration experiments, spraying or irrigating seedlings with viruses and detecting PFUs, suggested that phages could get into plants both by spraying and irrigation. However, these results should be supported also by molecular genetic studies. Determination of DNA sequence of the three phage strains is in progress. Apple trees were sprayed with a mixture containing the three phage strains and UV-protector agent at the time of blooming. Bacteriophage treatment caused a decrease of appearance of new fire blight cases with 77 %, in comparison with the untreated control. This difference between control and treated trees should be considered as significant.
Our results confirm that bacteriophage therapy may provide an effective solution for controlling fire blight.

SOYBEAN INOCULATION: HOST SPECIFITY AND EFFICIENCY

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Biologically fixed nitrogen covers about 50% of the nitrogen demand of agriculture (Caliskan et al, 2008). These fields fix approximately 60 teragrams (Tg) ($6 * 10^7$ tons) of atmospheric nitrogen annually (Kinzig and Socolow, 1994). 80 % of biologically fixed nitrogen comes from symbiotic nitrogen fixation (Graham and Vance, 2000). The aim of this research was to study the efficiency of soybean inoculation and host plant specificity of six different *Bradyrhizobium japonicum* strains.
A field experiment was set up on a chernozem soil in Hungary. The efficiency of inoculation was studied at full bloom. Soil and plant samples were taken from each treatment in five replicates. The results of field experiments were verified in a pot experiment. BOX-PCR method was used for comparative analysis of microsymbiota strains. Relationships between plant nutrient uptake and biological nitrogen fixation were studied.
No nodule formation was observed in the field experiment without inoculation. Seed inoculation of soybean was successful. On an average 6 - 7 composite, active nodules were developed per plant. Inoculation of soybean resulted in the increase of dry weight, height and number of flowers by 25%, 25% and 19%, respectively. High host-microsymbiota specificity was found between soybean and Bradyrhizobium japonicum strains. The BOX-PCR assay proved that from the six *Bradyrhizobium* strains of the inoculum only one was able to develop efficient symbiosis with soybean. Symbiotic efficiency of 25 strains, isolated from the field, was confirmed in a pot experiment. Linear relationship was found between nodule numbers and nitrogen content of the aboveground biomass. The phosphorous and boron uptake was significantly related to the nitrogen content of the biomass.
The high specifity of soybean for *Bradyrhizium* species is known for a long time. However, on the basis of our experimental results, this specifity could be characterised on subspecies level. From the six *Bradyrhizobium* strains of the inoculum only one was capable to infect the applied soybean variety. Our results draw the attention on the necessity of soybean variety specific inoculum development.
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REACTION KINETICS MODEL OF PROTEOLYTIC PROCESSING DURING HIV-1 VIRION MATURATION

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The cleavage of Gag-Pol polyproteins by the viral protease (PR) is crucial for the production of infectious HIV-1

virions, and inhibitors of the process have been among the most effective anti-HIV-1 drugs. The process has several layers of complexity (multiple cleavage sites and substrates; auto-cleavage), which calls for a systems level approach to identify key vulnerabilities and optimal treatment strategies. We here present the first full reaction kinetics model of proteolytic processing by the HIV-1 protease.
We developed a reaction kinetics model involving all canonical cleavage sites of Gag and Gag-Pol processing, partially cleaved substrates, intermediate enzyme forms, enzyme dimerization dynamics and the initial auto-cleavage of full-length Gag-Pol. Parameters of the model were compiled by extensive literature mining. The effect of uncertainties in the parameters and the relative importance of each parameter in the cleavage process were assessed by sensitivity analyses (both local and based on parameter space sampling). Virion maturation was identified by the concentrations of CA (required for capsid formation) and CA-p2 (considering its trans-dominant inhibition effect).
The model yielded a realistic time frame for virion maturation (~16 minutes) with the default parameters (gleaned from empirical data, rather than optimized for the outcome). The strongest effects on the time course of the cleavage reactions were associated with the rate of initial autocleavage and the rate of cleavage at the CA/p2 cleavage site. We also investigated the effect of varying combinations of parameters.
We identified key parameters that have the strongest effect on HIV-1 proteolytic processing and might therefore provide optimal targets for future drug development. The effect of combinations of drugs and/or mutations (with estimated effects on the kinetic parameters) can also be predicted in the model.

SPATIAL DISTRIBUTION AND DIVERSITY OF WATER AND SEDIMENT MICROBIOTA OF LAKE HÉVÍZ REVEALED BY DENATURING GRADIENT GEL ELECTROPHORESIS

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Lake Hévíz is the largest warm water natural lake of Europe. The curative mud and water of the lake harbors special bacterial communities. The microorganisms living in the water and sediment may play important roles in the preservation of the natural state and the curative effect of the lake, although their species composition is hardly known yet. The aim of the present study was to gain information about the spatial distribution and diversity of bacterial communities using cultivation independent methods. Filtration of water samples for DAPI cell count detection by microcopy was also carried out. First sampling was made vertically in the water column above the crater from the water surface to 29 m depth in November 2009. On the basis of Denaturing Gradient Gel Electrophoresis (DGGE) analysis no differences were found among the structure of bacterial communities from the different water depths probably due to the strong stirring effect of the water stream. In April 2010 14-14 water and sediment samples were taken from different locations of the lake. According to the results of DGGE analysis, community compositions of sediment and water samples were clearly distinguishable; furthermore samples from near the crater and shore could be separated from each other in case of both sample types. Five dominant DGGE bands were cut out and identified by sequencing. Two of the five sequences showed the highest similarity to Burkholderiales within Betaproteobacteria, other sequences were related to Cyanobacteria, Actinobacteria and Alphaproteobacteria.

NEW SCREENING METHODS IN MYCOTOXIN BIODEGRADATION AND METABOLISM RESEARCH

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Aflatoxins are the secondary metabolites of *Aspergillus* spp. Out of aflatoxins the B1 type is the most hazardous that have mutagenic, carcinogenic, teratogenic, immunomodulant and cytotoxic effects. This complex micropollutant can be found in food and feed as well, thus representing environmental and health safety problems. Therefore, detection and biodegradation of this mycotoxin are getting more important nowadays.
Besides several advantages of biodegradation, application-risks of the bioprocess also exist. Harmful cleave products are possibly created that can not be followed up by traditional methods. Parallel to expensive immunochemical and analytical methods biotests can be alternative screening methods to examine degradation

processes. For monitoring mycotoxin degrading microbes we adopted a luminescence and a colorimetric assay to analyse cytotoxicity and genotoxicity of aflatoxin B1. *A. fischeri* luminescent bacterium is one of the most sensitive ecotoxicological test-organism, however the standard test is not targeted at mycotoxins. Nevertheless, a modified *A. fischeri* method is suitable to detect aflatoxin B1 at as low as 1 μg/ml range. In addition, the SOS-Chromotest is appropriate to detect aflatoxin B1 yet in 0,078 μg/ml, hence it can be proper for food and feed safety purposes as well. In consequences these biotests are relevant methods to improve biodegradiation processes and to select the best mycotoxin degrading microbes with the less harmful end products. As these methods are cost-effective and rapid systems, they are suitable tools to investigate mycotoxin metabolism and to create genetically improved microbe clones by screening mutagenesis clone libraries. We adopted the *A. fischeri* system and the SOS-Chromotest to analyse degradation potential of different microbes including *Rhodococcus, Brevibacterium, Streptomyces, Pseudomonas, Microbacterium, Arthrobacter, Gordonia, Chryseobacterium, Paracoccus, Pseudoxanthomona* and *Arthrobacter* species, respectively. We could successfully select the best aflatoxin B1 degrading strains by the use of the two biotests. Moreover our results were confirmed by parallel chemical analytical and ELISA tests. Our results pointed out the necessity of such biotests as remained cytotoxicity and genotoxicity were revealed even after great toxin degradation. This finding also underlines the importance of using diverse biotests, since they analyse different effects: cytotoxicity and genotoxicity.
Based on these results we have already started a thorough screening project of thousands of clones from UV- and transposon-mutagenesis clone libraries. Our aim is the selection of clones with improved aflatoxin degradation ability and simultaneous identification of the key enzymes.
This study was supported by the NKTH TECH_08-A3/2-2008-0385 (OM-00234/2008) MYCOSTOP grant and KMOP 1.1.1.-07/1-2008-0002 project

INTERPRETATION OF SUSCEPTIBILITY RESULTS OF *STENOTROPHOMONAS MALTOPHILIA* CLINICAL ISOLATES

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Stenotrophomonas maltophilia is a multiresistant nosocomial pathogen causing infections typically in the lower respiratory tract. It represents a tremendous challenge since due to difficult intrepretation of susceptibility results it is very hard to successfully treat infections caused by this organism. Even ten years ago it was still established that all the strains were susceptible for sulfamethoxazole/trimethoprim. The susceptibility of S. maltophilia for sulfamethoxazole/trimethoprim together with its resistance to carbapenems used to be considered as a typical feature for phenotypic identification of this bacterium.
Seventy-two *S. maltophilia* clinical isolates (all of them isolated in our laboratory) were investigated with different methods: disc diffusion tests, E-tests and microdilution methods have been performed. In certain cases we got significantly different results with different methods which highlights the importance of choosing the adequate method. The incidence of *S. maltophilia* strains resistant to sulfamethoxazole/trimethoprim combination is highly increasing: the rate is 11,32 % among our isolates (between 2004 and 2009 it has increased from 3,2 to 8,7 %, respectively). About 80 % of strains showed resistance to ciprofloxacin by disc diffusion whereas all of them were resistant by microdilution method. For levofloxacin this ratio was 22 % by disc diffusion, but reading after 24 hours microdilution results showed resistance in approximately one half of the strains and after 48 hours over 90 % resistance based on EUCAST guidelines. These data suggest that levofloxacin should be suggested for treatment of infections caused by *S. maltophilia* (after careful susceptibility testing) and ciprofloxacin has much weaker activity against this organism. Based on preliminary results moxifloxacin seems to be as effective against *S. maltophilia* as levofloxacin.
Although in certain interpretation systems minocycline is suggested for testing, this drug is not available in Hungary now but tigecycline as a minocycline derivative should be tested in case of *S. maltophilia* infections since it may be used an effective agent against this organism in the future. Disc diffusion showed resistance to tigecycline in 37,1 % of the strains after 24 h and in 92,9 % after 48 h whereas microdilution results indicated resistance to tigecycline only in 20 % of our isolates, approximately.
In infections caused by extremely resistant strains polymyxins seem to be the last choice of therapy. The rate of resistance to polymyxin B and colistin is quite high and increasing as well: over 50 and 60 % by disc diffusion method. By microdilution over 80 % of our strains turned out to be resistant to polymyxins.
It is still not clear which susceptibility testing method would be the most reliable in the daily routine. Based on our results we suggest to perform E-test and microdilution method in routine microbiological laboratories in order to gain clinically relevant susceptibility results for S. maltophilia strains. We can suggest levofloxacin, moxifloxacin and sulfamethoxazole/trimethoprim for routine susceptibility testing.

INVESTIGATION OF POLYMYXIN-RESISTANCE IN *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES

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Due to worldwide use of antibiotics newer and newer resistance mechanisms are being developed in bacteria, especially among nosocomial pathogens. Gram-negative nonfermentative microorganisms such as *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Stenotrophomonas maltophilia* represent a tremendous therapeutic challenge. *P. aeruginosa* is an important opportunistic pathogen showing intrinsic resistance to a huge number of antibiotics: only piperacillin/tazobactam, ceftazidime and 4th generation cefalosporins, carbapenems, ciprofloxacin (and levofloxacin) and aminoglycosides may have therapeutic effect in infections caused by *P. aeruginosa*. Since the frequency of multiresistant strains among clinical isolates is highly increasing, polymyxins (i.e. colistin) have to be administered as last choice of therapy. Many *P. aeruginosa* clinical isolates turn out to be resistant even to polymyxin group of antibiotics resulting in infections that can not be specifically treated in the lack of effective antimicrobial agents. The main molecular mechanisms leading to elevated MIC-values for colistin are the following: (1) efflux pumps in plasma membrane that are able to remove polymyxins from the bacterial cell as well as other antibiotics, (2) alterations in outer membrane: covalent modifications of lipid A (addition of 4-amino-4-deoxy-L-arabinose), (3) production of protease enzymes directly degrading polymyxins and (4) overproduction of outer membrane protein OprH.

We have investigated the antibiotic susceptibility of 6 colistin-resistant P. aeruginosa isolates with different methods: by VITEK automated system, E-test, microdilution and agar dilution. Six of the investigated strains were resistant to colistin whereas two strains were multiresistant but susceptible for colistin. We also used two control strains: PAO1 and ATCC 27853 being susceptible for colistin. Characterization of molecular mechanisms resulting in polymyxin B and colistin-resistance in clinical *P. aeruginosa* isolates is ongoing: we have investigated the effects of inhibition of efflux pumps with different agents (CCCP and PAβN). MIC values for colistin were decreased at least with 2 orders of magnitude by CCCP in 6 of all strains, and in 5 of all strains by PAβN. MIC values for polymyxin B were decreased at least by CCCP or PAβN in 7 of all strains. Surprisingly in 2 strains PAβN has increased colistin MIC values with 2 orders of magnitude. In certain strains these efflux pump inhibitors showed significant effects on the MIC-values of other antibiotics (especially fluoroquinolones). Western-blot for the detection of OprH outer membrane protein overproduction and mass spectrometry for the analysis of covalent modifications in lipid A are under preparation.

Susceptibility results gained by using VITEK2 system or disc diffusion method should be verified in case of certain multiresistant bacteria. Microdilution method seems to be the most reliable in these cases; based on our results this methods seems to be adequate to double-check or even to control susceptibility results in routine laboratories. It is important to notice that one of our colistin-resistant strains showed susceptibility for some other antimicrobial agents; therefore we may have to reconsider the opinion that polymyxin-resistance occurs in strains resistant to all other anti-pseudomonal agents.

LABORATORY DIAGNOSIS OF HUMAN TOXOPLASMOSIS ET THE DEPARTMENT OF PARASITOLOGY NATIONAL CENTER FOR EPIDEMIOLOGY, BUDAPEST, HUNGARY

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Toxoplasma gondii is the organism responsible for toxoplasmosis. The natural life cycle of *T. gondii* occurs in cats and small rodents, although the parasite can grow in the organs (brain, eye, skeletal muscle, etc.) of any mammal or birds. *Toxoplasma* has worldwide distribution and 20%-75% of the population is seropositive without any symptomatic episode. 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. However, the infection poses a serious threat in immunosuppressed individuals and pregnant females. 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. Laboratory methods greatly improve the diagnosis. Routine *in vitro* diagnostic methods detect humoral immunity to *T. gondii* (ELISA IgG, IgM and IgA; 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)]. Evaluation and interpretation of serological results are not always easy and simple. At the Department of Parasitology NCE (Budapest, Hungary) we use the protocols for laboratory diagnosis of toxoplasmosis and *Toxoplasma* screening during pregnancy approved by National Advisory Board of Medical Microbiology.

A YEAST ORPHAN GENE

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As *Sch. pombe* is an attractive model organism for functional and comparative studies of eukaryotic cell processes, its genome has been sequenced and about 1200 genes out of the total 4,824 have been experimentally characterised. 344 uncharacterised genes are sequence orphans, whose functions cannot be predicted from similarity to genes in other organisms. Our earlier experiments have revealed, that one tiny orphan gene (SPAC212.02, chromosome I.) is a target of the transcriptional regulators med31 and med8 (1) which control the activity of large groups of genes of diverse functions. Computer analysis of its sequence suggests that SPAC212.02 does not have any homologue in other species. Interestingly, it has a copy on the Sch. pombe chromosome II. SPAC212.02 must be a functional gene, as its mRNA level is increased under stress conditions and in meiotic mutants (2,3). Its copy seems to be silent.
To reveal the function of SPAC212.02, we amplified it with flanking chromosomal regions and cloned the resulting PCR product into a *Sch. pombe* vector. We then deleted the coding region from the cloned fragment and replaced it with the ura4^{+} gene. The construct, which contained the chromosomal flanking regions of SPAC212.02 and ura4^{+}, was then transformed into a ura4-D18 strain to replace the chromosomal wild-type SPAC212.02 gene with the in-vitro constructed deletion allele. Transformants were selected for uracil prototrophy. Several transformants were picked and restreaked on selective media. Their examination is underway. Results of the phenotypic characterisation will be presented.
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OCCURENCE OF UNUSUAL AND VACCINE TARGETED ROTAVIRUS STRAINS IN A MULTICENTRIC SURVEY IN HUNGARY

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Vaccination is the main strategy to control severe dehydrating gastroenteritis caused by rotaviruses in early childhood. After recent introduction of new generation rotavirus vaccines in several countries worldwide, post-vaccination strain surveillance has a priority to assess the impact of vaccines on rotavirus strain prevalence. One of the most important open questions is the evolution of strains not included in the vaccines. The two current attenuated oral vaccines contain the globally most common human G1, G2, G3, G4, and P[8] genotypes. Nevertheless, another genotypes, such as G9 and G12 have emerged worldwide and spread throughout the world in a short period of time. A post-vaccination surveillance study was conducted in different part of Hungary, during the period between january 2007 and april 2010. Rotavirus positive stool samples were collected from patients mostly under 5 years of age with acute gastroenteritis. Extracted double-stranded RNA was subjected to

genotyping using multiplex RT-PCR assay. Type specific primers targeted to globally important G and P specificities were applied in our assay. Sequence analysis of VP7 and/or VP4 genes from selected nontypeable and unusual strains was performed to clarify the origin and the phylogenetic relatedness of these rotaviruses.
Of the 1712 rotavirus-positive samples, both G and P type specificities could be assigned for 1607 strains. Circulation of globally common genotype combinations was obtained with dominating prevalence of G1P[8] (42.23%) strains, followed by G4P[8] (26.52%), G2P[4] (12.21%) and G9P[8] (6.13%). Reassortments of common types (G1P[4], G2P[8], G9P[4]), minority endemic types (G3P[9], G4P[6], G6P[9]) and additional possible human-animal reassortant strains (G1P[6], G2P[6], G9P[6], G9P[4],G8P[8], G10 P[6], G12P[6], G12P[8]) were identified in the study period. Depending on the vaccination coverage achievable in the forthcoming years, rotavirus vaccines may help to decrease the socio-economic burden associated with rotavirus gastroenteritis. The sequencing and phylogenetic analyses of rotaviruses not covered by the recent vaccines can help us to understand the evolution of these strains and to answer the question, whether these rotavirus genotypes would be able to fill the evolutionary gap due to the pressure of vaccination.

STAPHYLOCOCCUS AUREUS CARRIAGE IN HEALTHY 3RD-YEAR STUDENTS OF SEMMELWEIS MEDICAL UNIVERSITY, BUDAPEST

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The carriage of pathogenic bacteria such as *Staphylococcus aureus* in healthy individuals is well known, with a 25-30% prevalence. In this study we surveyed the nasal carriage of students of a medical university, who already attend hospital wards, so they can be a potential source of infection. This is the first study of this kind in Hungary. Eighty-eight *S. aureus* isolates were collected from the nasal passages of the students attending Semmelweis University, Budapest, Hungary. The species identity was confirmed by colony morphology, catalase test, Pastorex test (Bio-Rad) and nucA PCR. MRSA strains were screened by mecA PCR. The antibiotic sensitivity was determined by E-test, applying the EUCAST breakpoints. The genetic relatedness of 56 strains was examined by PFGE. Altogether 300 3rd-year students (205 Hungarian and 95 non-Hungarian) were sampled on a voluntary base. The overall *S. aureus* carriage rate was 29.3%, little higher among the Hungarians (31.7%) than in the non-Hungarian group (24.2%). On one occasion carriage of two unrelated strains was detected. Out of the 88 strains, only 2 carried the mecA gene, but these had oxacillin MICs of only 0.75 and 2 mg/L, respectively. The strains were fully sensitive to gentamicin, ciprofloxacin and vancomycin. There were 9 isolates with high-level (MIC $\geq$ 256 mg/L), and 3 with low-level (MIC = 12-32 mg/L) erythromycin resistance. Only 1 isolate was resistant to clindamycin. Based on the PFGE pattern, 3 clones comprised approximately half of the strains (15, 14 and 6 strains, respectively), but the rest were rather diverse.
The *S. aureus* carriage rate correlates well with international data. Luckily there were only 2 MRSA strains (2.3%). The one with the higher MIC (the EUCAST breakpoint is >2 mg/L) was highly resistant also to erythromycin, but the other strain was fully sensitive to all tested drugs. The other isolates were generally very sensitive, which is characteristic in the case of carried pathogens. The strains proved to be genetically more diverse than the usual MRSA populations, with only a few smaller clones, indicating the presence of several independent strains. This, and the dissimilarity of isolates within the same groups, indicate that there is no extensive exchange between the students flora.

SELECTION FOR ACTINOMYCETES CAPABLE FOR BIOREMEDIATON OF HYDROCARBON-POLLUTED SOIL AND PROTEOMIC INVESTIGATION OF THE MOST EFFICIENT STRAIN

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The industrial use and transport of mineral oil and its derivatives are the most common sources of the hydrocarbon-pollution of natural habitats. The elimination of these pollutants is problematic and sometimes the applied processes are responsible for new damages. In this view we have to consider the self-regenerating abilities of nature. There are several microbes which are capable to degrade oil, and the selection of such natural strains and their introduction into polluted areas as members of a consortium can be the optimal solution in many

cases. Since Actinomycetes produce variable types of enzymes in order to degrade diverse classes of substrates like hydrocarbons, organic polymers, xenobiotic compounds, herbicides, they play an important role in waste removal and recycling of materials in nature. In our laboratory several hexadecane consuming bacterial strains - *Streptomyces* and *Rhodococcus* – had been isolated from soil contaminated with mineral oil. These different strains could decompose 33 to 80 % of hexadecane in polluted soil in laboratory scale experiments, depending on the applied strains or the type of soil. The most efficient decomposer was the strain ADB5, a *Rhodococcus* species, which proteome was also investigated. We isolated proteins from cells cultured in a control or in an n-hexadecane containing liquid medium and applied them onto 2-D gels. The differences of the two proteomes were analyzed by PDQest software (BioRad), and the proteins showing at least 2 times induction or reduction ratio were identified by mass spectrometry. We found that the use of hexadecane as a carbon source elevated the synthesis of several enzymes involved in the glyoxalate and tricarbonic acid cycle and also the synthesis of enzymes which are somehow in connection with fatty acid degradation, carbohydrate metabolism or other biosynthetic pathways using or liberating acetyl groups were up-regulated.

CHARACTERIZATION OF ESBL PRODUCING BACTERIA ACCORDING TO THE SPECIES AND ORIGIN OF SAMPLE BETWEEN 2005 AND 2009 IN THE INSTITUTE OF CLINICAL MICROBIOLOGY, UNIVERSITY OF SZEGED

ANDREA LÁZÁR, MARIANN PAPPNÉ ÁBRÓK, EDIT URBÁN

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Since the beginning of 1990-s., the increase in the number of the ESBL producing bacteria is getting greater and greater problem every year worldwide. In this sudy, we examined the frequency of prevalence the ESBL producing bacteria isolated in our lab between 2005 and 2009 according to the species and the prevalence of strains isolated from urine sample. We characterized separately the prevalence of ESBL producing strains isolated from inpatients and outpatients. ESBL production was examined using double-disc synergy test. The isolated strains were identified using conventional biochemical tests and VITEK 2 system. From one patient we considered one isolate.

	2005	2006	2007	2008	2009
Inpatient	91	37	57	140	261
Urine %	18%	32%	24%	57%	33%
Outpatient	36	16	20	44	78
Urine%	53%	75%	65%	72%	76%
Totall	127	53	67	188	339

	2005	2006	2007	2008	2009
Inpatient total	91	37	57	140	261
Klebsiella sp.%	68%	48,60%	56%	38%	43%
E. coli %	6%	16%	24,60%	32%	26%
Enterobacter%	23%	24%	12%	29%	26%
Other%	3%	11%	7%	1,40%	2%
Outpatient total	36	16	20	44	78
Klebsiella sp.%	70%	25%	35%	45%	59%
E. coli %	19,40%	50%	45%	48%	36%
Enterobacter%	11%	19%	5%	6%	5%
Other%	0	6%	15%	0	0

During five years, we experienced enormous increase in the number of the ESBL producing bacteria among inpatients and outpatients too. The prevalence of ESBL producing bacteria among the strains isolated from urine samples did not show alteration in this five years. We found that the prevalence of ESBL producing bacteria from isolated from urine samples was higher in outpatients than in inpatients. Among inpatients, the prevalance of *Klebsiella* spp. was decreased while the frequency of *E. coli* was increased.

Among the outpatients except for the year 2005 the frequency of *Klebsiella* spp. was sligthly increased while the prevalence of *E. coli* did not change especially. The ratio of *Enterobacter* spp. except for year 2006, when there was an increase remained costant.

STUDYING THE DYNAMIC BEHAVIOR OF STEREOISOMERIC FORMS OF THE ANTIMICROBIAL PEPTIDE INDOLICIDIN

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Indolicidin is an antimicrobial tridecapeptide with a remarkable primary structure (i.e. H-IL**P**WK**W****P****WW****P**WRR-NH_2), consisting of five Trp and three Pro amino acids, as well as of three basic (i.e. Lys and Arg) and two apolar (i.e. Leu and Ile) residues. This peptide was isolated from the bovine neutrophils, and it shows a broad spectrum of antibacterial and antifungal activities, however, it exhibits also hemolytic and antiviral effects. Since indolicidin contains three Pro amino acids, it exists as an equilibrium mixture of different stereoisomeric forms, and in accordance with the cis-trans isomerism about three Xaa-Pro peptide bonds, eight distinct stereoisomers of this peptide could be distinguished. In the present study, molecular dynamics (MD) simulations were performed on all stereoisomeric forms of indolicidin, in order to characterize their dynamic behavior, and to investigate the influences of cis-trans isomerism on the conformational features of this antimicrobial peptide. Based on the MD calculations, the alterations of structural properties were examined as a function of time, and it was characterized how the structural features change during the MD trajectories. The dynamic behavior of stereoisomers was studied by monitoring of the characteristic torsion angles of backbone and side-chains, furthermore, RMSD values defined on the different sets of atom types were calculated. In the case of each stereoisomeric form, the appearance of secondary structural elements (i.e. various β-turn and helical structures) and other non-conventional structural motifs was investigated. Nevertheless, the presence of intramolecular interactions played a stabilizing role in the case of different conformational states was examined, which were as follows: 1) H-bonds between the NH donor and CO acceptor groups; 2) aromatic-aromatic interactions between the side-chains of Trp amino acids; 3) proline-aromatic interplays between the pyrrolidine rings of Pro and the aromatic rings of Trp residues; 4) cation-π interactions between the positively charged side-chain groups of basic amino acids and the aromatic rings of Trp residues. On the basis of the MD trajectories, representative conformational states were identified for all stereoisomeric forms, and their three-dimensional structure was characterized in detail, especially with regard to the secondary structural elements and the stabilizing intramolecular interactions. The MD simulations carried out on eight stereoisomers of indolicidin indicated that conformational similarities and dissimilarities could be observed between the stereoisomeric forms, which proved to be dependent on the cis or trans nature of Xaa-Pro peptide bonds.
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STRUCTURAL CHARACTERIZATION OF PALINDROMIC ANTIMICROBIAL PEPTIDES RICH IN APOLAR AMINO ACIDS

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Several antimicrobial peptides (AMPs) possess a characteristic feature with regard to their sequences, namely they contain palindromic segments with various lengths. In the case of these AMPs, the palindromes can play an important role not only in the determination of their three-dimensional (3D) structure but also in their biological effects. Some of these palindromic peptides are rich in apolar amino acids, such as decoralin and a few members of the temporin peptide family. Decoralin (H-SLLSLIRKLIT-OH) was identified in the venom of the solitary eumenine wasp Oreumenes decoratus, while four temporins, i.e. temporin C (H-LLPILGNLLNGLL-NH_2), temporin 1Pra (H-ILPILGNLLNGLL-NH_2), temporin 1TSb (H-FLPLLGNLLNGLL-NH_2) and temporin 1DYa (H-FIGPIISALASLFG-NH_2), were isolated from the skin of frogs belonging to the genus *Rana*. For the palindromic segments of decoralin and temporins, a comprehensive structural characterization was carried out by means of three different molecular dynamics methods, including simulated annealing (SA), simulated annealing

coupled replica exchange molecular dynamics (SA-REMD) and molecular dynamics (MD) simulations. The SA protocol was first applied to explore the conformational spaces of palindromes, then SA-REMD calculations were performed to identify their low energy conformations, and finally MD simulations were carried out to investigate their evolving characteristic structures as a function of time. In the case of conformational states supplied by the MD calculations, the occurrence of various secondary structural elements, as well as the presence of different intramolecular H-bonds were examined. For each palindrome, types I and III β-turns appeared along the entire sequence of peptides, however, helical conformations with different lengths were also detected. Beside these secondary structures, in the case of the palindromic segments of temporin C, temporin 1Pra and temporin 1TSb, types II and II' β-turns were observed in certain tetrapeptide units, and additionally, Asx-turn structures were also identified. The majority of above-mentioned secondary structural elements were found to be stabilized by characteristic H-bonds. Among these intramolecular interactions formed between the backbone atoms, the i←i+3 H-bonds played a role in the stabilization of β-turn and 3_{10}-helical structures, while the i←i+4 H-bonds contributed to the structural stability of α-helical conformations. Nevertheless, the i←i+2 H-bonds evolved between the side-chain and backbone atoms played a stabilizing role in the case of Asx-turns. Based on the results of our MD simulations, it could be concluded that the 3D structure of the palindromic segments of decoralin and temporins could be characterized by turn and helical conformations.

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ENZYME PRODUCTION AND FIBER BIOCONVERSION OF *THERMOMYCES LANUGINOSUS* AND *PICHIA STIPITIS* ON RAPESEED CAKE SUBSTRATE

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Oil cake is a byproduct of the extraction of oil from oil seeds. Oil cakes have high nutritional value, as they have a high protein content. They are commonly used in animal feeding. The use of rapeseed cake in animal feeds is limited due to toxicity problems caused by the relative high glucosinolate content and digestibility problems due to the high fiber content[1]. Rapeseed cake is a potential substrate for microorganisms thus microbial conversion is an option to improve its digestibility. The fiber content can be decreased by hemicellulase-producing microorganisms. Thermomyces lanuginosus is a thermophilic filamentous fungus [2] which produces high levels of ß-xylanase both in shake–flask and bioreactor cultivations [3]. Pichia stipitis can also use xylose as substrate [4]. Filamentous fungi are potent tools in solid-state fermentation (SSF) also. Other fungi, like *Aspergillus* sp. and *Phanerochaete chrysosporium* can produce cellulases [5, 6]. Our aim was to examine if the mentioned fungus species are able to grow on rapeseed cake substrate in bioreactor cultivation and produce xylanase and proteolytic enzymes in SSF. Shake-flask and bioreactor experiments showed that the rapeseed cake provide sufficient nutrients for the growth of *T. lanuginosus*. *Thermomyces lanuginosus* NCAIM 001288 and Pichia stipitis NCAIM Y.00810 strains were cultivated on pretreated (boiled or NaOH-treated) rapeseed cake. To detect the helmicellulose and cellulose content of the submerged samples we measured their fiber fractions (ADF, NDF, ADL). A 20-60% decrease in the hemicellulose content was detected in the case of the following samples: boiled or alkali treated rapeseed cake inoculated with *P. stipitis*, and rapeseed cake fermented with T. lanuginosus in shake-flask (24 h) or bioreactor (48 h). A 30-40% decrease in the cellulose content was detected in the case of the following samples: boiled or alkali treated rapeseed cake inoculated with *T. lanuginosus*, and boiled or untreated rapeseed cake fermented with *P. stipitis*. Solid-state fermentation was carried out according to the method of Vig and Walia[1]. The media included ground rapeseed cake containing 5%, 10% and 20% agroindustrial lignocellulose material (ALM) and was inoculated with *T. lanuginosus*. During the one-week experiment xylanase and protease activities, reducing sugar content and fiber fractions of the 3, 6 and 7-day-old samples were measured. On the 7^{th} day of the experiment, a 25% decrease in the cellulose content and a 10% decrease in the hemicellulose fraction was observed. The highest xylanase(5700 U/g) and protease(11 U/g) activity was shown by the samples with 10% and 20 % ALM content.

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

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Rapeseed cake is a potential substrate for microorganisms. Our previous experiments showed that the rapeseed cake provide sufficient nutrients for the growth of Thermomyces lanuginosus in solid state fermentaiton (SSF). In laboratory trials Thermomyces lanuginosus produced high xylanase activity (5700 U/g) on rapeseed cake media containing 20% agroindustrial lignocellulose material (ALM). Scale-up studies were carried out in pilot plant trials under semi-sterile conditions. The rate of contamination was higher than in the case of the sterile laboratory trials. The appearance of contaminant microorganisms suppressed the development of the fungus and blocked the enzyme production (200-300 U/g).
Our previous results showed that the combined use of zinc- and iron- glicinate chelates has a synergic effect on the growth and enzyme production of the fungus probably by inhibiting the growth of unwanted microorganisms. SSF experiments were carried out adding zinc- and iron- glicinate chelates in 50 ppm zinc and 150 ppm iron concentration to the media. The fungal cultures tolerated the presence of metallic ions. The xylanase activity increased slowly but was higher on the fifth day than without the addition of trace elements.
According to these results solid state fermentation was accomplished in field trials. Admixture of trace elements suppressed the contaminant microorganisms and increased the xylanase activity (3000 U/g).
This work has been supported by the OMFB-01136/2009 (EUREKA) grant of the National Development Agency, Hungary.

ARE ITS SEQUENCES SUITABLE FOR DETECTING INTERSPECIFIC POLYMORPHISM OF *MONILINIA LAXA*?

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Monilinia spp is an economically important ascomycete fungal group infecting stone fruit crops. One of its members, *Monilinia laxa*, causes blossom and twig blights and fruit rot as well. As morphological variances among *M. laxa* isolates are well known in in vitro culture [1], genotypic differences may also exist among isolates. Therefore, aim of our study was to investigate that ITS sequences located between 18S and 28S rDNA regions (as a conservative region of the fungus) may suitable to manifest interspecific polymorphism of Hungarian populations of *M. laxa*. 24 isolates of *M. laxa* were collected from all over Hungary (2, 14, 3, 2, and 3 from Hajdú-Bihar, Békés, Szabolcs-Szatmár-Bereg counties, Sopron, and Balaton regions, respectively) originated from plum and peach trees. Each isolate was prepared from exogenous stromata of rotted fruit with sterile needle. Conidia were cultured on potato dextrose agar (PDA) incubated at 22°C. After the genomic DNA extraction, ITS sequences of the isolates were identified by fragment size of PCR products [2]. The 356-bp products for 24 isolates were sequenced. ITS sequences of our 24 isolates were identical. However, in the multiple alignment our ITS sequences were different from one isolate of the study of Petróczy [3]. In this case of

their sequences, there was a guanine at 215-bp while an adenine was present in all our isolates at the same site. The sequence difference is likely to be a sequencing error which might originate from inappropriate visual analysis of the sequencing chromatograms. According to our results we believe that there are no remarkable interspecific variations in the ITS sequences located between 18S and the 28S rDNA regions of M. laxa.
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LONG-TERM EFFECT OF BENTONITE TREATMENTS ON SOME QUANTITATIVE AND QUALITATIVE SOIL BIOLOGICAL PARAMETERS

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Bentonite, the three-layers motmorillonite type of clay, can be used as soil improving material in sandy soils. This soil type has a lack of organic and inorganic colloids, therefore the bentonite is suitable for improving the soil quality by increasing its clay content. The 0-5 cm ground bentonite fraction was applied in a small-plot experiment, at 0, 5, 10, 15 and 20 $t.ha^{-1}$ rates in the plough layer of a slightly acidic sandy soil in Nyíregyháza, Hungary. After three years of bentonite application the quantitative parameters of the colony forming units (CFU) of heterotroph, oligotroph, sporoform, and free-living N_2-fixing bacteria and the microscopic fungi were assessed on selective agar plates (normal or 100X diluted Nutrient agars, Nutrient with 95 ^oC, 10 minutes-treated soil-suspension, Ashby and Rose bengale agars), respectively. Beside this the activities of invertase and catalase soil enzymes were measured. The qualitative parameters, such as the identification of various bacteria from the selective plates by API tests, their growth characteristics and the CO_2 release from control and 10 $t\ ha^{-1}$ bentonite treated plots were measured by infrared gas analyser. The beneficial effect of bentonite amendments could be developed mainly on a long-term base, through the structural changes of the organo-mineral complexes of the sandy soil and the interactions between the clayi and sandi particles. The quantitative parameters were found to be less sensitive after the three-years of repeated applications, than the measured qualitative ones. The microbial counts (CFUs) and the enzyme activities were similar in the control and the treated plots while there were significant differences found in the community structure and the CO_2 release. The results suggested positive changes on the composition of microbial communities of sandy soil, by the increasing the numbers of bacteria species and genera, capable for the synthesis of some plant growth promoting (PGPR) and/or plant growth regulating (PGR) materials. The optimal amounts of the bentonite addition, therefore directly and indirectly can improve the low quality of the sandy soils.
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DEVELOPMENT AND EVAUATION OF METHODS FOR THE DETECTION OF *BRETTANOMYCES* IN WINE

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Yeasts belonging to the genus *Brettanomyces* are major cause of wine spoilage producing volatile phenols (4-ethyl phenol and 4-ethyl guaiacol), which impart a distinct odor (that winemakers describe as horsy, sweaty and like a wet-dog or simply as Brett). Such a wine faults of microbiological origin are highly prevalent and adds up to a substantial loss for the wineries. Although several techniques, such as sterilizing, filtering, use of sulphites, etc. exist for the elimination of Brettanomyces from wine, all methods can damage the quality, therefore, these treatments cannot be applied as preventive measures. By the time winemakers smell or taste Brett, it is usually too late to save the wine, thus development of diagnostic tests for the detection of *Brettanomyces* is crucial.
The primary objective of the present work was the development and evaluation of novel assays for the quantitative measurement of Brettanomyces and its chemical by-products in wine. For the detection of the Brettanomyces, a selective culture media and a new flow cytometric assay were developed. The flow cytometric

assay was compared to a commercial test (Partec OenoYeast kit) and to real-time PCR and plating results. Concentrations of the 4-ethyl phenol and 4-ethyl guaiacol were measured by GC-MS. A selective medium (yeast nitrogen base media supplemented with chemical compounds) was used for cultivation of different *Brettanomyces*/*Dekkera* strains. This medium completely inhibits the growth of *Saccharomyces* strains; therefore, the presence of *Brettanomyces* cells could be detected in the early phase of wine fermentation even if they represent only a small part of the yeast population. It also endows the colonies with pink color to facilitate detection. Plating on selective culture media is an easy-to-use approach for the winemakers to control the hygiene in the winery and to identify the possible sources of contamination. Flow cytometry offers a rapid, reliable and quantitative method for the analysis of wine samples; however, it is not suitable for the investigation of wine in the fermentation phase. GC-MS measurement of the concentration of phenolic by-products provides essential information for the winemakers by the selection of treatment techniques.
This comprehensive test system offers an efficient tool for the early detection of the spoilage problems as well as for the assessment of the microbiological quality of wines.

ANTIMALARIAL DRUG DISCOVERY AND DEVELOPMENT OF NEW IN VITRO ASSAYS FOR THE OPTIMIZATION OF ANTIMALARIAL THERAPY

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The spread of drug resistant *Plasmodium falciparum* malaria parasite has led to a significant increase of malarial morbidity and mortality, and a growing crisis in global public health. Thus, a major goal of malaria research is to find drugs with novel targets and a novel mechanism of action. In parallel, more research is needed to elucidate mechanisms of drug resistance, since new drugs should not only be effective, but should also evade mechanisms that contribute to parasite resistance.
The recently funded Add-Mal project is a French-Hungarian bilateral cooperation, which follows these general goals. The partners wish to characterize enzymes of the phospholipid synthesis in the plasmodiums identified as novel antimalaria targets, in order to discover new series of compounds that interact with these targets.
The fate of administered drugs may largely depend on their interactions with transporter proteins, which are present in all major pharmacologically relevant barriers. Such ADME considerations have to be taken into account also by the development of antimalarial drugs characterizing the interaction of candidate antimalarial agents with key human transporters influencing ADMETox properties. Furthermore, transporters are key determinants of antimalarial drug resistance of plasmodiums as well. Therefore, another objective of the project is the optimization of malaria treatment through the development of new in vitro test systems serving antimalarial drug discovery projects as well as the diagnosis of malaria. These new assays might facilitate antimalarial drug discovery projects and the characterization of plasmodium transporters.

WHOLE TRANSCRIPTOME ANALYSIS OF DIFFERENTIALLY TREATED *SALMONELLA TYPHIMURIUM* STRAINS USING SOLID NEXT GENERATION SEQUENCING

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Appearance of Next-Generation Sequencing technologies opened up many possibilities to perform global metabolomic analyses in an exceptionally accurate way. Due to its enormous throughput, the SOLiD platform is an excellent choice for whole genome resequencing projects and for gene expression analyses, either for whole transcriptome analyses (RNA-Seq) or SAGE applications (serial analyis of gene expression). The SOLiD platform can generate the highest number of reads, ten-millions of short tags of 35-75 nucleotides. Huge coverage can be achieved for microbial genomes, since the productivity of the system is 100 gigabases per run by now and increases rapidly (each base of an average bacterial genome of 5 Mbases is covered by 20 000 times in each run). This makes possible accurate quantitative analyses, which is essential for transcriptomics. SOLiD performs whole transcriptome analysis in digital way (digital gene expression analysis), the method is not dependent on previous knowledge of transcribed sequences in contrast to the microarray approach.
We have tested the capacity and accuracy of the system on various *Salmonella typhimurium* strains isolated from

industrial environment (meat factory). The strains were treated by various antimicrobial agents, like traditional antibiotics and novel antimicrobial peptides. The bacterial responses were analysed on different levels. Beside microbiological and biochemical assays (plating, MIC determination, membrane penetration, interaction studies), transcriptome analyses were also conducted using the SOLiD system. The results clearly showed rapid activation of stress-response functions. Detailed analyses of SOLiD data and conclusions will be discussed.

CHANGES OF SOIL PHOSPHATE CONTENT AND PHOSPHATASE ACTIVITY

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Soil phosphatases are essential enzymes in the cycling of phosphorus and indicators of soil changes.
Acid and alkaline phosphomonoesterase activity was examined on two soil types: calcareous sandy soil (Őrbottyán) and calcareous chernozem soil (Nagyhörcsök). The soil samples were taken from the 0–20 cm layer of the control plots (0 kg $N \cdot ha^{-1}$ and 100 kg $P_2O_5 \cdot ha^{-1} \cdot year^{-1}$) in November and May. The experiment was designed to detect changes in the AL-soluble P content and activity of acid and alkaline phosphomonoesterase as a function of soil type, sampling date and rape rhyzosphere. The AL-soluble P content of the sandy soil was greater in all cases than that of the chernozem soil, due to its smaller capacity for binding PO4 ions. The effect of the root system an increasing the AL-soluble P content could only be detected in the rhysosphere of chernozem soil, contrariwise seasonal differences could be detected on both soils. There were considerable differences in phosphatase activity between the soil types, the phosphatase activity of the chernozem soil being around three times that of the sandy soil. The positive effect of the rhyzosphere on the phosphatase activity was only perceptible in the May samples, when enhanced acid phosphatase activity was recorded on both soils, with increasing the AL-soluble P content in chernozem soil. Later results demonstrated importance of survived enzyme activity and role of other factors among others soil dissoluble carbon and moisture content.
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INFLUENCE OF THE POLLUTION TIME ON THE MICROBIAL BIOMASS C AND PHOSPHATASE ACTIVITY ON A HEAVY METAL POLLUTED SOIL

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Biological and biochemical properties, such as microbial biomass and enzyme activities are considered as indicator of soil contamination. There are increasing evidences of microbial biomass decrease due to metal contamination in soils. Generally both acid and alkaline phosphatase activities are inhibited by the heavy metal salts pollution. Different phosphatase reaction affecting heavy metals determined by metal or salt characters, metal concentration or solubility, and by term of pollution. Experimental site was located at a floodplain area along Toka-river south from Gyöngyösoroszi village (North-East Hungary) near an abandoned Pb/Zn mine. This area was flooded over and over with sediment contains heavy metals, and last previous sampling in 2004. Soil microbial biomass C and phosphatase activity was measured in 2004 and 2007 years.
Reduced soil microbial biomass C and enhanced acid phosphatase activity of polluted samples were recorded just after flooding, in 2004. Three years later in 2007, the soil microbial C was not differed between polluted and unpolluted soil samples, however biomass specific respiration showed an increasing, Cmic/Corg a decreasing tendency from unpolluted to polluted samples. Similarly the difference of the phosphatase activity between polluted and unpolluted samples decreased strongly. These results suggest an adaptation and resilience of soil microbial community over a subsequent sampling time.
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EVALUATION OF CLONAL DIVERSITY, BIOFILM FORMATION AND TYPE 3 FIMBRIA PRODUCTION BY EXTENDED SPECTRUM BETA-LACTAMASE POSITIVE *KLEBSIELLA PNEUMONIAE* ISOLATES

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Extended Spectrum Beta-Lactamase (ESBL) production by K. pneumoniae is a major health-concern world wide. Biofilm growth of bacteria is an important virulence factor; it promotes colonization of indwelling medical devices, hinders antibiotic therapy and may facilitate the spreading of antibiotic resistance genes via horizontal gene transfer. The type 3 fimbria plays a roll in the formation of the biofilm. In this study we performed molecular typing with the combination of ERIC-PCR, Random Amplification of Polymorphic DNA (RAPD) and plasmid profile analysis according to Kado and Liu to reveal the clonal relationships among 104 ESBL-positive K. pneumoniae strains isolated in the Laboratory for Diagnostic Microbiology, University of Pécs between 2004-2009. We also evaluated the capability of these strains to form biofilm in a static system and screened them for the presence of type 3 fimbria by agglutination of tannic acid treated bovine erythrocytes.
The 104 isolates could be arranged into 4 major clonal groups. The largest cluster (Kp-I.) contained 74 (71,2 %), the three smaller ones (Kp-II., Kp-III., Kp-IV.) included 12 (11,5 %), 8 (7,7 %) and 10 (9,6 %) isolates respectively. Most of the isolates harbored multiple plasmids. There wasn't any strain that carried no plasmids at all. The molecular weight of the plasmids ranged from 1 to 183 MDa. 77 (74 %) strains produced type 3 fimbria and 96 (92,3 %) formed biofilm. There was no significant difference among the four clusters in the aspect of biofilm growth. There were less isolates belonging to cluster Kp-II., Kp-III and Kp-IV being positive for type 3 fimbria production (50%, 25 % and 20 % respectively) than in the clonal group Kp-I. (90,5 %).
The detailed data of molecular typing based on ERIC-PCR and RAPD-analysis have already been presented in this forum in 2009. The present study has shown that the four clonal clusters of ESBL producing K. pneumoniae strains present in the Clinics of Medical School, University of Pécs differ in the aspect of at least one virulence factor, namely type 3 fimbria. Despite the differences in type 3 fimbria production, the majority of these strains irrespective of the grouping formed biofilm. This observation supports the assumption that type 3 fimbria is not the only factor involved in biofilm formation.

INVESTIGATION OF ANTIMALARIALS WITH TRANSPORTERS USING IN VITRO ASSAYS

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Options to control spread of malaria are increasingly limited due to emergence of parasites resistant to widely used antimalarials, therefore discovery of novel antimalarials appears crucial as ever. However, animal experiments are too expensive and laborous for the pharmacokinetic characterization of large number of compounds. The fate of administered drugs may largely depend on their interactions with transporter proteins, which are present in all major pharmacologically relevant barriers. Furthermore, transporters are key determinants of antimalarial drug resistance of plasmodiums as well. The aim of this study was to examine whether the high-throughput (HTS) membrane-based transporter assays can be applied to characterize the transporter interactions of candidate antimalarials.
Reference antimalarials, such as artemisinin, chloroquine, mefloquine, quinine, etc., have been tested for their interaction with the ABC-transporters MDR1, MRP1 and BCRP using the Solvo PredEasy ATPase kits. Results of the ATPase assays were correlated with the clinical observations on the tested antimalarials.
Our results showed that artemisinin is a substrate for MDR1, chloroquine is inhibitor of the MDR1 and substrate for the MRP1 and BCRP, mefloquine is substrate for the MDR1 but at higher concentrations is a not specific inhibitor of all the transporters and quinine is substrate for the MDR1. These results corresponded exactly to the clinical data on the antimalarials tested. We conclude that the membrane-based HTS in vitro assays can be applied to facilitate the ADME characterization of candidate antimalarials.

HHV-6 REACTIVATION IN RENAL TRANSPLANTATED PATIENTS

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Human herpesvirus 6 (HHV-6) is an important pathogen in renal transplanted recipients. It may cause disease or indirectly influence the immune functions and reactivation of other infections. 400 blood samples of 186 renal transplanted patients sent for human cytomegolvirus antigenaemia were studied to determine the frequency and the subtype of HHV-6 in blood samples of renal transplanted patients at different time after transplantation. Our aim was to reveal the possible connection of HHV-6 infection with HCMV reactivation and the clinical consequences. Presence, subtype and quantity of HHV-6 was determined by nested and real-time PCR. Connection of HHV-6 viremia to HCMV reactivation and clinical symptoms was analyzed statistically. HHV-6 viremia was detected in 26 samples (6.5%) of 13 patients (7 %).
Dominance of HHV-6A was observed [(23/26), 88.5 %]. HHV-6 DNA load in plasma samples ranged from 7.7 X 10^1 to 1 X 10^5 and 2 X 10^1 to 1.3 X 10^4 Geq / ml in DNA of leukocytes. Connection of HHV-6 viremia to HCMV reactivation and clinical symptoms was not proved with statistical analyses. HHV-6 viremia and the dominance of variant A was found early and late after transplantation. Low level of HHV-6 DNA load, negative HHV-6 PCR of leukocytes and higher proportion of HHV-6A positive patients then the incidence of chromosomally integrated HHV-6 suggest active HHV-6 replication.

COMPLEX MOLECULAR BIOLOGICAL INVESTIGATION OF TCE-CONTAMINATED MICROCOSMS TO REVEAL MICROBIAL COMMUNITY CHANGES WITH RESPECT TO ELECTRON DONOR AMENDMENTS

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We have successfully applied the multi displacement amplification (MDA) method to analyzing bacterial communities of chlorinated ethenes containing groundwater samples. Our results indicated that a pre-MDA step is useful for the better representation of community structure when molecular biological investigations are carried out. To validate the applicability of the method to a more complex system, we have also investigated a microcosm experiment with the objective to enhance reductive dechlorination of TCE contaminated groundwater using different electron donor amendments. The microcosms were monitored by geochemical and VOC parameters, as well as molecular biological methods. A detailed analysis was carried out for the initial groundwater sample and the different microcosms of day 210, which included VOCs and water chemical parameters, 16S rRNA gene based T-RFLP and DGGE, 16S rRNA clone library and group specific detection of dehalogenating bacteria, SRB and Archaea.
Our results can be summarized: (i) TCE was degraded to VC in all microcosms (ii) there was not a significant bio-degradative activity difference among the amendments, however sequence analysis indicated a remarkable difference of the key active members of the microbial communities (iii) statistical analysis revealed the very tight clustering of the pre-MDA and the DNA samples of each amendment (iv) the MDA analysis indicated a better correlation of the community structure and the group specific detections.
This work was supported by the Hungarian Scientific Research Fund (OTKA 75790).

TO IMPROVE ROUTINE SPECIES IDENTIFICATION AND DISCRIMINATION OF ANAEROBIC BACTERIA BY MALDI-TOF MS

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Anaerobic bacteria are important human pathogens most often causing mixed infections, which involve several aerobic and anaerobic bacteria in the same time. To isolate in pure culture and to identify them with classical

methods or with the help of automated micro-methods is time-consuming and may result in misidentification on species level. The Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has recently been introduced and validated in routine diagnostic laboratories for identification of aerobic bacteria isolated from clinical specimens. Only few studies deal with the use of this technique for human pathogenic anaerobic bacteria. Our aims were to investigate whether this technique can be used for identification of various anaerobic bacteria, how discriminative this technique is to differentiate closely related species and whether there are possibilities to type B. fragilis strains according to the presence of the cfiA gene responsible for carbapenem resistance. Altogether 504 anaerobic clinical isolates from different genera including *Bacteroides, Prevotella, Fusobacterium, Clostridium, Peptostreptococcus, Finegoldia* and some unidentified Gram-negative and Gram-positive anaerobic bacteria were investigated by MALDI-TOF MS in parallel with traditional identification methods and with rapid ID 32A ATB and API20 ANA (BioMerieux) identification systems. 95.2% of all isolates were unequivocally identified with MALDI Bruker Daltonic BioTyper software. Discrepant results were confirmed by 16S ribosomal DNA sequencing. Even difficult to distinguish species, such as B. fragilis and B. capillosus, were correctly identified. By including to the data-base of MALDI-TOF BioTyper newly sequenced anaerobic species, the discrepant results could be minimised. The careful analyses of the mass spectra of B. fragilis strains harbouring the cfiA gene and those which do not, could be distinguished. According to our investigation MALDI-TOF MS is a fast and reliable technique, which has the potential to replace conventional phenotypic identification of anaerobic bacteria in routine clinical microbiology laboratories and may be used to detect potential carbapenem resistant strains.

EXPRESSION AND ACTIVITY ANALYSIS OF HOX2 HYDROGENASE IN *THIOCAPSA ROSEOPERSICINA*

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The purple photosynthetic bacterium, *T. roseopersicina* harbours at least three functional [NiFe] hydrogenases. Two of them are attached to the periplasmic membrane (HynSL, HupSL), while the third one is apparently localized in the cytoplasm (Hox1EFUYH) (1).

Another soluble Hox-type hydrogenase enzyme - named Hox2 - was discovered recently as a result of the ongoing genome sequencing project. The coding sequences of the structural subunits are organized in one operon as hox2FUYH. Hox2 is a heterotetrameric enzyme composed of two hydrogenase (YH) and two diaphorase subunits (FU). Detailed comparative in vivo, in vitro activity and expression analyses of the heteropentameric Hox1 (HoxEFUYH) and the newly discovered Hox2 enzyme were performed. Our previous investigations indicated that the in vivo activities of the Hox1, Hyn and Hup hydrogenases were dependent on the nature and quantity of the electron sources used in the growth medium (2). Thiosulphate – being the primary energy and electron source - is one of the key factors connected to the hydrogen metabolism of T. roseopersicina. However, changing only the thiosulphate concentration did not induce Hox2 activity, although in vivo hydrogen production by Hox1 can be apparently driven by this compound. We concluded that Hox2 may utilize electrons deriving from components distinct from thiosulphate. Glucose proved to be the inducer of the in vivo hydrogen production by Hox2. In order to get better insight into the metabolic routes requiring the Hox2 enzyme, quantifications of hox2 expressions (hox2FU, hox2YH) under various growth conditions were performed. Functional differences were disclosed; Hox1 is connected to both sulphur metabolism and dark/photofermentative processes, while the bidirectional Hox2 hydrogenase was shown to have a physiological role in tuning the NAD^+/NADH balance under photomixotrophic conditions at the stage when cells enters into long term stationary phase.

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MICROBIAL DIVERSITY OF ARSENIC-RICH THERMAL SPRING OF KÖRÖM, BORSOD REGION, HUNGARY

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Microbial communities in a hot spring, namely Köröm-spring, at Borsod region, Hungary were examined using culture dependent and culture independent strategies. Chemical analysis of the hot spring (84 ^{o}C) water showed a high arsenic concentration and neutral pH value. Six pools were chosen to represent a thermal range (84, 80, 75, 65 and 60^{o}C) of the spring for detailed microbial community analyses.

Microbial diversity was analyzed using 16S rRNA gene clone library analyses. Primers for the main lines of archaea and the universal eubacterial 27f-1492r primer set were used. Significance and cluster analyses of bacterial communities were performed using the UniFrac program. A phylogenetic tree, containing the 16S rDNA sequences from this study, was constructed with neighbour-joining method, then exported from MEGA4 and annotated to indicate the environment of origin for each sequence. Information in the phylogenetic tree was used to measure the difference between bacterial communities of the samples. Archaeal sequences were not able to detect from the investigated samples- all the sequences belonged to eubacterial phylum.

With the exception of one pool with the highest temperature, eubacterial diversity was quite high, with *Thermotrix azorensis*, *Hydrogenobacter* and *Thermus* spp. as dominating bacterial components at most pools. The sequences recovered from culture independent method were identified as a member of Caldilineae, Actinobacteria, Alphaproteobacteria, Clostridia, Deinococci, Betaproteobacteria, Planctomycea, Thermotogae, Desulfobacterales, Deltaproteobacteria, Gammaproteobacteria and Thermodesulfobacteria class. However, the majority of sequences did not belong to certain species or even genus- at least twenty new taxa were identified.

Meanwhile, the 16S rDNA sequences from culture-dependent samples yielded strains belonging exclusively to Firmicutes. Axenic cultures were obtained on special gelrite- solidified medium. The seven isolates from the 60 ^{o}C pool represent two new species candidates from the recently described *Anoxybacillus* genus.

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ASSAY THE AFFECTING FACTORS OF NITRIFICATION PROCESS IN DRINKING WATER NETWORKS

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The drinking water quality by the 201/2001 (X. 25.) edict refers that the accepted limit value of the ammonia and nitrite is 0.5 mg/L, and of the nitrate 50mg/L in the drinking water network. In the point of the water entering in the drinking water network is important to eliminate ammonia, because the biological oxidation of ammonia – nitrification- generates nitrite and nitrate, and these ions cause public health problems.

The detection and quantitative determination was carried out of the microorganisms involved in nitrification and denitrification processes, in five towns which had different drinking water network. The microorganisms were analyzed by molecular methods as Polymerase Chain Reaction (PCR) - for the gene of 16S rRNA, amoA, NirS and NirK - and Terminal Restriction Fragment Length Polymorphism **(**T-RFLP**)**. The above-mentioned organism was enumerated by most probable number (MPN) analysis.

The heterotrophic plate count increased in the consecutive points of the drinking water networks of the towns, the highest number of bacteria was in the end point of the systems. The number of the ammonia-oxidizing bacteria (AOB) was low and they were detected only a few sampling points of the drinking water networks of the towns and there was not any AOB in the groundwater. The number of the nitrite-oxidizing bacteria (NOB) was higher than AOB and NOB was detected all of the sampling points. The cell number of both groups

increased in the end points of the networks, in line with increasing concentration of nitrite and nitrate. *Nitrospira moscoviensis* and *Nitrobacter vulgaris* were the determining species of the NOB. The T-RFLP method confirmed the presence of the two genuses in the samples and other not yet identified members of genuses were detected in some samples. Denitrifying bacteria was detected all of the sampling points, but it is less typical in drinking water networks. The plate count of the denitrifying bacteria in respect of each drinking water network of the towns there were significant differences. The total inorganic nitrogen was decreased by the activity of the denitrifying bacteria in two town drinking water network; while the other three networks the denitrifying bacteria presence only potentially was considered. Four drinking water network for were disinfected by chlorine-dioxid, its concentration and period of its use has influence the bacterial plate count. Its concentration was decreased the further points of drinking water network in line with increasing bacterial plate count. The disinfectant may have played role the inhibition of the denitrifying bacteria in three of the drinking water networks.
Overall nitrification process was observed in the examined drinking water networks, while in some networks denitrification process as well.

EFFECT OF CHANGES IN HEAT RESISTANCE OF *SALMONELLA* SPP. DURING PASTEURIZATION ON THE EFFICIENCY OF LONG TERM HEAT TREATMENT

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In this study, a specific heating procedure was applied during which native homogenized liquid egg white, liquid egg yolk, and liquid whole egg samples were kept at 55°C for 24 h. The method proved to be more efficient than did the conventional liquid egg pasteurization process because the bacteria tested were completely destroyed by the end of thermal treatment. With this technology, the heat treatment of packaged liquid egg products can be performed, thereby preventing post-infections from the environment. It has also been found, however, that the applied long term heat treatment is not always efficient. Our results showed that the use of heat shock (e.g., pasteurization of products) prior to long term heating may increase the thermal resistance of surviving microorganisms.

PREVALENCE OF DOBRAVA-BELGRADE AND SAAREMAA HANTAVIRUSES AMONG *APODEMUS* MICE IN HUNGARY AND NORTHERN CROATIA

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Dobrava (DOBV) and Saaremaa (SAAV) hantaviruses belong to the genus Hantavirus, family Bunyaviridae and carried by yellow necked (*Apodemus flavicollis*) and striped field (Apodemus agrarius) mice. The goal of this study was to determine the real prevalence of DOBV and SAAV in *Apodemus* rodents in Hungary and in Northern Croatia. Rodents were trapped in seven different locations during the summer and autumn seasons of 2005-2007. Rodents were dissected and lung tissues were used for hantavirus detection by SYBR Green-based real-time PCR. A representative DOBV strain (DOB/Pécs/242Af/06) was selected for protein expression. Truncated nucleocapsid protein (rNP50) was expressed in BL21 Rosetta (DE3) pLysS Escherichia coli cells using pET28a expression vector. Presence of antibodies against DOBV and SAAV in the collected rodents was determined by ELISA reaction. During the study period a total of 125 Apodemus sp. (67 A. agrarius, 58 A. flavicollis) was tested for the presence of hantaviruses. DOBV and SAAV were detected from 21 *Apodemus* rodents by RT-PCR and/or ELISA. Five rodents were RT-PCR and ELISA positive and also 5 animals were positive by RT-PCR only. 11 small mammals were ELISA positive only, while 104 rodents were negative with both methods. The real prevalence based on the results of these two detection techniques was 17% (21/125). In this study we concluded that prevalence of DOBV and SAAV is much higher as it was expected before, and the parallel use of molecular and serological techniques is the most reliable way to estimate the real prevalence of hantavirus infections.

HUMAN PAPILLOMAVIRUS ONCOGENES DOWN-REGULATE INVOLUCRIN EXPRESSION

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The productive phase of the human papillomavirus (HPV) life cycle is closely linked to keratinocyte differentiation. The E6 and E7 oncogenes of high-risk HPVs can transform the infected cells and disturb the differentiation procedure. The expression of involucrin is activated in the upper layer of the epidermis due to rising extracellular and intracellular calcium. It is found in the cytoplasm, cross linked to membrane proteins by keratinocyte transglutaminases and it is a precursor of the keratinocyte cornified envelope. Involucrin can be used as a marker for keratinocyte differentiation. The aim of this study was to investigate the effects of HPV16 E6 and E7 oncogenes on the expression of involucrin in keratinocytes.
Primary human foreskin keratinocytes were maintained in serum free, low calcium medium and transduced by LXSN (control) retrovirus or virus vectors expressing HPV16 E6, E7 or E6/E7 genes. These cells were induced to differentiate by culture in high calcium and serum containing medium for 24h. The expression level of involucrin in differentiating and non-differentiating infected cells was estimated using real-time RT-PCR. The involucrin protein was detected by western blotting using monoclonal anti-human involucrin antibody. Primary human keratinocytes and MCF-7 cells were co-transfected by a luciferase reporter plasmid carrying involucrin promoter, different fragments of the promoter and vectors expressing HPV 16 E6 or E7 genes. Luciferase assay was used to measure the effect of the HPV oncogenes on involucrin promoter activity.
The differentiation of keratinocytes by serum and calcium highly increased the transcription level of involucrin. The E6 and E7 oncogenes of HPV16 together caused down regulation of the involucrin mRNA both in differentiating and non-differentiating cells. The effect of HPV16 E6 and E7 on cellular involucrin protein level was similar to that found for mRNA level. High calcium and serum stimulated the level of involucrin protein and the presence of the two oncogenes reduced it. In order to verify the effect of HPV oncogenes on the involucrin promoter, we made transient transfection assays and found that the HPV E6 and E7 repressed involucrin promoter activity in primary keratinocytes and in MCF-7 cells. The effect of HPV oncogenes was localized to the proximal region of the involucrin promoter (-231 from transcription start site). In this proximal region of the involucrin promoter, there are some important transcription factor binding sites (AP1, C/EBP and Sp1). Using electrophoretic mobility shift assay (EMSA), we try to determine which transcription factors are involved in the regulation of the involucrin promoter by the HPV oncogenes.

INCIDENCE AND ANTIBIOTIC RESISTANCE OF *UREAPLASMA UREALYTICUM* CULTURED FROM GENITAL DISCHARGES OF SEXUALLY ACTIVE INDIVIDUALS

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The aim of this investigation was to study the frequency and antibiotic susceptibility of *Ureaplasma urealyticum* strains cultured from cervix and urethra of sexually active individuals attended to our STD outpatient division.
Specimens were taken from the cervix and urethra with universal swab (Biolab®) into Urea-Myco DUO kit (Bio-Rad®) and cultivated in ambient air for 48 h at 37 C^{o}. The determination of antibiotic resistance was carried out in U9 broth using SIR *Mycoplasma* kit (Bio-Rad) under the same conditions. Only one strain per patient was taken into consideration. Between May 2008 and October 2009 a total of 169 isolates were obtained from urethra (38) and cervix (131). Most isolates were sensitive to tetracycline (95%), doxycycline (97%), pristinamycin (96%), azithromycin (95%), josamycin (95%) and ofloxacin (93) but not to erythromycin (13%) and clindamycin (22%). 75% of the strains were simultaneously resistant to clindamycin-erythromycin combination also, suggesting that administering ex juvantibus any of them can select cross-resistant strains to both antibiotics. Not only cultivation but also prior antibiotic resistance testing is required for successful treatment of symptomless or manifest *U. urealyticum* infection since none of the tested strains was fully sensitive to all examined antibiotics. In case of U. urealyticum infections or *Chlamydia trachomatis* and *U. urealyticum* mixed infections, azithromycin seems to be the drug of choice since it is active against both

pathogens. However, since clarithromycin inhibits biofilm formation of *U. urealyticum*, it is also recommended.

COMPARISON OF TPPA AND TPHA METHODS IN DIAGNOSING EARLY SYPHILIS AND IN BIOLOGICAL FALSE-POSITIVITY

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Diagnosis of syphilis is based on clinical observation, direct detection of the *Treponema pallidum*, and confirmation by serological tests.The aim of this study was to compare the specificity and sensitivity of two screening methods *Treponema pallidum* particle agglutination (TPPA) and *Treponema pallidum* haemagglutination (TPHA) in syphilis diagnosis. In our department the Rapid Plasma Reagin (RPR), TPHA, TPPA have been carried out from twofold dilution samples of sera, and from every sera we made syphilis Enzyme Linked Immunosorbant assay (ELISA) as well as IgM and IgG immunoblot. Between May 2008 and October 2009 a total of 6847 serum samples 2743 were realy positive with all the treponemal tests, but in 52 cases of not T. pallidum infected individuals the TPHA test was false-positive. In very early nearly seronegative infections the TPPA was really positive in 26 cases, while TPHA remained false-negative.
In our study TPPA proved to be more sensitive and specific than TPHA. As screening method of syphilis infection, together with the non-treponemal tests (RPR, VDRL), as treponemal test the ELISA or the TPPA is rather reliable and recommended than the TPHA.

Q-RT-PCR ASSAYS FOR THE DETECTION OF *ASPERGILLUS* SPECIES

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Despite of the rapid development in antifungal therapy during the past decade, the incidence of invasive mold infections, especially those caused by *Aspergillus* species remains a major cause of the infection-related morbidity and mortality in developed countries. The reasons behind this phenomenon are the use of immunosuppressive agents (corticosteroids) in recipients of an allogenic stem/bone marrow cell and solid-organ transplantation or hematopoietic stem cell transplantation.
Mayor causative agents of the highly devastating systemic mycoses are mainly the opportunistic filamentous fungi of the *Aspergillus* genus, such as *Aspergillus fumigatus, A. terreus, A. flavus* and *A. nidulans*. The saprophytic *Aspergillus* species are ubiquitous in our environment. The exposure to *Aspergillus* spores or conidia is therefore almost constant. Due to the immunocompromised state of these individuals, aspergillosis can become invasive and in spite of the fact that in the status of the primary disease may appear to get better, the secondary evolved infections could lead to death. The only means of the survival is the antifungal therapy initiated early enough. The most reliable microbiological and histopathological methods are time consuming, signs and symptoms of systemic diseases caused by *Aspergillus* species are non-specific and patients are often unable to undergo invasive diagnostic procedures.
The current, commercial aspergillosis diagnostic methods are based either on detection of DNA or being serological or the combination of these. However these hybrid methods that unite the high specificity of DNA based PCR techniques and the high sensitivity of serological methods are time consuming.
We have developed species specific, highly sensitive quantitative real-time PCR diagnostic assays for detecting specific markers and identifying *Aspergillus fumigatus* and *A. terreus* species.
The principle of our novel invention is based on the finding that the orthologous of *Streptomyces* facC gene is present in certain human pathogenic filamentous fungi. It is worth to mention that the advantages of our quantitative real-time PCR diagnostic assays are that they are rapid, cheap, reliable, highly sensitive and reproducible. Due to the fact that detectable markers are almost exclusively found in *Aspergillus fumigatus* and *A. terreus*, the high rate of false positive results that is given from the presence of other pathogen species obtained by using other multi copy target genes in DNA based assays will be reduced to zero.
The sensitivity of these tests was measured and they do not only range with those of other commercially available Q-RT-PCR diagnostic methods, but top them. They are able to detect 30 CFU/10 µL. Due to the fact that such detectable markers are not present in other fungi, the level of discrimination is maximum high (100%). The possibility of false positive results that originate from the presence of other species is neglectable.
This Q-RT-PCR diagnostic method is elaborated on basic research level. The clinical settings are in progress.

POTENTIAL UTILIZATION OF *BACILLUS CLAUSII* IN YOGHURT MANUFACTURING

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The *Bacillus clausii* is an endospore-forming species and unlike the majority of probiotic strains it does not belong either to the *Bifidobacterium* or *Lactobacillus* genus. Clinical experiments confirmed its beneficial effects on the human health and there is a commercial product that contain *B. clausii* endospores in a high number.
Most of the probiotic food stuff are yoghurts or milk-based products. Traditionally lactic acid bacteria are used for yoghurt making and these products are enriched by probiotic Bifidobacterium or *Lactobacillus* strains.
In our experiments the main goals were the following: determine whether it was technically possible to make probiotic yoghurt by B. clausii endospores, is viability of endospores decreased by the technological steps of yoghurt manufacturing and does the *B. clausii* supplementation change the features of the yoghurt?
The yoghurts used in our experiment were inoculated by the starter cultures in the dairy farm and then delivered to our laboratory. At this point we added various amount (25-900 µl) of *B. causii* endospore suspension to the samples then incubated them for 4 hour at 44 °C. Acidity and pH of the samples were measured after 1 and 4 hour of incubation, then the samples were stored at 5 °C. Colony counting was performed at inoculation and after one week of storage at 5 °C; total colony count was made on PCA (Plate Count Agar) plates, endospore number was counted on DTA (Dextrose Tryptone Agar).
After inoculation the *B. clausii* spore number was slightly lower than in the initial suspension, we found about one order of magnitude decrease in the number of spores. After one week of storage at 5 °C the spore number decreased again by about one order of magnitude. Neither the pH nor the acidity values changed in the inoculated samples when compared to the controls. These values were not affected by the *B. clausii* spores, independently from the amount of added suspension. We did not find significant differences in the texture, odour, colour and taste between the control and inoculated samples.
The results of our experiments showed that the *B. clausii* can be used in yoghurt to produce probiotic food, since it did not have any disadvantegous effect on the yoghurt and the endospores survived the storage in high number.
The research was financed by the NKTH and NFÜ.

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

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

FIRST GASTROENTERITIS OUTBREAK CAUSED BY *SAPOVIRUS* IN HUNGARY - PART OF AN INTERNATIONAL EPIDEMIC?

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Sapovirus belonging to Caliciviridae is one of the known pathogen of sporadic gastroenteritis infections in infants, children and in elderly. Sapoviruses have a linear, single-stranded, positive-sense RNA genome from approximately 7300nt to 8500nt in length that is organized into two major ORFs. Since the beginning of molecular monitoring of caliciviruses (mid 1990's) sapovirus has been described rarely, once in approx. 5 years, as source of an outbreak. Circulation of caliciviruses has been monitored with molecular epidemiological methods by the authors for 10 years in Hungary. Sapovirus has not been detected yet in the approximately 800 examined non-bacterial gastroenteritis outbreak. Based on the informal data supported by the international calicivirus surveillance study group, the number of outbreaks caused by sapovirus was increasing in Europe in 2008. Supposedly these outbreaks can be linked to genotype GI/2 sapovirus. Our aims were to describe the first verified detection and molecular epidemiological description of a gastroenteritis outbreak caused by sapovirus in Hungary. Stool samples were originated from Bács-Kiskun County, from a mental deficiency day care center, where a gastroenteritis outbreak occurred in September, 2008. Amplification of the RNA polymerase gene of sapovirus was performed by RT-PCR method and the product was directly sequenced and phylogenetically analyzed. Clinical and epidemiological data were collected by epidemiological investigation. 17 of the 135 exposed people (12.6%) had gastroenteritis with vomiting and diarrhea in the period of September 11–22, 2008. Bacterial pathogens, rotavirus, adenovirus and norovirus were not detected, but sapovirus could be identified in 1 out of the 4 (25%) stool samples. The source of the outbreak was presumably the ill nurse and the virus spread with direct contact among the mentally deficient patients. Based on the continuous 4390nt long ORF1 ($NS5^{VPg}$, $NS6^{Pro}$ and $NS7^{Pol}$), ORF2 (capsid) and 3' Untranslated regions, the virus belongs to genotype GI/2 sapovirus. Furthermore, as the longest available genotype GI/2 sapovirus sequence it has been deposited to GenBank under Hu/GI.2/Kecskemet/HUN3739/2008/HUN (FJ844411). This study reports on the first detection of sapovirus from gastroenteritis outbreak in Hungary. Epidemiologic and clinical characteristics of the outbreak in the mental deficiency day care center are described in details to prove that not every case is "calicivirus" infection, and epidemic is caused by norovirus, which is another calicivirus examined by diagnostic methods. The outbreak caused by genotype GI/2 sapovirus might be the part of an international epidemic, extended into a larger geographic area

PHYLOGENETIC ANALYSIS OF SIMIAN ADENOVIRUSES

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Simian adenoviruses (SAdV) are members of *Mastadenovirus* genus in the family Adenoviridae. Five of the officially accepted species (Human adenovirus B, C, E and G) include or totally consist of simian adenoviruses (SAdV-A). Our goal was to perform a preliminary phylogenetic analysis based on a partial amino acid sequence (288 amino acids) of the adenoviral DNA-dependent DNA polymerase gene. Subjects of this assay included 20 previously deposited strains from ATCC (American Type Culture Collection), and three recently sequenced types. Host species of these 23 viruses were Old World monkeys. We also examined one adenovirus from a New World monkey (red-handed tamarin, *Saguinus midas*), which died in a Hungarian private zoo. Compared to adenoviruses of apes and humans, the phylogenetic relationships of monkey adenoviruses are yet to be clarified. To date, eight complete genome of the inspected viruses were determined. In order to gain target sequences from the remaining 16 SAdVs, nested PCRs were performed with two different sets of oligonucleotides. After determining the short sequences, we performed phylogenetic analysis. The results of our analysis were compared with that of an earlier study, based on a short partial hexon gene nucleotide sequence. By summarising the topology of the two phylogenetic trees, out of 24 virus total, 13 were found to be representatives of the taxa accepted so far: Simian adenovirus A (n=6) and Human adenovirus G (n=7). For 10 viruses, we suggest the establishment of 4 novel adenovirus species, including the type detected in the New World monkey. The phylogenetic status of one type (SAdV-19) remains unclear after the investigation, and needs the determination of further genome sequences.

ZEARALENONE CAUSED CITOTOXIC EFFECT AND ADAPTATION IN *SCHIZOSACCHAROMYCES POMBE*

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The mycoestrogen zearalenone (ZEA) produced by different *Fusarium* species is present in numerous mycotoxicoses especially in farm animals. Its cytotoxic effect was demonstrated on S. pombe ura4D-18 h^- cells utilized as a model-system. Minimal inhibitory concentration was 1000 µM, determined by microdilution method (M27A) according to the NCCLS standard. The growth inhibitions at 100 µM, 500 µM and 1000 µM ZEA were measured in shaken cultures in supplemented minimal broth at 30 °C. After 20 hours 100 µM ZEA treatment did not induce any remarkable inhibitory effect. In the presence of 500 and 1000 µM ZEA, the growth inhibition was 83 % and 84 %, respectively, and it decreased to 40 % and 38 % after 36 h treatment. 100 µM ZEA did not affect the survival rates but it was approximately 0 % after 30 min treatment in the presence of both 500 µM and 1000 µM ZEA. The retarded growth kinetics indicated adaptation was investigated, where 250 µM ZEA pretreatment was used as defined as sublethal concentration from survival rates. The kinetics of ZEA uptake was also determined and showed time dependent changes. To demonstrate whether ZEA causes membrane alterations and loss of membrane barrier function, the efflux of 260 nm absorbing substances was determined. In mammalian cells, ZEA binds to oestrogenic receptors and causes well characterized symptoms. However, *S. pombe* does not contain oestrogenic receptor homologue sequences, yet ZEA has cytotoxic effect on these microorganisms, so the mode of action of ZEA in *S. pombe* cells needs further investigation.

CHANGES IN THE MICROBIOLOGICAL STATUS OF WHEAT GRAINS AND SPROUTS DURING SPROUTING PROCESS

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Vegetable seed sprouts have become popular in many countries as a nutrient supplement due to their high content of proteins, minerals and vitamins. During sprout germination, complex compounds comprised of lipids, carbohydrates, and storage proteins are broken down into simple and digestible nutrients. Besides their nutritional value to people, sprouts can be a good food source of pathogenic and spoilage microorganisms.

Bacterial species on the surface of grown sprouts are often related to soil bacteria. They are members of pseudomonades, enterobacteria, lactic acid bacteria and yeast species. There is only limited information available on the growth of contaminating and spoilage microorganisms during the germination of sprouts in Hungary. The purpose of this study to investigate the changes in the microbiota and microbiological status of germinated grains during the process of sprouting (from grain to ready-to-eat sprout).

Organic wheat grains were bought from herbalist shops. Sprouts were grown under hydroponic conditions at 20 °C in sterile germinating dishes. We examine the microbiological quality (total aerobic plate count, coliform count, yeast and mould count) of grains before soaking, and after soaking in sterile water for 12 hours, and of the one-, two- and three-day sprouts. Enumeration of total aerobic plate count was performed using Plate count agar, coliform count using Violet Red Bile Lactose agar, furthermore yeast and moulds count using Chloramphenicol Glucose Yeast extract agar. During the analysis of the microbiological quality of organic wheat grain samples before soaking we found that, mean total aerobic plate counts were 4.9 $\log_{10}$ cfu/g, coliform count 3.9 $\log_{10}$ cfu/g, yeast and mould count 3.5 $\log_{10}$ cfu/g. After soaking, total aerobic plate count of grains increased to 6.2 $\log_{10}$ cfu/g, coliform count to 6.0 $\log_{10}$ cfu/g, yeast and mould count to 4.0 $\log_{10}$ cfu/g. In the first three day of sprouting, microbial populations on grains increased approximately 1.5-2 logs. During sprouting process, the total aerobic plate count of ready-to-eat (three-day) sprouts increased to 7.9 $\log_{10}$ cfu/g, coliform count to 7.4 $\log_{10}$ cfu/g, yeast and mould count to 6.6 $\log_{10}$ cfu/g.

These high microbial levels per se reduce the shelf-life and safety of sprouts. Initial microbial load should be controlled, for minimizing microbial contamination in the sprouts prior to consumption. On the basis of our results, we recommend the decontamination of seeds before sprouting.

HOW TO TRANSFER INFORMATION EXTRACTED FROM GENOME ANNOTATION AND TRANSCRIPTOME ANALYSES TO WET LAB? THE *ASPERGILLUS NIDULANS* ATFA STORY

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Our team took part in the 2008 update of the *Aspergillus nidulans* genome annotation performed by the Eurofungbase community (Wortman et al. 2009). We annotated 3908 stress-responsive proteins encoded in eight fully sequenced *Aspergillus* genomes, and set up the publicly available *Aspergillus* Stress Database (http://193.6.155.82/AspergillusStress/; Miskei et al. 2009). Stress signal transduction and regulatory pathways were analyzed using yeast-based models and we found that the complex and robust stress response systems harbored in the aspergilli are closer to that found in fission yeast than that possessed by budding yeast. The centerpiece of the fission yeast's stress response system is the Atf1 bZIP-type 'all-purpose, general stress'' transcription factor with high homology to the aspergilli's putative AtfA factors. The evaluation of oxidative stress-initiated global transcriptome changes also supported the view that AtfA may play a prominent role in the orchestration of the stress response in A. nidulans (Pócsi et al. 2005). The successful complementation of the osmotic and oxidative stress sensitive phenotypes of the Δatf1 *S. pombe* mutant with the heterologous expression of *A. nidulans* atfA highly supported the view that Atf1 and AtfA are true functional orthologs (Balázs et al. 2010). The deletion of atfA resulted in an oxidative stress sensitive phenotype in A. nidulans and, as demonstrated by RT-PCR experiments, the expressions of a number of oxidative and osmotic stress responsive genes were under the control of AtfA (Balázs et al. 2010). Data collection and mining have been continued since then by the involvement of further available fungal genomes, the improvement of bioinformatics tools, and by the analysis of stress-triggered changes in the proteome of A. nidulans (Pusztahelyi et al. 2010). Data-driven wet lab studies are now in progress to gain a deeper insight in the organization and orchestration of the stress response system of this euascomycete.

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DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN A LONG TERM MAIZE MONOCULTURE

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The community structure of arbuscular mycorrhizal fungi (AMF) was assessed in a long-term field experiment, where maize has been grown in exclusive monoculture since 50 years. Root samples collected from plots, where (i) normal mineral fertilization was used every year (MIN), (ii) corn stalk residues were yearly incorporated into the upper 20 cm of the soil by plowing (RCR) and (iii) no soil cultivation, no fertilization occurred (NON) were subjected to molecular analyses. By using nested PCR, 18S rDNA fragments were amplified, cloned, and sequenced. Of the 257 sequences recovered, 203 belonged to *Glomeromycota* AM fungi that were grouped into 22 operational taxonomic units (OTU) comprising 1 to 39 unique sequences. The majority of the 203 *Glomeromycota* sequences belonged to the family *Glomeraceae*; altogether 197 *Glomeraceae* sequences were identified, with a 194:3 distribution between groups *Glomus* A and *Glomus* B. Only one and five sequences were assigned to families *Archaeosporaceae* and *Paraglomeraceae*, respectively. AMF sequences were most frequently recovered from root samples collected from NON plots and diversity of clones was also the richest in these samples. PCR amplifications of DNA samples originating from roots from MIN and RCR plots resulted in significantly smaller number of AMF clones as compared to clones amplified from samples collected from NON plots, indicating that both mineral fertilization and crop residue recycling (accompanied with tillage-induced disruption of hyphae) reduce the frequency and diversity of AM fungi. Clones belonging to subgroup *Glomus* Aa were absent in roots collected from MIN plots, but they were frequently detected in samples from the other two treatments (RCR, NON) suggesting that these AM fungi are sensitive to mineral fertilization, but successfully

tolerate soil disturbance. In general, clones belonging to subgroups *Glomus* Ab, Ac, and Ad rarely occurred in samples from RCR plots indicating the disturbance-sensitivity of these phylotypes.
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PARASPORAL PROTEIN CRYSTALS OF *BACILLUS THURINGIENSIS* TYPE STRAINS: MICROSCOPIC OBSERVATIONS AND PROTEIN PATTERNS

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Among the entomopathogenic microorganisms the *Bacillus* genus has particular importance in the biological pest control. Numerous *B. thuringiensis* subspecies provide valuable source for development of effective biopesticides against various pests responsible of great damage in crop production. Bioinsecticides composed of *B. thuringiensis* strains are utilized world-wide on a significant scale for more than 30 years.
The entomopathogenic activity of the species is principally due to its ability to synthesize delta-endotoxins [crystal (Cry) proteins] in the sporulation growth phase, forming parasporal crystal inclusions. The inclusions can be distinguished as distinctively shaped and sized crystals. Delta-endotoxins are effective against various insects, including Lepidoptera, Diptera and Coleoptera. Additionally, *B. thuringiensis* produces a number of extracellular compounds, such as phospholipases, proteases, and chitinases, and other toxins, such as beta-exotoxin and vegetative insecticidal proteins (Vip), that may contribute to virulence.
In this study we compared several well-known *B. thuringiensis* type strains, regarding their sporulated cultures, parasporal crystals and protein patterns of their spore-crystal preparations.
Type strains were *B. thuringiensis* ssp. *kurstaki* NCAIM B.01262, *B. thuringiensis* ssp. *israelensis* B.01289, *B. thuringiensis* ssp. *kumamotoensis* DSMZ 6070 and B. thuringiensis NCAIM B.1292. The subjects of investigation were liquid cultures grown in various sporulation media and growth conditions. Sporulated cultures were observed by phase contrast and scanning electron microscopy. Adequate methods were developed for the separation of spore-crystal fractions and differential solubilization of crystal proteins. Polypeptide components of spore-crystal suspensions were revealed by SDS-PAGE. Our results showed that cells with endospores, free spores, crystals and vegetative cells in various amounts could be observed under phase contrast microscope in all sporulated cultures. In all type strains, SDS-PAGE patterns of the spore-crystal preparations and the corresponding vegetative cell suspensions were essentially different, regarding the number and size of detected polypeptide bands. In the spore-crystal patterns polypeptides resembling Cry proteins in molecular weight were detected. Besides, scanning electron microscopy observations confirmed the presence of distinctively shaped parasporal crystals in some sporulated cultures. However, correlation between the presence of crystals and detection of Cry protein resembling polypeptides varied in the type strains.
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DETECTION AND CHARACTERIZATION OF KOBUVIRUSES (IN FAMILY *PICORNAVIRIDAE*) IN NEW HOST SPECIES

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Picornaviruses (family Picornaviridae) are small, non-enveloped viruses with single-stranded, positive-sense genomic RNA which is divided – at present - into twelve genera. Kobuvirus genus consists of 2 officially recognised species, Aichi virus and Bovine kobuvirus. Aichi virus has been first isolated in stool samples from human with oyster related gastroenteritis outbreaks in Japan, in 1989. Bovine kobuvirus (strain U-1) has been detected in fecal samples collected from clinically healthy cattle in Japan, in 2003. Up to 80-95% of the populations at the age of 30-40 have antibodies against Aichi virus. Serendipitously, new kobuvirus species were found in Hungary from fecal samples collected from clinically healthy domestic animals by molecular methods. Porcine kobuvirus (Kobuvirus/swine/S-1-HUN/2007/Hungary, EU787450) was found in healthy pigs and complete nucleotide (8,210nt), amino acid (2,488aa) sequences and genetic organization were determined. The structure of the S-1-HUN genome, VPg–5'UTR–leader protein–structural proteins (VP0, VP3, VP1)–non-structural proteins (2A-2C, 3A-3D)–3'UTR–poly(A) tail, was found to be typical of picornavirus. The 5'UTR

region forms a unique hepacivirus/pestivirus-like type IV internal ribosomal entry site (IRES) element. A tandem repeat (a 30 amino acid long motif) was detected in region 2B. The genetic identity on coding region between Aichi, U-1 and S-1-HUN viruses are between 35% (L-protein) and 74% (3D region). By follow up study, high incidence (65% and 53%) and endemic circulation of porcine kobuvirus was found at the tested farm (in vivo evolution rate for VP1 is $6.75x10^{-3}$ substitutions/nucleotide/year). Porcine kobuvirus RNA was also identified in pig sera samples indicating that porcine kobuvirus escaped the gastrointestinal tract into the circulatory system in immunocompetent virus-infected hosts, resulting viremia. Bovine kobuvirus (2/32; 6.25%) was detected in healthy cattle at the first time outside Asia. Kobuvirus sequences related to bovine kobuvirus were also found in newborn sheep (5/8; 62.5%). Aichi virus shedding was detected in a 3-year-old child (1/65; 1.5%) with enteric (diarrhea) and extraintestinal symptoms. Kobuviruses were identified in 4 host species. Our knowledge's (about diversity, pathogenesis, geographical distribution, transmission, epidemiology, virus organization etc.) are not complete related to enterically transmitted viruses especially kobuviruses.

ADVENTAGES OF INTRAMUSCULARLY ADMINISTERED PROLINE RICH PEPTIDE *A3-APO* OVER EXISTING THERAPIES IN EXPERIMENTAL *ACINETOBACTER BAUMANNII* WOUND INFECTIONS

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The designer proline rich antibacterial peptide A3-APO is effective in mouse models of *Escherichia coli* and *Acinetobacter baumannii* systemic infections. We compared the effectiveness of this peptide with that of colistin and imipenem in *Acinetobacter baumannii* wound infections after burn injury. In three series of independent experiments, mice were inflicted with burn wounds and different inocula of *A baumannii* isolated from an injured soldier were inoculated into the wound sites. The antimicrobials were added intramuscularly 1-5 times. The systemic toxicity of A3-APO and colistin were studied in healthy mice.While toxicity of colistin was observed at 25 mg/kg bolus drug administration, the lowest toxic dose of A3-APO was as much as 75 mg/kg. In the *A. baumannii* blast injury models, 5 mg/kg A3-APO improved survival and reduced the bacterial counts in the wounds and improved wound healing significantly better than other antibiotics. Peptide A3-APO with an i.m. therapeutic index of 15 is more active and less toxic than any other burn injury infection therapy modalities against multi-drug resistant Gram-negative bacteria.

FREQUENCY AND ANTIBIOTIC RESISTANCE OF *STREPTOCOCCUS AGALACTIAE* CULTURED FROM GENITAL DISCHARGES OF SEXUALLY ACTIVE INDIVIDUALS

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The aim of this study was to examine the incidence and antibiotic sensitivity of *Streptococcus agalactiae* isolated from genital samples of sexually active individuals attended to our STD outpatient division.
Specimen was taken with universal swab into transport medium then cultured simultaneously on blood agar and CROMagar in 5% CO_2 incubator at 35 C° for 24 h. S. agalactiae classification was made with conventional tests. Disc diffusion method was applied for the determination of antibiotic susceptibility. Only one isolate one patient was considered. Between May 2008 and February 2010 a total of 607 *S. agalactiae* isolates was obtained from urethra (237), cervix/vagina (235), glans-wound (85), ejaculate (16) and urine (34). Resistance to erythromycin (44%), clindamycin (44%) and tetracycline (88%) proved to be extraordinary high. 36% of the isolates proved to be simultaneously resistant to macrolide-lincosamide-tetracycline combination, therefore, ex juvantibus administration of any of them may select the cross-resistant strains to all these three classes of antibiotics. 98-99% of the isolates were sensitive to moxifloxacin and all beta-lactams, however, penicillin-resistant S. agalactiae appeared in Hungary in our outpatients, too.
Successful treatment of colonisation, carrier stage or manifest infection caused by *S. agalactiae* requires not only cultivation but also a prior antibiotic sensitivity testing, since near half of the isolates is multiple resistant. In case of coinfections with *S. agalactiae* and *Chlamydia* or *Ureaplasma*, moxifloxacin seems to be the drug of choice since it is active against all of them.

COMPARISON OF THE HUMORAL AND THE CELLULAR IMMUNE RESPONSE FOLLOWING HUMAN INFLUENZA VIRUS IN INFECTION

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The level of serum antibody to HA and NA correlates with resistance to illness and with restriction of the influenza virus replication in the respiratory tract of humans. This has been shown after experimental infection with influenza virus or after natural infection. HA antibodies can prevent infection by neutralizing the infectivity of the virus, whereas NA antibodies mediate their antiviral effect primarily after infection has been initiated by limiting virus release from cell and hence restricting spread of virus within the respiratory tract of the host. The role of cellular immunity in clearance of influenza virus has been well defined in the murine model but less well in humans. When the immune system fighting pathogens, cytokines signal immune cells such as T-cells and macrophages to travel to the site of infection. In addition, cytokines activate those cells, stimulating them to produce more cytokines. Anti-influenza antibody synthesized was detected and quantitated by a virus neutralization assay, inhibition of haemagglutination assay, inhibition of neuraminidase assay and immunoenzymetric assay for the in vitro quantitative measurement of human IL-6, IL-4, TNF-α and IFN-γ.

INTERACTIONS OF *SALMONELLA ENTERICA* SEROVAR *ENTERITIDIS* AND *GALLUS GALLUS*

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Although epidemiological situation may differ in years and from country to country, human cases of salmonellosis in EU countries are the most frequently caused by *Salmonella enterica* serovar *Enteritidis* and major source of this zoonotic agents is poultry and poultry products. This is the reason why we are interested in the interaction of this particular serovar and host aiming at the development of a new generation vaccine. Since live attenuated vaccines are of better performance in the protection of poultry against *Salmonella* infections than the inactivated ones, we are focused on the development of live vaccine. First we analysed chicken's response to *S. Enteritidis* infection and found out that newly hatched chickens are highly sensitive to the infection, however chickens as old as one week are already 10x more resistant to the infection than the day-old chicks. Day-old chicks responded to oral *S. Enteritidis* infection by upregulation of IFNγ, TNFα, IL-18, iNOS, IL-8, IL-1β, IL-17 and IL-22 in caecum while one-week-old chickens responded only by upregulation of IL-8, IL-1β, IL-17 and IL-22. In adult 50-week-old hens barely any response to oral *S. Enteritidis* infection was recorded in caecum. Immune response in adult hens however could be recorded when intravenous infection with *S. Enteritidis* was used. In this case, the birds responded by significant suppression of IL12β in liver, spleen, lungs, caecum and colon. On the other hand, IL-17 and IL-22 were highly induced in liver and spleen but suppressed in lungs indicating refocusing of immune response to the organs which were preferentially colonised by *S. Enteritidis*. Our results indicate that chickens and hens respond to *S. Enteritidis* by the activation of NK cells (IL-22) and Th17 branch (IL-17) of immune system and it will be interesting to compare the response to the wild type strain with the response to selected attenuated mutants and also to a secondary exposure to wild type *S. Enteritidis* in vaccinated birds.

EFFECT OF DIFFERENT HERBICIDES ON SOME MICROBIAL PARAMETERS OF A CALCAREOUS CERNOZEM IN A MICROCOSMOS EXPERIMENT

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For the sustainable agricultural production we have to pay attention to environment-friendly cultivation-technologies; but at the same time make an effort to product good quality and economical costly products. Today, agricultural production (in spite of many efforts) is unthinkable without the use of pesticides (herbicides, insecticides and fungicides). On the other hand, these chemicals contribute to the pollution of the atmosphere, surface and underground waters and agricultural soils, especially if they are applied improperly. The herbicide

usage is inseparable part of the plant production, but beside the exemption from weeds we have to count another secondary effect of chemicals on the soil life and on the so called "not purposed" soil organisms. Such herbicides should be used which have only a minimal secondary effect on soil microbes, besides their weed killing effect.
In the course of experimental work the effect of herbicides on soil biological properties were examined in different maize (*Zea mays*) culture. We would have liked to know that how affect the herbicides on the quantity change of soil microorganisms, and the activity of microorganisms. In 2008 a small pot experiment was set up in the breeding house of the Department, where the soil moisture and the nutrient supply was secured in optimal level. In the small pot experiment two herbicides were used, they are the Merlin 480 SC and Acenit A 880 EC.
In small-pot experiment the effects of herbicides were studied on the number of total bacteria, and microscopical fungi, on the quantitative changes of aerobic cellulose decomposing and nitrifying bacteria, on the soil respiration, and the nitrate mobilization. Besides we examined the effect on the quantity of fumigation-incubation biomass carbon, and fumigation-extraction biomass nitrogen.
At the bases of results the following can be stated: the two herbicides and their all doses affected negatively to the number of total soil bacteria, the inhibiting effects were significant. The quantity of microscopical fungi increased by the effect of Merlin 480 SC and decreased in the treatments of Acenit A 880 EC; the Acenit A 880 EC had stimulating effect on the nitrate mobilization. The CO_2-production was stimulating by the basic doses of herbicides; the other treatments did not influence the CO_2-production significantly; the quantity of microbial biomass-carbon –except only one treatment- decreased significantly by the effect of herbicides. Besides it, the quantity of microbial biomass-nitrogen increased significantly in the treatments of Acenit A 880 EC; the biomass of test plant decreased in the treatments of herbicides, their quantities were smaller than in the control. In the pots treated by Merlin 480 SC, parallel with the increase of doses decreased the quantity of plant-biomass.

HORTICULTURAL APPLICATION OF A COMMERCIAL MYCORRHIZAL PRODUCT IN HUNGARY

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Arbuscular mycorrhizal fungi (AMF) give a significant contribution to plant nutrition promoting mostly the uptake of phosphorus and water. Thereby, mycorrhizal inoculation can make cultivation cost-efficient in the horticulture. The alternative technology based on mycorrhizal inoculation usually allows achieving similar yield and food quality as the conventional ones. However, the high level of nutrients commonly used in commercial horticultural potting media often limits the mycorrhizal formation, reducing the potential beneficial effect of the inoculations. Thus, it is highly required to provide more information about the proper application conditions of AMF. Furthermore, completion of deficient field experiment data are also demanded in Hungary.
In order to get more complex picture about the possibilities of commercial mycorrhizal product "Symbivit", inoculation trials in plot experiments were constructed with two Hungarian spice peppers (*Capsicum annuum* L. var. *longum* cv. *Szegedi* and *Capsicum annuum* L. var. *longum* cv. *Kalocsai*), cucumber (*Cucumis sativus* L. var. *Perez*) and strawberry (*Fragaria ananassa* cv. *Elsanta*). Experiments clearly showed positive effect of "Symbivit" on yield of Kalocsai pepper (increase by 10%), Szegedi pepper (by 65%) and cucumber (by 10%).
Inoculations significantly increased the root dry weight of strawberry (*Fragaria ananassa* cv. *Elsanta*), however no significant difference in fruit yield was observed. Changing the nutrient supply of strawberry plants to a lower nutrient amendment, significantly higher root fresh weight and colonization were measured without increased crop production. Beyond plant growth parameters and crop production, fungal inoculants also improve food health properties (antioxidant compounds, sugars, etc.). Our aim is in the next to reveal the economical benefits of "Symbivit" in that point of view.
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BIODIVERSITY OF FUNGAL COMMUNITY IN SANDY SOIL TREATED WITH COMPOST FROM WASTES OF BIOMASS-ENERGY PRODUCTION

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RELEVANCE OF THE BIOLOG SYSTEM FOR THE IDENTIFICATION OF THE MEMBERS OF *PASTEURELLA* SENSU STRICTO

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Pasteurella species commonly colonise the oropharynx of healthy and diseased wild and domestic animals. They are well recognized as widespread veterinary pathogens and recently their importance has been increasing in human infections followed cat and dog bites and scratches. Saliva of carnivores could contain multiple *Pasteurella* species simultaneously, such as *P. multocida, P. dagmatis, P. canis*, and *P. stomatis* that have also been cultured from the human wounds.

In clinical laboratories a large number of automated, semi-automated and manual phenotypic methods and systems have been accepted for rapid and accurate identification of bacterial pathogens. Since the occurrence and morphological features of the *Pasteurella* species, especially members of *Pasteurella* sensu stricto, are very similar their identification by traditional methods is laborious and sometimes misleading. The automated techniques offer fast, standardised, easily reproducible results based on computer database of different bacteria. They generally perform well for identifying common bacterial species but they are often not proper enough for detection of the rare or unusual pathogens. Furthermore, their sensitivity and specificity of identification could be highly variable in the different bacterial families at the genus or species level.

The aim of our study was the accurate taxonomical identification of 50 *Pasteurella* isolates originated from canine or feline oropharynx and oral cavity. The primary identification was carried out by Biolog Microstation™ ID System (Biolog, CA, USA). It is based on tests for the utilization of 95 different carbon sources in a 96-well microtiter plate. The Biolog substrate utilization system clearly identified all of our isolates at genus level (as *Pasteurella* sp.) but the definition of certain species was not totally accurate in a number of cases. Identification of *P. multocida*, the most frequent pathogen in animal and human infections, was correct. The separation of other species was queried. The non - *P. multocida* isolates were identified as *P. dagmatis* although - according to molecular analysis - the isolates from dogs belonged to *P. canis* and several feline isolates represented unique monophyletic group within *P. dagmatis*. Verification of the Biolog results was resolved by sequence analysis of sodA gene (Mn-dependent superoxide dismutase) of representative isolates. The variability detected in sequences of sodA genes is more useful for recognition of closely related species within family Pasteurellaceae, than the 16S rRNA gene analysis. We conclude that the carbon source utilization assay has the ability to identify *P. multocida*, at the species level. But the identification of other members of *Pasteurella* sensu stricto based on only metabolic fingerprinting is often misleading. Thus, this technique must be combined other available methods and database of this system is need to wide with results of genetically accurately characterized isolates for better application of Biolog Microstation™ ID System in diagnostic work.

FAGE (FAST ADAPTIVE GENOME EVOLUTION) OF *SACCHAROMYCES* DURING WINE FERMENTATION

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Although grape wine is the product of microbial activity, the fermenting grape must is a rather hostile environment for most microorganisms. Very few yeast species can adapt to the harsh conditions and survive until the completion of fermentation. One of these species is *Saccharomyces cerevisiae*. The physiological adaptation of this yeast is mainly based on transcriptional up- and downregulation of genes which, however, has limits. Therefore changes in the genome may also be necessary (adaptive genome evolution). Due to its unique plasticity, the wine yeast genome can easily change both during vegetative propagation (mitotic divisions) and in the sexual cycle (meiosis-sporulation-conjugation). During fermentation, the genomes of certain vegetatively propagating yeast cells undergo multiple, recurrent changes (mutations and gross genomic rearrangements) resulting in a variety of clones with slightly modified genomes. In each phase of fermentation, the clone(s) with the highest fitness outgrow(s) the other clones, but to cope with the even harsher conditions of the next phase its/their cells will have to further modify their genomes. The cost of this process is a concomitant accumulation of recessive lethal and deleterious alleles in heterozygous state. Upon completion of fermentation, starvation triggers meiosis and sporulation in the cells which results in segregation of their heterozygous genomes. The spores that received the deleterious alleles will die; their death eliminates most of the deleterious mutations

(genome purification by meiosis-sporulation). The viable spores will germinate to produce vegetative cells capable of conjugation. Conjugation of sister cells results in homozygous diploids (autodiploidisation, "genome renewal"), whereas conjugation of non-sister cells generates new genomes by combining the genomes of two different spores (heterodiploidisation). These auto- and heterodiploids will then compete with each other and evolve during the new fermentation.

INVESTIGATION OF THE ROLE OF PENICILLIN-BINDING PROTEINS IN THE CEFOXITIN AND CARBAPENEM RESISTANCE OF *BACTEROIDES FRAGILIS* STRAINS

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Cephamycins and especially carbapenems are among the most effective antibiotics in the treatment of anerobic infections. The antimicrobial resistance mechanisms in these cases mainly include the action of β-lactamases and the decreased affinity of the penicillin-binding proteins (PBPs). The PBP species of *B. fragilis* NCTC9343 have previously been determined by bioinformatic searches of the sequenced genomes and functional assays. Additionally, we recorded the minimal inhibitory concentrations of *Bacteroides* strains to cefoxitin and imipenem, and detected the presence of β-lactamase genes (cfxA, cephamycins; cfiA, carbapenems), which implicated a substantial role for other resistance mechanisms, presumably for the PBPs. To investigate the role of PBPs in cefoxitin and carbapenem-resistant cases, cell-wall membranes were prepared from selected *B. fragilis* strains and competitive binding assays for cefoxitin and imipenem were carried out using bocillin, a flourescent derivative of penicillin, as a detection agent. From the resultant data, IC_{50} values were calculated to characterize each PBP species in the strains examined. It was a general trend that the PBPs of the different B. fragilis strains examined were either largely insensitive (PBP-A or PBP3) or largely sensitive (PBP1ab or PBP1c) to these antibiotics in the applied concentration range (0-256 μg/ml). An exponential connection was revealed between the cefoxitin MICs and the binding affinity of PBP2 or the sum of all PBPs for cefoxitin in the cases of cfxA-negative strains. Our data suggest that some PBPs have a major, but not exclusive role role in cefoxitin and carbapenem resistance of B. fragilis.

EFFECTS OF PH AND SUBSTRATE CONCENTRATION ON BIOSYNTHESIS OF FRUCTO-OLIGOSACCHARIDES

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Nowadays, fructo-oligosaccharides (FOSs) are one of the most important and widest applied prebiotics, because the number of interesting properties. Clinical evidences are available that FOSs stimulate the growth of the probiotic bifidobacteria resident in the colon improving health balance. However, FOSs offer important physiological properties. They decrease the levels of serum cholesterol, phospholipids, and triglyceride. Additionally, FOSs have a low sweetness intensity since they are only about one-third as sweet as sucrose. They are non-cariogenic. Through decreasing of pH of colon mucosal they increase the absorption of several mineral matters. Chemically, FOSs mainly consist of fructose units joined to chain by β(2-1) glucosidic linkages. The length of chains may be variable (DP_3-DP_{10}), which have a glucose unit at the end with b(1-2) bond. The three mostly used FOSs are 1-kestose [β-D-fructofuranosyl-(2→1)2-α-D-glucopyranoside, GF_2], nystose [β-D-fructofuranosyl-(2→1)3-α-D-glucopyranoside, GF_3] and fructosyl nystose [β-D-fructofuranosyl-(2→1)4-α-D-glucopyranoside, GF_4). Recently these oligosaccharides are produced from sucrose applying transferase activity of fructofuranosidase. However, commercial FOS contains high levels of glucose, fructose and sucrose which are released as by-products. Thus, in commercial products, FOS can account for only 55–60% of the total dry weight and relatively contain high amount of glucose molecule that limit the consumption of these product by diabetes. In this research work the effect of pH and substrate concentration on biosynthesis of fructo-oligosaccharides were studied with application of mono-substrate (sucrose) and multi-substrate (sucrose and fructose). Commercial available invertase from *Candida utilis* was used to create transfructosyl bioconversion. The enzyme reaction was carried out on the optimal temperature (55 □C). Samples were taken at time intervals and boiled for 10 minutes for stop the reaction before analysis. Saccharides were monitored by HPLC. Effects of ratio of substrate to enzyme were investigated in range of substrate concentration from 10 (w/v) % up to 60 (w/v)

%. At the 2nd day of bioconversion with 1 U/ml hydrolytic activity the FOS concentration increased significantly. Maximum FOS concentration was detected at 50 (w/v) %. After that increasing of substrate concentration did not result any increase of FOS. Effects of pH (from pH 3.0 to 7.5) on transfer reaction were also investigated. In the case of monosubstrate, bioconversion carried out at pH 6.0 giving the best result with DP_3 content (1.28 g/100ml).
To reduce amount of glucose molecule in final product, bioconversion of multi-substrates was created and applied. Media containing different ratios of sucrose and fructose were prepared in total dry matter 50 (w/v) %. Optimal fructose:sucrose ratio was fixed to be 10g:40g in 100ml sodium-acetate buffer. Applying this medium, the FOS concentration reached 0.57g/100ml. Effects of pH on bioconversion with multi-substrate system were also investigated. Invertase enzyme showed the maximum transfer activity at pH 6.5 (0.84 g/100ml). More studies are needed dealing with multi-substrate system to improve FOS content.
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SIZE CONTROL IN FISSION YEAST: EXPERIMENTS AND SIMULATIONS

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Size control maintains size homeostasis in cell populations; it enables that size distribution does not change in consecutive generations. The most important requirement is that there must be at least one cell cycle event, which cannot be performed unless the cell has reached a critical size. Generally, two events are known to be controlled by cell size, namely initiating DNA replication (S phase) and the onset of mitosis (M phase). As a consequence, two size checkpoints exist in the eukaryotic cell cycle, one in G1 phase, and another one in G2. The molecular mechanisms of these size checkpoints are still obscure, however, they seem to be evolutionary conserved from unicellular microorganisms up to even human cells. Since the late 1970s fission yeast is an attractive model organism in size control studies. The cylindrically shaped fission yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. As a consequence, cell length is an easily measurable parameter, which characterizes cell size. Early time-lapse microphotographic studies with fission yeast proved that there is a strong negative correlation between cycle time and birth length, i.e., the larger the cell at birth, the shorter its cell cycle. In wild-type cells, G2 phase is long and size-dependent, meanwhile G1 is short and constant, therefore, size control seems to operate exclusively in G2 in fission yeast. Later, it was discovered that length growth in fission yeast follows a bilinear pattern during the cycle, i.e., growth is linear with a point in mid G2 where growth rate increases. Separating the cell cycle into a preRCP and a postRCP period, indicated that size control acted in the first part of G2 rather than near the G2/M transition (as previously thought). The wee1 mitotic inhibitor was found to be mainly responsible for this size checkpoint. The general view is that in small cells wee1 keeps the cdc2/cdc13 complex (also known as M-phase promoting factor or MPF) in an inactive form. After reaching a critical size, wee1 itself becomes inactivated by some mitotic activator(s) and the cell starts to prepare for mitosis. Former models suggested that MPF accumulated (proportional to the increasing cell size) in the constant sized nucleus, which hypothesis was able to describe quantitatively the phenomenon of size control for many years. However, in 2007 Paul Nurse showed that the nucleus extended during the fission yeast cell cycle, parallel to cell volume, ruling out the above hypothesis. Recent experimental data seems to give us a new clue how size control might even operate. Namely, a spatial gradient of a mitotic inhibitor (pom1, indirectly acting on wee1) is generated along the cell cortex, having a maximal value at the cell tips, and a minimum at the centre. As the cell grows, local pom1 concentration at the cell centre decreases and finally in late G2 it drops below a critical level, which is no more able to delay mitotic onset. As a consequence, initiation of mitosis is connected to reaching a threshold in cell size.
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MEROPENEM SENSITIVITY OF MYCOBACTERIA

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Tuberculosis is one of the most persistent human diseases. No new anti-TB drugs have been introduced in the past 40 years, even though their development is becoming increasingly important to face new challenges posed by multidrug-resistant and extensively drug-resistant strains and by acute infection with *Mycobacterium tuberculosis* of HIV positive patients. β-lactam antibiotics were regarded as ineffective against tuberculosis. This widely used class of antibacterial drugs targets the 4 →3 transpeptide linkages in the peptidoglycan layer. In the cell wall of nonreplicating *M. tuberculosis* the nonclassical 3→3 linkages predominate. Recently, meropenem-clavulanate was shown to be effective against drug resistant *M. tuberculosis*. We are studying the sensitivity of different bacteria against meropenem. Among bacteria examined, *Escherichia coli* was the most sensitive with a mic value of 0.023 μg /ml, the mic value of *M. bovis* BCG was 0.19 μg/ml while *M. smegmatis* was even more resistant at 2 μg/ml. The meropenem resistance gene (blaIMP-6) was cloned from *Pseudomonas aeruginosa* and expressed in *E. coli*. Cell lysates were separated in SDS-polyacrylamide gels. The overexpressed protein (metallo-beta-lactamase IMP-6) showed a strong band, which was identified by mass spectrometry. Its calculated molecular weight is 27 kDa. The mic value of mycobacteria containing the cloned IMP-6 gene was 30 μg/ml. Plasmids carrying the meropenem resistance gene could be valuable tools in mycobacterium research.
This work was supported by OTKA NKTH 69132.

AMINOGLYCOSIDE MODIFYING ENZYME GENES IN *ESCHERICHIA COLI* ISOLATES COLLECTED IN A UNIVERSITY HOSPITAL AND IN HUNGARIAN ESBL POSITIVE *E. COLI* ISOLATES

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Aminoglycosides are frequently used antibiotics, but their utility is severely threatened by spread of resistance. The aim of this work to seek for the five aminoglycoside resistance genes coding for modifying enzymes most commonly found in *Escherichia coli*. Out of these three genes confer clinically important resistance, i.e. aac(3')-IIa to gentamicin, tobramycin and netilmicin, aac(6')-Ib to tobramycin, netilmicin and amikacin and ant(2")-Ia to gentamicin and tobramycin, while the remaining two cause clinically less relevant resistance,
ant(3")-Ia to streptomycin and spectinomycin and aph(3')-Ia to kanamycin and neomycin.
Altogether 127 *E.coli* isolates, collected between 2005 and 2007 were examined; 37 were ESBL producers collected from different regions of Hungary by the National Center for Epidemiology (NCE), while 90 isolates were collected from the Surgery Department of the University of Debrecen (UD). Aminoglycoside susceptibility tests were performed by the CLSI disk diffusion method. Genes were sought for by means of specific PCR assays, results were confirmed by sequencing.
In the NCE isolates the aac(3')-IIa, aac(6')-Ib, ant(2")-Ia, ant(3")-Ia and aph(3')-Ia genes were detected in 12/37 (32.4%), 10/37 (27.0%), 2/37 (5.4%), 8/37 (21.6%) and 4/37 (10.8%) isolates, respectively. At least one gene was detected in 26/37 (70.3%) isolates and multiple genes were carried by 9/37 (24.3%) isolates. Nineteen isolates (51.4%) harboured at least one gene conferring clinically relevant resistance. In six cases (16.2%) the gene patterns found could not explain the resistance phenotype, while demonstration of resistance genes indicated that phenotypic susceptibility testing yielded a false susceptible result for ten isolates (27.0%).
In the UD isolates detection rates for the genes aac(3')-IIa, aac(6')-Ib, ant(2")-Ia, ant(3")-Ia and aph(3')-Ia were 8/90 (8.9%), 8/90 (8.9%), 4/90 (4.4%), 21/90 (23.3%) and 16/90 (17.8%), respectively. At least one gene was found in 46/90 (51.1%), but out of these only 20/90 (22.2%) were associated with clinically relevant resistance. Carriage of multiple genes was detected in 10/90 (11.1%) isolates, but only one of the harboured genes was responsible for clinically relevant resistance in these isolates. The resistance phenotype was not explained by the gene pattern found in three isolates, while false susceptibility was demonstrated also in three isolates (3.3%)

The most common gene among the NCE isolates was aac(3')-IIa, a clinically relevant gene, while among the UD isolates ant(3")-Ia. Though the latter gene confers resistance to drugs used rarely in human medicine, the carriage rates are relatively high, drawing attention to the risk of long-term maintenance of resistance genes. Gene detection frequently indicated false susceptible results of the phenotypic tests, especially for amikacin susceptibility; therefore strict observation of the EUCAST expert rules is justified.

MOLECULAR VIROLOGY ASSAY AS PCR FOR RAPID AND SENSITIVE DETECTION OF INFLUENZA VIRUSES DURING 2009/2010 SEASON IN HUNGARY

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The most important respiratory infection in human caused by Influenza virus belongs to family of Orthomyxoviridae. Our laboratory used nested PCR assay for detection with hemagglutinin specific primers. A new method was developed as real-time reverse transcriptase PCR because of pandemic H1N1 in 2009. Since the genome of Influenza A and B are single-stranded RNA. A DNA copy which is complementary to viral RNA must be synthesized prior. Specific primers and probes were designed by recommendation of several laboratories for real-time PCR. Our developed PCR system use to identify subtypes of Influenza virus (H1, H1swl, H3, H5) but we carry out a PCR assay also for detection of neuramindase, nucleoprotein and matrix genes. Sensitivity of PCR was high because the copy number was approximately 1.8 x 10^2 copies/ µl. Due to Influenza Surveillance the samples from patients and conformation of H1swl were traceable. Our developed system turned out a success, reliable and sensitive.

DETECTION OF TOXINOGENIC *FUSARIUM* STRAINS IN PLANT TISSUES BY MOLECULAR BIOLOGICAL METHODS

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Several *Fusarium* species, which can be inhibited in soil, produce mycotoxins belonging to the trichothecenes, such as deoxinivalenolt (DON), and other toxins e.g. zearalenon (ZEA) and fumonisin (FB1). Fusariums in general can be found in the cereals grown in the temperate zones of America, Europe and Asia.
Cereals can be infected by mycotoxin-producing moulds in Hungary. Wheat, barley and maize give the two third of the world's crop production and these plants are the most exposed of *Fusarium* infection. These cultures take significant parts of the sowing area of Hungary. Food quality is highly influenced by the microbial factors of the food chain stage soil-plant-animal product. Occurrence of *Fusarium* toxins in products intended for animal consumption can be harmful to any species of animals and can cause significant damage in livestock breeding what can be observed in the decrease of the ability of reproduction, the utilization of fodder and the capability of resistance against the different infections. Even frequent diseases and death can occur according to the quantity and type of mycotoxin. To prevent the occurrence of *Fusarium* toxins in products intended for animal consumption or at least to decrease it to the minimal level on behalf of the avoidance the negatively effect is highly important. Beside the identification of *Fusarium* isolates on the base of phenotypical features the development of fast and reliable molecular biological methods inclusively effective detection of toxinogenic genes is also required. During our research, such a PCR method was used that was suitable for the detection of toxinogenic fungi species by amplifying the genes responsible for the production of toxins. The PCR reaction was optimized by testing the efficiency of the primers in vitro, using isolated DNA from cultures of DON, zearalenone and fumonisin producing species. Then we used these optimized PCR systems to test the DNAs from different plant tissues infected with Fusarium in order to determine the sensitivity and specificity of the PCR based detection when used with different matrixes. The results are compared with those concluded using classic isolation and fusario toxin detection techniques.
This study was supported by the NKTH TECH_08-A3/2-2008-0385 (OM-00234/2008) MYCOSTOP grant.

FERMENTATION OPTIMIZATION OF THE *FUSARIUM VERTICILLIOIDES* STRAINS FOR THE FUMONISIN PRODUCTION

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Fumonisins are mycotoxins produced primarily by *Fusarium verticillioides* in corn, however, their production has also been proved for several *Aspergillus* isolates came coffee beans, grapes as well as dried vine fruits. Fumonisins can cause both animal and human health problems, and fumonisin B_1 was considered by International Agency for Research on Cancer to be class 2B carcinogen to humans. Recent surveys identified about 100 different isomers and stereomers of fumonisins, but the toxicological effects of these compounds are currently unknown. In order to evaluate the toxicological and carcinogenic effects of fumonisins, large amounts need to be purified, which start with fermentation of the toxicological compounds and requires optimal conditions for production in culture. In this study, we investigated the effect of cultivation period and water amount on the fumonisin B_1, B_2, B_3 and B_4 production.

Six Hungarian *F. verticillioides* isolates were compared for their ability to produce fumonisins on solid rice culture in three cultivation period. Inoculations were made with agar plugs of the precultured strains and the incubation was at 28 °C in dark for 2, 3 and 4 weeks. After the initial examinations, one of the isolates was selected to monitor the effect of the water amount added to rice at start of the fermentation. The fumonisins were extracted from the dried culture material after the cultivations with acetonitrile/water and were analyzed by reversed phase high performance chromatography ion-trap mass spectrometry.

Both the profiles and the amounts of the produced toxins were different among the isolates and was influenced by the applied fermentation conditions. In some cases the toxin production decreased in time or remained in about same level at the weekly sampling depending on the isolates. However, the amount of water correlated well with the production, higher water content usually caused increased toxin secretion.

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IDENTIFICATION OF BACTERIUM STRAINS OF DIFFERENT ORIGIN ON THE BASIS OF 16S RDNA

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Development of functional foods is mainly based on the utilization of probiotic microbes, which strains principally belong to the group of lactic acid bacteria. These bacteria are responsible for the fermentation process of certain foods and some of them might be found in the human colon, helping to maintain the microbial balance. Nowadays there is a growing demand towards the probiotic products from the manufacturers' and costumers' side, as well. In the course of the development of probiotic foods the first step is the isolation and identification of potential probiotic bacterial strains, followed by the evaluation on the bases of strict standard rules.

Probiotic products in Hungary contain only a few different probiotic bacterial strains. Our aim is the development of such functional foods that contain newly isolated bacteria, hence these products would be unique and novel in the Hungarian and regional food market.

In our experiments we investigated different milk samples (non-pasteurized cow's milk, sheep's milk, goat's milk, sheep cheese, goat cheese and butterfat) of various origin, fermented products (bred beverage and boza), honey stomach of Apis mellifera and bee bread. In order to select the lactic acid bacteria, we used BSM and MRS media as selective medium. For DNA extraction, enrichment of bacteria was made in TPY and MRS broth. 30 strains were isolated from the different milks and other products. At first we identified and differentiated as many strains as possible by the traditional microbiological methods, and then we performed restriction analysis (RFLP) on the strains. We used the E8F-E1115R primer pair that amplifies an 1100 bp long fragment of the 16S rDNA. This product was digested by the mix of three restriction enzymes (AluI, HhaI and RsaI) overnight at 37 °C. Each restriction enzyme has a 4 bp recognition site and works in the same buffer. Restriction fragments were separated on 2 % agarose gel. Standard restriction patterns were made by the use of known bacterial strains and the pattern of the new isolates was compared to these. PCR fragments from of each bacterium were sequenced and the data were used to set up a database for identification of new bacteria.

An advantage of the RFLP method when compared to the microbiological identification is that even those strains can be distinguished, which have very similar morphological and biochemical characters. The 30 isolates we found showed 16 different restriction patterns. We continue the search for new probiotic strains and our database can be used for the rapid identification of bacteria.
The research was financed by the NKTH and NFÜ .

ANTIMICROBIAL ACTIVITY OF POTENTIALLY PROBIOTIC BACTERIA

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Research of probiotic bacteria gets more and more attention from the consumers and producers side, as well. In addition to their well-known effects some probiotic strains have another useful feature: production of bacteriocin, which prevents the growth and multiplication of pathogenic Gram-positive bacteria, thus may represent alternatives to artificial preservatives. Nowadays, the nisin is the only authorized bacteriocin, but there is a growing interest towards the bacteriocins. Lactic acid bacteria are widely used in the production of fermented dairy and meat products and vegetables. The primary use of bacteriocin producing lactic acid bacterial strains is to supplement the commonly used starter cultures with them as natural ingredients and functional food components in order to produce antimicrobial agents; hence, artificial preservatives, which may harm not only pathogenic but useful bacteria, too, may be replaced or their amount can be decreased. The other possibility is to add bacteriocin to the food, thus it can inhibit food contaminating pathogens and spoilage microorganisms. Proteolytic enzymes in the human digestive tract usually inactivate bacteriocins and break them down as any other protein. Bacteriocins are not toxic and their physicochemical properties (colourless, odourless, tasteless substances and can be used simultaneously with artificial preservatives) further increase their potential in the food industry. Until recently, bacteriocin production ability of several *Lactobacillus* and *Bifidobacterium* strains was investigated. In our experiments we examine the bacteriocin production of our own bacterium isolates. A long-term goal of our work is to identify bacteriocin producing lactic acid bacteria to inhibit the growth of pathogenic and spoilage bacteria in different food matrices, and hereby form a new collection of useful bacteria for the rapidly developing food industry. We started our investigations with two probiotic strains, the *Lactobacillus acidophilus* DSM 20079 and *Lactobacillus delbrueckii* DSM 20076. The *Lb. acidophilus* is a bacteriocin (Acidocin 20079) producer, while the *Lb. delbrueckii* strain shows sensitivity against the Acidocin 20079. The *Lb. acidophilus* was grown in fermentor for 18 hours at 37 °C at pH 6.0 under anaerobic conditions. After cultivation the pH was set to 5.0 and the bacteriocin was extracted from the broth. We tested the effectiveness of the Acidocin by agar diffusion method against different *Lactobacillus* strains.
As a second step we have started to investigate the bacteriocin production ability of *Bifidobacterium* and *Lactobacillus* strains that we isolated from various sources (milk and bee products and fermented beverages). The effectiveness of bacteriocins is tested against *Listeria*, *Staphylococcus* and *Streptococcus* indicator strains. In the next phase of the work we try to map those genes that are responsible for the production of bacteriocins.
The research was financed by the NKTH and NFÜ.

EPIGENETIC REGULATION OF THE HUMAN MICRORNA MIR-146A IN EBV POSITIVE AND NEGATIVE CELL LINES

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MicroRNAs are 20- to 23-nt-long RNA molecules expressed in plants and metazoan animals. MicroRNAs affect both posttranscriptional and transcriptional regulatory processes. The human miR-146a micro RNA, encoded by an individual gene, is an important regulator of the TNF receptor mediated signaling pathway. MiR-146a is upregulated in various neoplasms and plays a role in innate immunity. It affects TRAF6 and IRAK1 negatively and it is overexpressed in numerous pathogenetic processes (e.g. rheumatoid arthritis, acute coronary syndrome). The Epstein-Barr virus (EBV) encoded latent membrane protein 1 (LMP1) can induce the expression of miR-146a via the NFkB pathway. The miR-146a promoter contains several transcription factor binding sites, for example NFkB and c-Myc recognition sequences, based on in silico examinations.
Epigenetic regulatory mechanisms play an important role in the control of gene activity. We wished to analyse,

therefore, the role of DNA methylation using bisulfite modification and direct sequencing, activating histone modifications using chromatin immunoprecipitation, and protein-DNA interactions using in vivo DMS footprinting, in the regulation of the human miR-146a microRNA promoter. To characterize the promoter activity we determined the level of different maturation forms of miR-146a using Northern blot and Nuclear run-on assay in EBV positive and negative cell lines.
Nuclear run-on experiments showed that LMP1 expression did not correlate with miR-146a promoter activity. The promoter was silent in epithelial cell lines and hybrid cell lines, and showed a variable activity in lymphoid cells. Bisulfite sequencing showed that the promoter region of the miR-146a micro RNA gene is unmethylated in most of the cell lines with a few exceptions, where the elevated level of DNS-methylation correlated well with promoter inactivity. A single CpG dinucleotid was found to be un- or hypomethyalted in most of the cell lines. Using chromatin immunopecipitation we found only an incomplete correlation between promoter activity and the level of activating histone modifications. NFkB and c-Myc binding to the promoter could be verified in all cell lines except one, independently of the EBV gene expression pattern, using DMS in vivo footprinting.
Further studies are needed to understand the cell type specific regulation of the miR-146a promoter.

CROSS-PROTECTION AND IMMUNE RESPONSE PROVOKED BY LIVE *SHIGELLA* MUTANTS LACKING MAJOR IMMUNODETERMINANTS

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Shigella elicits approx. 164 million dsentery cases annually. As proper hygienic conditions alone are not enough for the prevention of diseases in the endemic areas, the need for a vaccine has been declared since decades. As during a natural infection the protective immunity is specific to the highly variable serotype determining O-antigen, the protection is restricted to the homologous strains. However an effective vaccine should provide protection against numerous serotypes. To obtain broad spectrum coverage current vaccine strategies rely on the combination of several *Shigella* serotypes, whereas a cross-protective vaccine strain could be an optimal approach. To examine the cross-protective capacity of the conserved antigens expressed by the *Shigella* strains, we tested immunogenic potential of mutants lacking both the O-antigen and the invasion plasmid.
We selected a phase II. form of a prototype *S. sonnei* strain, lacking both the invasion plasmid antigens and the O-antigen encoded on the same virulence plasmid and examined the attenuation and cross-protective capacity of this mutant. In order to identify the protective shared antigens we performed Western-blot with the sera and bronchoalveolar lavage of immunised mice. Furthermore to gain deep insight on the immune response provoked by the mutant we identified the white blood cells in the lung and the blood samples of mice infected with either the phase I. or the phase II. form strain with flow cytometry.
Similarly to the previously presented data with a non invasive rough *S. flexneri* 2a mutant, the phase II. form of *S. sonnei* was highly attenuated. The repeated immunisation with the mutant provided significant protection in the mouse lung model against a heterologous *S. flexneri* 6 strain. The identification of the proteins detected as shared antigens with the Western-blot is in progress. The ratio of the lymphocytes, granulocytes and monocytes in the lung and blood of the mice was slightly different following an infection with the phase II. form mutant compared to the wild type strain. To clarify the effect of the booster immunisation on the protective immunity we intend to perform the same analysis of the white blood cells following repeated immunisation.

IN VIVO EFFICACY OF FLUCONAZOLE AND CASPOFUNGIN AGAINST *CANDIDA PARAPSILOSIS* AND *C. ORTHOPSILOSIS* IN TEMPORARILY AND DEEPLY NEUTROPENIC MOUSE MODELS

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We tested the in vivo efficacy of fluconazole (FLU) and caspofungin (CAS) against three *C. orthopsilosis* isolates and against two *C. parapsilosis* isolates for comparison. BALB/c male mice were given a single or two 200 mg/kg cyclophosphamide doses (temporarily and deeply neutropenic model, respectively). Temporarily neutropenic mice were infected intravenously with 5-6x10^6 CFU/mice. Intraperitoneal treatment with 1, 5 and 10 mg/kg FLU and 1 and 2 mg/kg CAS daily doses was started 24h postinfection and continued for 5 days.
In the deeply neutropenic model the inoculum was 2x10^6 CFU/mice. Groups of 7-8 mice were treated with daily 1 and 2 mg/kg or 5 and 10 mg/kg single CAS doses or used as untreated controls. Drug efficacy was assessed by

determining the number of CFUs per kidney pair. For statistical analysis we used Kruskal-Wallis test with Dunn's post testing. FLU was active against all *C. orthopsilosis* and both *C. parapsilosis* isolates at 10 mg/kg and ≥5 mg/kg doses, respectively ($p<0.05$). Two mg/kg CAS was effective against *C. orthopsilosis*, but not against *C. parapsilosis*; the lower dose was ineffective against both species. In the deeply neutropenic model 2 mg/kg daily and the single 5 and 10 mg/kg CAS were effective ($p<0.01$) against *C. orthopsilosis*, but not against *C. parapsilosis*. *C. orthopsilosis* seems to possess decreased susceptibility to FLU in vivo. CAS is a good alternative against *C. orthopsilosis*, but not against *C. parapsilosis*.
This study was supported by Richter Gedeon Nyrt.

AUTOLYSIS AND EXTRACELLULAR GLUCANASE PRODUCTION IN *ASPERGILLUS NIDULANS*

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Autolysis of filamentous fungi can be defined as a natural process of self-digestion occurring as a result of hydrolase activity, causing vacuolization and disruption of organelle and cell wall structures. With other cell wall biopolymers (primarily chitin) ß-1,3-glucans are important structural components of the fungal cell walls and ß-1,3-glucanases are needed to degrade their complex structure.
A ß-1,3-endoglucanase was purified from carbon starving cultures of Aspergillus nidulans by a two-step purification procedure consisting of $(NH_4)_2SO_4$-precipitation and chromatofocusing. This enzyme was found to be encoded by the engA gene (locus ID: AN0472.3). EngA belongs to the glycosyl hydrolase family 81 (family of eukaryotic ß-1,3-glucanases). The molecular mass of the EngA was proved to be 91±3 kDa (SDS-PAGE) and 230 kDa (native gel electrophoresis) so it is likely that EngA forms a homodimer. EngA was active over a wide range of pH (3.5–10.5) with an optimum activity measured at pH 6.5, and also preserved its activity when incubated at pH 6.5-8.5 without laminarin for 24 h. It was active up to 95 ^{o}C incubation temperature with highest activities recorded between 45-65 ^{o}C incubations but it was quite unstable when pre-incubated in the absence of laminarin at temperatures higher than 55^{o}C.
The regulation of engA was found to be dependent on the FluG/BrlA asexual sporulation signaling pathway in submerged culture, however, neither the mutations affecting heterotrimeric G protein signaling pathways, e.g., fadA^{G203R} and ΔganB, nor the ΔcreA mutation had any significant effect on the β-1,3-glucanase production.
The autolysis-related physiological function of EngA was verified by generating ΔengA single and ΔengA ΔchiB double deletion mutants. The absence of engA gene resulted in a considerable blockage in the autolytic loss of biomass and hyphal fragmentation triggered by carbon-starvation. The ΔengA mutation also affected negatively the extracellular ß-1,3-glucanase, chitinase, ß -glucosidase and proteinase activities and the mRNA accumulation of the genes pepJ (encoding the metalloproteinase PepJ), prtA (encoding the serine proteinase PrtA) and chiB (encoding the endochitnase ChiB). It is worth mentioning that the ΔchiB mutation alone also decreased the extracellular ß -1,3-glucanase, ß -glucosidase and proteinase activities.
Our results demonstrate that the production of cell-wall-degrading enzymes was coordinately controlled in a highly sophisticated and complex manner. EngA appears to have a pivotal role in fungal autolysis and activities of both EngA and ChiB are necessary to orchestrate the expression of autolytic hydrolases.

ENVIRONMENTAL MONITORING OF DANUBE WATER QUALITY IN BUDAPEST REGION

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Water quality monitoring of international rivers has always been a difficult task in Worldwide. Monitoring the environment with biological and chemical systems is absolutely essential to identify human health and ecosystem hazards, to assess environmental cleanup efforts, and to prevent further degradation of the ecosystem. Biomonitors and biomarkers combined with chemical monitoring offer the best approach to making these assessments. According to changing in the environment monitoring parameters of Water Monitoring System, our objectives are going to select the most important parameters that investigate physico-chemical and microbiological assessment for water quality of the main Danube River in Budapest region.

The monitoring assessment of the Danube water was conducted in three replicates. The samples were collected in sterile dark bottles of 500 ml capacity from three different zones between Erzsébet and Petőfi Bridges from the Pest and Buda sides. A comparative studies were carried out during the time intervals 1998, 2005 and 2010. The surface water test parameters were: 1. Physico-chemical conditions: transparency, temperature, pH, electroconductivity, dissolved oxygen content, oxygen saturation, BOD_5, COD_{Mn}, NH_4^+, NO_3^-, NO_2^-, total N content, total phosphorus, alkalinity (K^+, Na^+, Cl^-, SO_4^{2-}); total hardness (Ca^{2+}, Mg^{2+}), heavy metals, total iron, total manganese; total dissolved organic materials; 2. Biological status: Biological indicator (chlorophyll-a), and 3. Microbiological status: Number of coliforms, faecal coli, faecal streptococci and total bacterial count at 20°C.
According to the investigated environmental parameters, our results demonstrates that the water quality of the main Danube River has become unstable during the last 12 years subsequent to the diversion which could be witnessed particularly in the unfavorable oxygen saturation levels. It was found that dissolved organic matter, nutrient pollution, and microbiological parameters as well as the chlorophyll-a were higher in 2005 than in 1998 and 2010. Monitoring of the river section began after the underwater weir began operating. The data showed no significant differences between transparency, temperature, pH and Electroconductivity in 1998, 2005 and 2010 as well as the between the both sides of the River.
With large numbers of new chemical substances entering river ecosystem, continuous monitoring systems for their detection will become increasingly important with respect to environmental effects they produce, in addition to other toxic effects. Much effort has to be directed towards the detection of such pollutants in rivers. Thus the challenges to continuous chemical and biological monitoring will be immense. Finally the importance of integration of biological and chemical monitoring has to be emphasized.

INVESTIGATIONS ON THE TRANSCRIPTION PATTERNS OF AUJESZKY DISEASE VIRUS MUTANTS

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OPTIMIZATION OF THE FERMENTATION CONDITIONS FOR TOXIN PRODUCTION OF *BACILLUS THURINGIENSIS* SP.

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The corn is one of the most important food and feed crop. Its utilization is versatile, such as forage feed, industrial applications and human consumption. One of the most important corn pests is the Western corn rootworm (*Diabrotica virgifera virgifera* LeConte). In Hungary, its presence was detected for the first time near Szeged, in 1995. Till than it became the main enemy of the corn and thereby of the agronomists and farmers. The main damage, manifested on the root, is caused by the larvae. That is the reason why scientists are making efforts to develop a technology for the efficient control of larval damage and spread. *Bacillus thuringiensis* is a Gram-positive, soil-dwelling bacterium that is capable producing endospores and also occurs in the gut of caterpillars and larvae. As well known some strains of *Bacillus thuringiensis* are able to produce endotoxins

(crystal proteins, Cry toxins) lethal to corn rootworm larvae. Thus, the bacterium itself, or alternatively, the Cry toxins can be used as biopesticides. Efficient production of Cry toxins by biotechnological fermentation is crucial. Our aim was to develop a fermentation technology that optimizes the growth, sporulation and crystal protein production of various *B. thuringiensis* reference strains (*Bacillus thuringiensis* var. *kumamotoensis* DSMZ 6070, *Bacillus thuringiensis* var. *kurstaki* NCAIM 01262, *Bacillus thuringiensis* var. *israelensis* NCAIM 01289). The effect of different fermentation media was investigated in order to reach high cell density (CFU/ml) and intensive crystal protein forming. Various carbon and nitrogen sources as media components were applied in the fermentations. The optimal harvest time of the crystal proteins production was determined. Spore and crystal contents of the fermentation cultures were investigated by phase-contrast microscopy, while protein patterns of the endospore + crystal preparations were revealed by SDS-polyacrylamide gel electrophoresis. Cultivation temperature was also optimized. Fermentations were run at different temperatures, crystal forming was ascertained and protein profiles of the sporulated cultures were compared.
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CHARACTERIZATION OF ACTIVE MICROBIAL COMMUNITY OF HYPOXIC, AROMATIC HYDROCARBON CONTAMINATED GROUNDWATER: DETECTION AND DIVERSITY OF CATECHOL 2,3-DIOXYGENASE MRNA AND 16S RRNA TRANSCRIPTS

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Defensins are an important family of natural antimicrobial peptides. *Chlamydophila pneumoniae*, a common cause of acute respiratory infection, has a tendency to cause persistent inflammatory diseases such as atherosclerosis, which may lead to cardiovascular disease or stroke. As endothelial cells are related to the physiopathology of stroke, the effects of in vitro *C. pneumoniae* infection on the expression of human β-defensin 2 (HBD-2) in brain capillary endothelial cells BB19 was investigated.
A time-dependent increase in HBD-2 mRNA was observed by means of RT-PCR in BB19 cells following *C. pneumoniae* infection, with a maximum increase at 24 h. A gradual induction of HBD-2 protein in the *C. pneumoniae*-infected endothelial cells was detected by immunoblotting. Immunofluorescence revealed the staining of HBD-2 in the cytoplasm of endothelial cells following *C. pneumoniae* infection. The secretion of HBD-2 (confirmed by ELISA) was significantly elevated 24 h after *C. pneumoniae* infection.
These novel results indicate that HBD-2 is expressed and produced in the human brain capillary endothelial cells upon infection with *C. pneumoniae*, and provide evidence that HBD-2 plays a role in the early immune responses to *C. pneumoniae* and probably in the immunopathogenesis of atherosclerosis.

DISTRIBUTION OF VIRULENCE-ASSOCIATED GENO- AND PHENOTYPIC CHARACTERS IN PULSED FIELD GEL ELECTROPHORESIS (PFGE) CLUSTERS OF *KLEBSIELLA PNEUMONIAE* ISOLATES FROM URINARY TRACT AND BLOOD STREAM INFECTIONS

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The genome plasticity is a well-established phenomenon among the microbes and one of the most effective tools for the adaptation to the environment including the host organisms as well. This process reflects back when new genes or mobile genetic elements are identified in a pathogen causing serious, life-threatening diseases such as meningitis and primary liver abscess, which are not characteristic for classic *K. pneumoniae* infections. Some studies have shown the presence of new virulence-associated genes at low frequency in strains isolated from classic *K. pneumoniae* infections e.g. pneumonia, urinary tract and blood stream. Previously, we have already reported the results of our study on phenotypic and PCR based genotypic characterisation of *Klebsiella* isolates at this forum. In our present study, the previous characterisation data of the isolates were analysed in context of pulsed field gel electrophoresis (PFGE) patterns in order to reveal relations between clonality and virulence.

Both clinical sample groups included 88 *K. pneumoniae* isolates, which were analysed by PFGE according to the Standardized Laboratory Protocol (CDC, Pulse Net). Former phenotypic characterisation data (presence of type 3 and type 1 fimbriae, production of enterobactin and aerobactin, biofilm formation, and string test positivity) and PCR based genotypic characterisation data (presence of irp1 and irp2, kfuB, allS, rmpA, k2A and magA genes) of the isolates were analysed according to the clusters given by the PFGE dendrograms. The data evaluation revealed three PFGE clusters in the group of urinary tract isolates (i.e. UTI irp2-1, UTI kfuB I, and UTI kfuB II.), and two clusters in the group of blood stream isolates (i.e. BSI kfuB I and BSI kfuB II.). The kfuB gen showed significantly higher incidence rate ($p<0,001$) among the isolates of clusters UTI kfuB I.,-II. and BSI kfuB I,-II. than the rest of the isolates. The frequency of irp1, irp2 genes was significantly higher ($p<0,001$) in the cluster of UTI irp2-1 compared to all the other isolates in either sample types. Type 3 fimbriae showed significantly higher incidence ($p<0,005$) in BSI kfuB II cluster compared to the rest of the isolates irrespectively whether they were associated with the kfuB I cluster or not in the group of the BSI isolates. The biofilm production capability (mean of optical density 3,3) was significantly higher ($p<0,001$) in the BSI kfuB II cluster than in other isolates regardless whether they were associated with the designated cluster or not in BSI group.
Complex analysis of these data may reveal new relationships among the different characters that may help us to assess the importance of a certain virulence-associated character in the pathogenesis of *K. pneumoniae*.

INDUCIBLE EXPRESSION OF HUMAN B-DEFENSIN 2 BY *CHLAMYDOPHILA PNEUMONIAE* IN BRAIN CAPILLARY ENDOTHELIAL CELLS

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Defensins are an important family of natural antimicrobial peptides. *Chlamydophila pneumoniae*, a common cause of acute respiratory infection, has a tendency to cause persistent inflammatory diseases such as atherosclerosis, which may lead to cardiovascular disease or stroke. As endothelial cells are related to the physiopathology of stroke, the effects of in vitro *C. pneumoniae* infection on the expression of human β-defensin 2 (HBD-2) in brain capillary endothelial cells BB19 was investigated.
A time-dependent increase in HBD-2 mRNA was observed by means of RT-PCR in BB19 cells following *C. pneumoniae* infection, with a maximum increase at 24 h. A gradual induction of HBD-2 protein in the *C. pneumoniae*-infected endothelial cells was detected by immunoblotting. Immunofluorescence revealed the staining of HBD-2 in the cytoplasm of endothelial cells following *C. pneumoniae* infection. The secretion of HBD-2 (confirmed by ELISA) was significantly elevated 24 h after *C. pneumoniae* infection.
These novel results indicate that HBD-2 is expressed and produced in the human brain capillary endothelial cells upon infection with *C. pneumoniae*, and provide evidence that HBD-2 plays a role in the early immune responses to *C. pneumoniae* and probably in the immunopathogenesis of atherosclerosis.

CHANGES THE NUMBER OF MICROORGANISMS AND THE ENZYME KINETICS OF BIOGAS PRODUCTION AT DIFFERENT SUBSTRATES

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The combustion of greenhouse-gas-emitting fossil fuels plays a part in global climate chane, and the amount of theese energy resources is decreasing fast, therefore the importance of using renewable energy sources has been grown lately. Hungary has great biomass potential, hence biogas production can be a good way to use alternative energy sources. Kaposvár Sugar Factory of Hungarian Sugar Ltd. has a large-scale biogas plant using by-product of sugar industry (spent beet pulp) as raw material. In addition, the sugar factory has a pilot-scale biogas plant to test the parameters. The aim of the research was to determine the best fermentig temperature, and the optimal proportions of raw materials in order to optimizes the biogas process. Furthermore the aim was to come to know (partly) the microbiological processes of biogas production.
We used as raw materials the spent beet pulp and the sludge, which were taken from the pilot-scale biogas plant of Kaposvár Sugar Factory. We measured the gas yield with OxiTop OC110 when changing two parameters: the fermenting temperature (20°C, 37°C and 55°C) and the proportion on raw materials. The measuring head of this Oxitop system was collecting the pressure data derived from the gas (methane) production. The results shown, that the optimal fermenting temperatue is 37°C, and te best beet-sludge proportion is 1:1. The total aerob

bacterial number of the sludge before digestion was $1*10^9 – 3,1*10^8$ /g dry matter. After mixing the sludge with the spent beet pulp and fermenting, the amount of this bacterias was approximately 10^7 /g dry matter. The amount of total fungi was approximately 10^4 /g dry matter. Besides, the total aerob, anaerob and sporulated aerob bacterial number were determined. %. The anaerob total bacterial number was changed from $1,15*10^6$/ cm^3 to $1,9*10^7/cm^3$ during the thermofil fermentation using beet pulp-sludge mix. Furthermore, dehydrogenase enzyme activity of experimental sludge was determined in terms of mg formazane/ g sludge/ 24 hours, was 1,287± 15.
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INSTABILITY OF CYTOLETHAL DISTENDING TOXIN TYPE IV LOCUS IN AN AVIAN PATHOGENIC *ESCHERICHIA COLI*

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In pathogenic bacteria virulence genes are almost always located on mobile genetic elements. Therefore, these genes can be spread by horizontal gene transfer leading to new geno- and pathotypes. Cytolethal distending toxin represents an emerging toxin family, widely distributed among pathogenic bacteria. So far five types of CDT-s, have been identified in *E. coli*. The cdt operons are either part of the genome of lytic prophages or framed with phage genes. Earlier it was demonstrated that cdt-IV, similarly to cdt-I genes, are framed by lambdoid prophage genes suggesting that the cdtABC--IV operon might be part of a phage genome.
In order to investigate the stability and the transfer potential of the cdt-IV locus the cdtB gene was replaced by chloramphenicol (Cm) resistance encoding cat gene in the avian pathogenic *E. coli* strain 250. After passages in non-selective medium at 37 °C 7.6% (219/2900) of the investigated colonies of 250::cat strain became Cm-sensitive. In order to estimate the rate and mechanism of this instability sixty-eight Cm^S colonies were analyzed for presence or absence of cdtA, cdtC and two flanking genes by PCR. Fifty-four of these colonies (79.4%) lost part or the whole investigated locus. These segregants showed several deletion patterns confirming the mosaic structure of the cdt-IV locus. The failure of in vitro self-transfer of the cdt-IV locus suggests that investigated cdt-IV locus could be either part of a defective phage or of a pathogenecity island. To clarify either possibility further transfer and mobilization experiments have to be conducted.

ECHINOCANDIN SENSITIVITY OF *ASPERGILLUS NIDULANS* VAR. *ROSEUS*

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Due to its echinocandin B (ECB) production, *Aspergillus nidulans* var. *roseus* ATCC 58397 is an industrially important strain (Boeck, and Kastner, 1981). ECB is a lipopeptide type antifungal compound used as a precursor of semisynthetic antifungal drug anidulafungin. The taxonomical status of this isolate is questionable, but morphological, physiological and molecular features suggest that it is closely related to *A. nidulans* and *A. rugulosus* (Boeck, and Kastner, 1981, Klich et al. 2001). The resistance against echinocandin type antifungal compounds is still rare but the number of resistant isolates expectedly increases in the future. We study how *A. nidulans* var. *roseus* ATCC 58397 as an ECB producer model organism can tolerate echinocandin type antifungal drugs. Surprisingly the minimal effective concentration (MEC) for caspofungin of *A. nidulans* var. *roseus* ATCC 58397, tested by a standard microdilution method, was even smaller than it was obtained with *A. nidulans* FGSC A4, an ECB non-producing strain (MEC values were 1 μg/ml and 5 □g/ml, respectively). The results were independent of the temperature (24 ºC and 37 ºC were tested). At 37 ºC both strains grew equally fast and neither of them produced ECB, while at 24 ºC *A. nidulans* var. *roseus* ATCC 58397 produced ECB but grew markedly slower than the *A. nidulans* strains (Boeck, and Kastner, 1981). Sensitivity tests were repeated using partially purified ECB in an agar diffusion method and the same difference was observed between the two strains. The results of the agar diffusion method clearly indicated that the "paradox effect" (increasing drug concentration causes less inhibition) can be observed with *A. nidulans* var. *roseus* ATCC 58397 but not with *A. nidulans* FGSC A4. Our preliminary experiments on the background of echinocandin tolerance demonstrated

that the transcription of the β-1,3-glucan synthase (the target of echinocandins) catalytic subunit was significantly lower in the *A. nidulans* var. *roseus* ATCC 58397 strain than it was observed in *A. nidulans* FGSC A4. Besides β 1,3-glucan synthase gene further genes involved in cell wall biogenesis also showed significantly different expression patterns in the two strains.

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FUNGICIDE RESISTANCE OF *BOTRYTIS CINEREA* IN HUNGARIAN VINEYARDS

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Botrytis cinerea (de By.) Pers. (teleomorph: *Botryotinia fuckeliana*, Whetz) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants in the temperate zone worldwide by infecting various tissues. The agricultural producers, using either traditional or integrated or ecological pesticide management, make huge efforts to control the disease, which causes large outgivings for them. In practice, protection usually means the application of chemicals. Because the infection is endemic, and the fungus is extremely variable, the appearance of resistant strains against almost all the fungicides used is a frequent phenomenon. The widespread employment of fungicides increases the risk of environment pollution. In order to maintain the effectivity of chemicals and decrease the risk of environment pollution, it is necessary to determine the general parameters of *B. cinerea* populations and the status of fungicide resistance in the local populations. Reasonable and effective plant protection technologies can be built based on this knowledge. In grapewine *B. cinerea* is feared by wine growers because of its qualitative and quantitative effects on wine production . Chemical control remains the main way to reduce the incidence of gray mould. Recently several highly active fungicides have been introduced but with some of them failures of disease control have been observed in vineyards. The present study was carried out to test the in vitro sensitivity of *B. cinerea* isolates, obtained from Hungarian vineyards towards various fungicides, they include members of the main botryoticide families.

Strains of *B. cinerea* were collected from treated Hungarian vineyards located in Badacsony, Eger, Tokaj and Villány. They were isolated from infected berries in 2007 year at the harvest (September - October). Single-spore isolates were prepared for following works. . Potato dextrose agar (PDA, Sharlau) was used as a base medium, fungicide added minimal medium and fruit rotted test for resistance levels. Isolates were tested for iprodione (Rovral 50WP, BASF), fenhexamide (Teldor 500SC, Bayer) and pirimethanil (Mythos 30 SC, BASF).

In summarises the segregation of fungicide resistance do not show a high variability between vineyards in case of fenhexamide and dicarboximide, but there are a great difference in case of pirimethanil. In the case of pirimethanil in all territories almost half of the isolates showed resistance against the fungicide in spite of the fact that this fungicide it is just used for some years. Due to the well known high genetic variability of *Botrytis cinerea* and in behalf of effective plant protection should not be used the fungicides without due foresight and also with the same mode of action in the sequential years.

THE OPTIMIZATION AND APPLICABILITY OF THE TERMINAL RESTRICTION FRAGMENT LENGTH POLYMORPHISM (T-RFLP) DATA PROCESSING

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The description of microbial community structure(s) is a basic requirement for the better understanding of the role of these communities in the given environment. Molecular fingerprinting techniques give major help in such work. One of them, the T-RFLP (Terminal Restriction Fragment Length Polymorphism) has gained increased popularity in recent years due to its relative ease, fidelity, reproducibility and high resolution power. Unfortunately, many researchers do not have adequate technical knowledge for the application, particularly in the field of data processing. Thus, our objective was to optimize T-RFLP data processing and to create a standardized T-RFLP data processing protocol.

During optimization, firstly the optima of T-RFLP chromatogram parameters were determined. Mainly due to the cost and time limitations, only one run from each sample was used for analysis. Therefore, the extent of error due to neglecting the variance between parallel runs of the same sample was determined. To reduce this kind of error, first the data of the AluI and Hin6I T-RFLP runs were combined; second, on the basis of the variance among several parallel runs, the maximum difference in base pairs between two peaks was determined, when two peaks can still be aggregated into the same category (bin). For noise filtering, the method of „statistical determination of the threshold" was chosen. Then the T-RFLP data were uploaded to the T-REX on-line evaluation program and the alignment of the T-RFLP profiles from different runs was carried out with the built in T-Align module using the variable bins option determined through the analysis of parallel runs. Based on the resulting data matrix, the similarities/dissimilarities among the samples were visualized with principal component analysis, supplemented with hierarchical clustering based on Bray-Curtis similarities and Simper analysis, together with the identification of Simper50 peaks with clone libraries. The resulting T-RFLP data processing protocol is directly applicable to the analysis of other samples, or with the decision-making mechanisms provided more appropriate protocols can be determined.
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THE ROLE OF *ASPERGILLUS* SPECIES IN MYCOTOXIN CONTAMINATION OF AGRICULTURAL PRODUCTS IN HUNGARY

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Aspergillus species are able to produce a range of mycotoxins, including ochratoxins, aflatoxins, fumonisins and patulin. Ochratoxin contamination of grapes and grape-derived products is usually caused by black *Aspergilli*, especially by *A. carbonarius* and *A. niger*. Due to the climatic conditions of Hungary, these species have only relatively rarely encountered in Hungarian vineyards. However, black Aspergilli are frequently isolated from imported dried vine fruits, and ochratoxin contamination of these samples has also been observed. Aflatoxins are mainly produced by members of *Aspergillus* section *Flavi*, and contaminate several agricultural products including maize in several parts of the world. However, aflatoxin-producing *Aspergillus* species have not been identified yet in maize in Hungary. Recent surveys clarified that aflatoxins occurred in concentrations exceeding the EU limit in several regions of Central Europe including Serbia, Slovenia, Croatia, Northern Italy and Romania. We examined the presence of potential aflatoxin-producing Aspergilli in maize samples collected around Szeged. According to their calmodulin genes sequences, all isolates were found to belong to the A. flavus species. Examination of aflatoxin producing abilities of the isolates is in progress.
Fumonisins are produced mainly by Fusarium species, and by the recently identified producers *Aspergillus niger* and *A. awamori*. We examined fumonisin producing abilities of *A. niger/A. awamori* isolates collected from a variety of substrates including raisins, figs, dates, maize, pistachio and onions. Species assignment of the isolates was carried out using sequence analysis of part of the calmodulin gene. The range of fumonisin isomers present in the various substrates, and produced by *A. niger* isolates collected from dried vine fruits was also examined. Among the *A. niger/A. awamori* isolates identified, about 65% were found to be able to produce fumonisins. The average fumonisin content of the dried vine fruit samples contaminated by potential fumonisin producing black Aspergilli was 7 mg kg^{-1}. The isolates produced several fumonisin isomers also present in the dried vine fruit samples. Besides, strains collected from figs, dates and onions were also able to produce fumonisins, and preliminary data indicate that figs and onions are contaminated by lower but significant fumonisin levels than raisins. Interestingly, fumonisin producing black Aspergilli could not be identified in maize and pistachio samples. Further studies are in progress to examine the occurrence of fumonisin isomers in other products including Hungarian wines and grape juices.

CHARACTERISATION OF THE MURINE ADENOVIRUS 2 GENOME AND PARTIAL SEQUENCES FROM SIMILAR RODENT ADENOVIRUSES

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The genome of murine adenovirus 2 (MAdV-2) strain K87 was sequenced and found to be 35,203 bp long being considerably longer than that of MAdV-1 (30,944 bp) and MAdV-3 (30,570). The genome analysis showed that the size difference between MAdV-2 and the other two MAdVs is caused by the size and not the number of the genes. The homologues of the E1B 19K gene seem to have very different size in the three MAdV types. It is only 23 amino acid (aa) long in MAdV-3, 175 aa in MAdV-1, and 330 aa in MAdV-2, where only the N-terminal (150 aa) shows homology to MAdV-1 19K. In the E3 region, the homologue of the 12.5K gene is present in MAdV-2, but is missing from MAdV-1 and -3. The single E3 ORF of MAdV-1 and -3 do not show homology to the second E3 ORF of MAdV-2 either. In the E4 region, only three ORFs are in MAdV-2. One is a homologue of the 34K gene, but the other two ORFs do not show any similarity to their positional counterparts in the other MAdVs (ORFA to E in MAdV-1 and ORFA, C, D, E, F in MAdV-3). Phylogenetic analyses revealed that the three murine AdVs are monophyletic, and seemingly more ancient than the other studied mastadenoviruses. Phylogeny and genome organization confirm that the MAdVs are distinct enough to represent three different species: *Murine adenovirus A, B, and C*. In two environmental samples, collected in the zoo, viruses very similar to MAdV-2 were detected with a general, consensus PCR (supposed to detect all AdVs). We speculated that mice living in the area could be the source of the contamination. In another case, we detected a similar virus in the sample (containing intestines) of a common buzzard (*Buteo buteo*). This was the only occasion of recovering a mastadenovirus from avian (or any non-mammalian) sample. This AdV has a phylogenetic place between MAdV-1 and -2. We suppose that the amplified DNA might have originated from the prey of the bird. An additional novel AdV was detected by PCR in a hamster (*Cricetus cricetus*). This virus seemed to be monophyletic with the earlier mentioned rodent AdVs suggesting a close common ancestry for these AdVs originating from the group of Muroidea. This is a new proof for the co-evolution of AdVs with their hosts. We amplified AdV sequences also from vole, red squirrel and other rodents (agouti and guinea pig) that are phylogenetically more distant. Their AdVs appeared on more distant branches on the phylogenetic tree.
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STANDARDIZATION AND VALIDATION OF ASSAYS DETERMINING CELLULAR IMMUNE RESPONSES AGAINST INFLUENZA

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Influenza vaccine efficacy does not always correlate with humoral immune responses. Recent results indicate that the cellular immune response also contributes to protection.
Our laboratory participated together with four international laboratories in a standardization and validation process with assays for detection of human influenza-specific cellular responses. The production of granzyme B as marker of T cell-mediated cytotoxicity and release of Th1 and Th2 cytokines were evaluated. The granzyme B and cytokine assays were specific, accurate, precise, and robust. Replicate stimulations with PBMC from the same donors showed an intralaboratory robustness (coefficient of variation) for quantitation of granzyme B of 33% and for cytokines – including IFN-γ, TNF-α, IL-2, IL-10, IL-4, IL-13, GM-CSF and including the log IFN-γ/IL-10 ratio – of 52%. The inter-laboratory robustness for detection of granzyme B was 29% and for detection of all cytokines was 49%. The assays can now be used for determining cell-mediated immunity and explored as correlates of protection. Moreover, the precision and robustness of these cellular assays allow the reliable detection of cellular responses even in small study populations.