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MONITORING THE IMPACT OF *ENTEROBACTER CLOACAE* ON THE BIOGAS PRODUCING CONSORTIA

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The improvement of productivity of the biogas producing systems is crucial in order to raise the competitiveness of this renewable energy carrier in the energy market. One way to achieve increased biogas yield is to identify the limiting steps in the microbiological chain of events and to widen these bottlenecks. A sensitive limiting factor is the concentration of hydrogen available for the microbial consortium. Numerous studies have corroborated the importance and regulatory role of hydrogen in the activity and syntrophic relationship between acetogens and methanogens and the key role of the hydrogenotrophic methanogens in biogas formation has been recognized. The positive effect of a pure culture of a hydrogen producing bacterium strain to the biogas generating consortium had been previously demonstrated in our research group using various substrates both under mesophilic and in thermophilic conditions. Significant increase in the biogas production could be detected shortly after inoculation with the selected strains. To understand the biochemical processes in more details the microbial partners interacting in the digester, and their fluctuation in time, should be monitored. A powerful semi-quantitative DNA fingerprinting method, T-RFLP, has been used to address the related questions, which enables the rapid detection of various eubacterial and archaeal groups present in the samples. In this set of experiments two continuously stirred and daily fed mesophilic biogas fomenters were set up. The input substrate was maize silage. One digester served as a control while the other was inoculated with the hydrogen producing strain after steady state operation was reached. The process parameters (pH, temperature, redox potential, FOS/TAC, TOC, TON, gas composition) were followed in both fomenters up to six weeks.
The inoculation with the hydrogen producing bacterium culture (*Enterobacter cloacae*) resulted almost 20% more biogas yield relative to the control fermenter. T-RFLP results revealed that the eubacterial groups went through a significant rearrangement as a result of the externally added bacterium. The presence of the inoculated strain was verified with the help of real-time PCR using specific primers during the whole experiment. The identity of the dominant strains involved in the biogas formation before the inoculation and at the sixth week, was specified using two separate clone libraries. Each of them contained more than 200 clones.

STUDY OF HXNR, THE TRANSCRIPTION FACTOR OF THE NICOTINIC ACID DEGRADATION PATHWAY IN *ASPERGILLUS NIDULANS*

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Despite the nicotinic acid degradation is widely studied in prokaryotes, genetics and biochemistry of the eukaryotic pathway is unknown. Many organisms, including the model organism *Aspergillus nidulans* are able to utilize nicotinic acid as sole nitrogen source, therefore an enzymatic route must exist for its degradation. Our workgroup aimed to unravel the genetics and the biochemical background of the nicotinic acid degradation pathway in *Aspergillus nidulans*. We have identified

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three genomic clusters (Nicotinic acid Degradation Clusters -NDC1, NDC2, NDC3), where the expression of the clustering genes are induced by nicotinic acid and repressed by ammonia. In NDC1 (composed from *hxnS*, *hxnR*, *hxnP*, *hxnT*, *hxnY* and *hxnZ* genes) we identified *hxnR* as a gene for a Zn-finger type protein. Through Northern analysis and qRT-PCR experiments we revealed that HxnR is essential for the expression of all the genes of the NDC clusters. Here we show the construction of an HxnR-GFP expression vector and a substitution cassette which were transformed into an hxnR deletion strain. The former construction was made for obtaining strains with multi-copy integration of native promoter driven hxnR-gfp in trans and the latter was made in order to obtain a strain with a single copy insertion of a substitution cassette containing a native promoter driven hxnR-gfp fusion. By fluorescence microscopy we studied the intracellular localization of the GFP-fused transcription factor under non-inductive, inductive and repressed conditions.
Support: Hungarian Scientific Research Fund (OTKA-K101218) and TÁMOP-4.2.2.A-11/1/KONV-2012-0035.

BIOFILM AND WATER BACTERIAL COMMUNITIES OF DIANA-HYGIEIA THERMAL SPRING FOUND IN THE BUDA THERMAL KARST SYSTEM

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The Buda Thermal Karst System is an active hypogenic karst area where microbes may participate in mineral precipitation and hydrothermal cave forming processes. The aim of the present study was to gain information about the diversity of biofilm and discharging water bacterial communities inhabiting the Diana-Hygieia Thermal Spring using cultivation-based and molecular cloning methods. The biofilm bacterial community proved to be somewhat more diverse than that of the water sample and the distribution of the dominant bacterial clones was different between biofilm and water samples. The phyla Actinobacteria, Firmicutes and Proteobacteria were represented by both bacterial strains and molecular clones but phyla Acidobacteria, Chlorobi, Chlorofexi, Gemmatimonadetes, Nitrospirae and Thermotogae only by molecular clones which showed the highest similarity to uncultured clone sequences originating from similar environmental sources. The majority of biofilm clones was affiliated with Deltaproteobacteria and Nitrospirae while the largest group of water clones was related to Betaproteobacteria.
Comparing our cultivation-independent results to others conducted in cave environments, it can be stated that in the cave biofilms, members of the phylum Proteobacteria occurred most frequently and in the highest proportion.
This research was supported by the Hungarian Scientific Research Fund (OTKA) Grant NK101356.

THE EFFECT OF IONISING RADIATION ON THE CONTAMINATING MICROFLORA OF SPICE PAPRIKA POWDER

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The EU-FP7 project SPICED "Securing the spices and herbs commodity chains in Europe against deliberate, accidental, or natural biological and chemical contamination" focuses on pathogen agents based on e.g. frequency of natural occurrence, possible impact on human health, and relevance for food terrorism. One of its tasks is the evaluation of available means and techniques for the decontamination of spices. As irradiation of spices has been approved by EFSA as being a safe treatment, it will be compared and assessed with other treatments on microbial contaminations and the quality of spices and herbs with regard to e.g. their colour and other sensory attributes. As during processing of spice paprika there is a severe heat treatment, the microflora of the end product consists mainly of Gram-positive bacteria (mostly spore-formers) 10^5-10^6 cfu/g and moulds 10^2-10^4 cfu/g, though also coliforms 10^3 cfu/g might be present in spite of the low water activity. Our control (guaranteed untreated) paprika powder had $6.9x10^6$ cfu/g mesophilic aerobic total count, from which $6.8x10^6$ cfu/g was spore forming bacteria, $5.1x10^3$ cfu/g coliforms, though no *E. coli*, $1.9x10^3$ cfu/g Enterobacteriaceae, and $6.0x10^3$ cfu/g mould contamination. *Salmonella* could not be detected in the sample. Ionising radiation of spices and herbs is authorised in the EU for microbial decontamination up to the dose of 10 kGy. Irradiation with 5 kGy ensures the appropriate reduction of cell count by 2-3 orders of magnitude. For our experiments untreated spice paprika powder was irradiated with ionising radiation (Co-60) with 1, 5, and 10 kGy in the Institute of Isotopes Co. Budapest, Hungary. Molecular methods were also applied in the detection, enumeration, and identification of the dominant bacteria and moulds.

All bacteria are spore forming rods of the family Bacillaceae. *Bacillus cereus*, *B. licheniformis*, *B. mycoides*, *B. subtilis*, and other *Bacillus* spp. represented 85% of the dominant bacteria.

This work was supported by the EU-FP7-SEC-2012-1-312631 SPICED project.

HORMONAL ACTIVITIES OF FOUR UV FILTERS MEASURED BY BIOLUMINESCENT YEAST BIOREPORTERS

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Organic UV filters are used in personal care products and technical materials to protect skin from cancer or materials from deterioration caused by UV radiation. However, UV filters were detected in lakes, rivers, sludge, animals and even in human milk at trace-level concentrations. UV filters have different hormonal activities *in vitro*, so it is important to develop sensitive biological assays appropriate for detecting the hormonal effect of the UV filters. The aim of this work was to adapt yeast bioreporters to measure the hormonal activities of 4 selected UV filters. In addition the effective concentrations (EC50/IC50) were also determined and were compared to effective concentrations from other *in vitro* experiments using the hERα or hAR. In order to detect hormonal activities and cytotoxicity of the UV filters bioluminescent yeast strains of *Saccharomyces cerevisiae* (BLYES, BLYAS and BLYR) were applied. The BLYES/BLYAS strains serve to measure estrogenic/androgenic effects whereas BLYR strain to detect the cytotoxicity. The antiestrogenic/antiandrogenic activities could be measured indirectly by adding to each well 17ß-estradiol or 5α-dihydrotestosterone in concentration corresponds with EC65 so the decreasing of bioluminescence could be followed up. For determining the dose-response curves of the UV filters serial dilutions were made in 3 parallels. According to the dose-response curves all UV filters can

be characterized by antiandrogenic activity whereas none of the UV filters had androgenic potential in spite of *in vitro* results in the literature. Benzophenone-3 (BP-3) and 4-methylbenzylidene camphor showed submaximal dose-response curve in the estrogen assay, however ethylhexyl methoxycinnamate and octocrylene were not estrogenic. Antiestrogen activity could be observed at 3 UV filters. Only BP-3 was cytotoxic to the yeasts in the applied concentration interval. These results confirm that these UV filters have more and different types of hormonal activities.
It may be hypothesized BP-3 can pose significant environmental and human health risk due to its high antiandogenic and cytotoxic activity.
Supported by the Research Centre of Excellence - 8526-5/2014/TUDPOL and the KTIA-AIK-12-1-2013-0017 project. Csilla Krifaton is a grantee of János Bolyai Scholarship of the Hungarian Academy of Sciences. We thank for the BLYES, BLYAS and BLYR test organisms (The University of Tennessee, Knoxville, Tennessee).

BACTERIUM-FUNGUS INTERACTIONS IN AN OYSTER MUSHROOM CULTIVATION MODEL SYSTEM

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Oyster mushroom (*Pleurotus ostreatus*) is an edible, saprotrophic white rot fungi with a versatile enzymatic profile. In the previous years our research group studied the bacterial succession during the substrate preparation. The aim of the present work was to study bacterium – fungus interaction during colonisation and fruiting body production with the characterisation the growth and nutrient consumption of the mushroom partner with determining its lignocellulose degrading enzymatic activities. A model system was created with autoclaved, reinoculated autoclaved (with bacteria from the original mushroom substrate, with bacterium free filtrate from the mushroom substrate, with inhibitory strain for oyster mushroom, with sterile water) and normal substrate. These substrates were filled into tubes, inoculated with oyster mushroom spawn at the end. Half of the tubes were incubated for 1 month until almost total colonisation, whereas the other half was incubated further for fruiting body development. After the incubation time the tubes were cut into slices. Lignocellulose decomposing enzymes (endoglucanase, cellobiohydrolase, β-glucosidase, endo-1,4-β-xylanase, 1,4-β-xylosidase, laccase, manganese peroxidase and N-acetyl-β-glucosaminidase) were extracted from the slices and reducing sugar content were determined. Enzyme activity pattern of the tubes was compared by Combined Cluster and Discriminant Analysis. The different developmental regions showed different enzymatic pattern. Activity of laccase was the highest in the region of the front hyphae, whereas the manganese peroxidase showed largest activity in the 3-week old colonised region. The activity of cellulases and hemicellulases reached their peak in the 1-month older region which took part later in the development of fruiting body. The enzyme pattern was similar on the autoclaved substrate reinoculated with the original bacteria or with its filtrate and on the normal substrate. Oyster mushroom grew also on these tubes the fastest. Other type of substrate tubes (autoclaved, autoclaved reinoculated with inhibitory strain or with sterile water) were contaminated by mould during the colonisation. The bacterial partners played a role in avoiding the contamination during the colonisation of oyster mushroom; respectively they affected the growth and the lignocellulolytic enzymes production of *P. ostreatus*.
This research was supported by the Hungarian Scientific Research Found (OTKA K 83764).

NOVEL AVIPOXVIRUS SPECIES DETECTED IN TURKEY (*MELEAGRIS GALLOPAVO*)

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Poxviruses are large pleomorphic, brick-shaped, cytoplasmic DNA viruses infecting numerous animals including both invertebrate and vertebrate species. The latest ICTV report classify poxviruses of vertebrates into the Chordopoxvirinae subfamily which is further divided into nine genera, such as *Avipoxvirus*, *Capripoxvirus*, *Cervidpoxvirus*, *Leporipoxvirus*, *Molluscipoxvirus*, *Orthopoxvirus*, *Parapoxvirus*, *Suipoxvirus*, and *Yatapoxvirus*. Avipoxviruses infect birds. Recently, accumulation of avipoxvirus infections with typical skin lesions was reported among 38-week-old turkeys in a flock. Clinical signs were observed exclusively among female turkeys on average in 15-20% of animals in the four roosts of the farm. The disease was characterized by various severity of proliferative skin lesions (including thicken skin and cauliflower-like cutaneous extrusions). No reduction in egg production and no excess mortality were observed. Histopathological examination of the skin and mucosal lesions revealed proliferation of the multilayered epithelium and cytoplasmic inclusions in the epithelial cells. Microbiological examination of the affected tissues identified no pathogenic bacteria or fungi. However, consistent with the gross pathological and histopathological findings, which were consistent with poxvirus infection, the presence of poxvirus DNA was confirmed by gene specific PCR assays. The virus was isolated by hen-egg inoculation. The whole genome was determined by next-generation sequencing. The assembled genome was found to consist of 188.5 kbp. The extremities of the genome were not validated by Sanger sequencing, therefore the starting nt position was arbitrarily assigned. Characteristic to many poxvirus strains, the turkey origin strain was also AT rich (70.2%). The central genomic region was flanked by two identical, very short ITRs that measured ~1.5 kbp, respectively. Gene annotation algorithms identified 171 putative ORFs. Of interest, the short ITRs contained only a single duplicated gene. Similarly to other avipoxviruses (and also in Chordopoxviruses), the ORFs located in the middle region of the TPV genome encode proteins related to viral DNA and RNA metabolism, the structure of virus particles, and virion morphogenesis. Phylogenetic analysis of the DNA polymerase gene clustered the turkey avipoxvirus into a novel clade. Our data indicate that the turkey origin novel poxvirus strain may be the representative strain of a novel avipoxvirus species.

MULTIDRUG- RESISTANT *SERRATIA MARCESCENS* STRAIN ISOLATED IN A UROLOGY UNIT- CASE REPORT

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Serratia marcescens is a member of the Enterobacteriaceae family and is known as a cause of severe nosocomial infections in pediatric and neonatal intensive care units, and also in urology sections. Recent data show appearance of multidrug- resistant strains which are also resistant to trimethoprim

/ sulphametoxazole and are only susceptible to phosphomycin. The aim of our work is to present the case of a 67-years old man known with left double J stent for urethral stenosis, complains of pain and terminal gross hematuria. Imagistic examination and urine culture revealed a urinary tract infection (UTI) with ESBL- producing *Klebsiella pneumoniae* and calcifications on the bladder double J stent. After changing the urethral catheter and antibiotic treatment with meropenem for 7 days the patient's symptoms persisted. The urine samples revealed UTI with a carbapenemase producing multidrug- resistant *Serratia marcescens* strain. Identification was performed by biochemical tests and confirmed by Vitek 2 Compact System. The strain's susceptibility to antibiotics was determined by disk diffusion tests and by Vitek 2 Compact System and revealed sensitivity only for Phosphomycin. Usually this drug is only recommended for UTIs caused by *Escherichia coli* strains. In our situation, because of resistance to all the antibiotics used in UTIs, namely ampicillin, cephalosporines, amoxicillin, tazobactam / piperacillin, gentamycin, tetracyclin, nitrofurantoin, ciprofloxacin, norfloxacin, amikacin, trimethoprim / sulphametoxazole, and carbapenems there was no other substance left. This medication healed the patient and follow-up after 2 and 4 weeks, respectively, evidenced sterile urine samples and the patient had no symptoms. In conclusion, multidrug- resistance of *Serratia marcescens* strains makes these infections very difficult to treat and it is very important to isolate and monitor patients in order to prevent the propagation of nosocomial infections.

COMPLEX GENETIC VARIABILITY TESTING OF *ASPERGILLUS FLAVUS* ISOLATES FROM FOUR VARIOUS HABITATS

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Aspergillus species are filamentous fungi which are widespread on agricultural products and they are among the most common organisms causing fungal keratitis in subtropical and tropical areas of the world. Aspergilli are able to produce a range of mycotoxins which can be harmful to animals or humans, including aflatoxins, ochratoxins, fumonisins and patulin. According to recent studies, climate change accompanied by global warming affects the occurrence of fungi and their mycotoxins in our foods and feeds. A shift has recently been observed in the occurrence of *Aspergillus* species, especially aflatoxin producers, mainly *A. flavus* in Europe. This species is frequently isolated from indoor air, particularly in subtropical and tropical areas. Recently this species was also identified in large quantities in indoor air in Croatia and Hungary. In this study, 60 *Aspergillus flavus* isolates from four various populations (clinical isolates from India, environmental isolates from India, isolates from indoor air from Croatia and Hungary, and isolates from maize from Serbia) were examined. The isolates were determined at the species level using sequencing of part of their calmodulin gene. We also investigated the genetic variability of the isolates with UP-PCR analysis, microsatellite typing methods, and determined their mating types. Phylogenetic and population genetic analysis of the data is in progress. For the examination of the aflatoxin producing abilities of the isolates thin layer chromatography was used. Further investigations of the aflatoxin producing abilities of the isolates using ELISA and HPLC methods are in progress.

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PRO- AND PREBIOTICS RESEARCH FOR DEVELOPING NEW SYNBIOTICS

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Probiotics are living bacteria which mainly colonize the large intestine and convey health benefits to the host. Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the intestinal tract. The term synbiotic refers to nutritional supplements combining probiotics and prebiotics in a form of synergism. The focus of our joint project is the development of synbiotics for animal feed, where lactobacilli represent the probiotic part and mannooligosaccharides are the prebiotics. During the project more than a thousand bacteria with lactobacillus morphology were isolated on MRS and MRS containing xylan/mannan plates from the exotic herbivorous animals of the Budapest Zoo & Botanical Garden. This strain collection was targeted to growth experiments to study their carbohydrate utilising abilities on mannan and xylan carbon sources. Molecular taxonomical identification of the investigated Lactobacillus strains was performed by combined methods: cluster analysis with repetitive PCR followed by sequence analysis of the 16S rDNA and housekeeping genes (*recA, gyrB*).For prebiotic production, prokaryotic mannan-hydrolase enzymes were selected based on genome projects of earlier sequenced *Thermobifida* species. Fusion proteins were prepared and the endomannanase enzymes were expressed in *E. coli* host. After purification and biochemical characterization, terminal hydrolysis of different low cost mannan sources (LBG, KM) was done, and the whole hydrolysate or fractionated oligomannan products were used as prebiotic fraction. In order to detect the effects of our prebiotics, control mice DSS-treated animals with inflammatory symptoms were applied. Effects of tetrameric mannooligosaccharide (MOS4) on the expression of proinflammatory cytokine IL-1b and TNF in mouse model of inflammatory bowel disease were investigated. According to the molecular taxonomical identifications, our isolates belong to 22 valid *Lactobacillus* and 9 *Enterococcus* species. We have isolated a new *Lactobacillus* species, and from the porcupine we have a new genus, new species candidate. The formal species descriptions are in progress. From the preliminary tests of MOS4 oligosaccharide was proved to be an effective prebiotic as it could decrease the level of interleukin 1beta in mouse model of inflammatory bowel disease.

RELATIONSHIP BETWEEN SOIL RESPIRATION AND SOIL FERTILIZATION

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Soil respiration has a major pathway of flux in the agroecosystem C cycle. The measurement of soil CO_2 respiration is a means to measure biological soil fertility. A CO_2 production is a strong indicator of the soil metabolism and ecological soil functions. It reflects the intensity of the soil organic matter decomposition and mineralization and the incidence of the microbiotas in soil, and it is often used for the biomass determination. In addition to altering root growth and turnover rates, fertilization could affect soil processes. In this contribution, an experiment was conducted to study the relationship between soil respiration activity and organic, mineral fertilizers or their combination to two soil types (sandy loam Kovárvány brown forest, Nyíregyháza and clay loam chernozem meadow, Szeged). Soil amendments were: no fertilizer, NPK (165 kg N, 82.5 kg P_2O_5, and 82.5 kg K_2O/ha), 45 kg/ha wastewater sludge as organic fertilizer or treated with a mixture of organic and mineral fertilizer. Soil respiration rate was measured by the 0.1 M NaOH absorption (titration) method. The respiration activity was measured after incubation of soil samples in dark brown glass vessels at 28 °C for 1 day (potential respiration), 3 days (reactive respiration), after the 7 days (basal respiration) and 28 days (cumulative respiration). Results showed close relationships between the soil respiration activity, microbial biomass C and total organic C content in the investigated soils. The three day CO_2 release from all treatments was highly correlated to both basal soil respiration and cumulative respiration. Organic, mineral and combined fertilisation increased the reactive respiration activities in relation to the controls in both soils. Combined fertilisation increased the respiration activity in relation to the control and to single fertilisation and reflect additive effects in both soil types. Finally, soil respiration activity frequently can be used to evaluate soil quality, soil fertility or soil contamination with organic pollutants or heavy metals. However, soil respiration activity is depending on a number of ecological factors, abundance and diversity of the soil microbiota, substrate availability, aeration, etc. Due to the high variability and seasonal dynamics of the respiration activity, long-term investigations are needed to evaluate the effect of different agroecosystems on the respiration activity.

RESPONSE OF SOIL MICROBIAL DYNAMICS AND EXTRACELLULAR ENZYME ACTIVITIES TO VARIOUS FERTILIZATIONS

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Microbial activity is qualitatively and quantitatively associated with the presence of extracellular hydrolytic enzymes which are important in the process of decomposition and mineralization of organic matter. An experiment was conducted to study how the relationship between microbial communities and extracellular enzyme activities can be affected by adding organic, inorganic fertilizers or their mixture to various soil types (sandy loam Kovárvány Brown Forest and clay loam chernozem meadow). Soil amendments were: no fertilizer, NPK (165 kg N, 82.5 kg P_2O_5, and 82.5 kg K_2O/ha), 45 kg/ha wastewater sludge as organic fertilizer or treated with a mixture of organic and inorganic fertilizer. Microbial (bacterial, actinobacterial and fungal) composition were determined by culture enrichment technique. Microbial biomass (MB) C, N, P and S were

determined. Enzyme (β-glucosidase, cellulase, urease, phosphatase and aryl-sulphatase) activities were estimated. This study recognized that organic fertilizer addition tends to increase the MB, though the responses of specific groups such as Gram-positive, Gram-negative bacteria and fungi vary, also, increased or altered fungal populations, shifts in Gram-positive and Gram-negative bacteria, and increased fungi/bacteria ratios. Although it was shown that NPK could increase the soil MB C, N, P and S but no significant change in the microbial characteristics of the soil. The activities of β-glucosidase, urease and phosphatase were reported to be highest in the clay loam soils. Fungal biomass has been demonstrated to be low in comparison with the bacterial biomass in sandy soil. Generally, soil microbial biomass and extracellular enzymatic activities in all treatments with applications of either organic, organic and inorganic mixture or inorganic fertilizer increased significantly by 112.6%, 73.5% and 50.0%, respectively, compared with control. Total counts of bacteria and actinobacteria in soil amended with wastewater sludge were highest among different fertilizer amendments. Finally, soil microbial population and enzymatic activities were very responsive to organic fertilizer application. Enzymatic activities were positively and significantly correlated with content of soil organic C. Soil enzymatic activities can be used as an index of soil fertility and microbial functional diversity.

PURIFICATION OF OPHIOBOLIN I PRODUCED BY *BIPOLARIS ORYZAE*

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Ophiobolins are a remarkable family of sesterterpene-type secondary metabolites produced by certain filamentous fungi belonging into the genera *Bipolaris*, *Cochliobolus*, *Drechslera* and *Aspergillus*. Until now more than 30 of these compounds have been described and assigned into several subgroups based on their characteristic structure. Ophiobolins have various biological activities such as antimicrobial, cytotoxic, nematocid and calmodulin antagonist. In our continuing search for bioactive secondary metabolites a multi-step semi-preparative HPLC method was developed for the purification of different ophiobolin analogues produced by a selected *Bipolaris oryzae* isolate. Fermentation was carried out in rotary shaker for 21 days at 28°C. The filtered ferment broth was extracted three times with ethyl acetate and was concentrated at reduced pressure. The resolved material was fractioned by a semi-preparative normal phase HPLC system using ethyl acetate/n-hexane (1:1, V/V) eluent and the collected fractions were checked for ophiobolin content by analytical HPLC runs. The fractions showing promising chromatograms were pooled and separated by two consecutive semi-preparative reverse phase chromatographic runs. The mobile phase of the first separation was the mixture of H_2O:MeCN (3:7). The resulting subfractions were pooled and further purified on the same HPLC system using H_2O:MeCN (5:5) as mobile phase. The purified compound has been identified as ophiobolin I based on mass spectrometric and NMR experiments. The purity of the isolated ophiobolin I was determined by an isocratic HPLC technique and proved to be 99%.

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NOVEL HPLC METHOD FOR RAPID ANALYSIS OF TRICHOTECENES FROM *FUSARIUM* INFECTED RICE CULTURES

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Trichothecenes are a family of sesquiterpenoid type mycotoxins mainly produced by certain species of *Fusarium*, *Trichoderma* and *Trichotecium*. Trichotecenes are divided into four groups (A-D) based on their characteristic functional groups. The most prevalent members of type B trichotecenes are nivalenol (NIV), deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON). DON frequently occurs in maize, wheat, rye, barley and oats. The toxicity of DON has been extensively studied and several toxic effects have been reported including immunosuppressive, teratogenic, embryotoxic or neurotoxic effects. In this study a rapid HPLC method was developed for detection and quantification of these secondary fungal metabolites. The separation was carried out using a Shimadzu modular HPLC equipped with Phenomenex Onyx Monolithic C18 column. The optimal mobile phase proved to be the mixture of water and acetonitrile (95:5) at 4 ml/min flow rate and the detection wavelength was 218 nm. The separation of these four mycotoxins was carried out within 13 minutes. The retention times of NIV, DON, 3-ADON, 15-ADON were 0.84 min, 1.47 min, 9.78 min and 10.82 min, respectively. Eighty *F. graminearum* strains were isolated from wheat grown in Hungary.

Rice cultures were infected with them for test their B-type trichotecene productions, which were analyzed by the developed HPLC technique after a simple sample extraction step. Characteristically different trichothecene profiles were obtained within the culture extracts analysed, where the DON showed the highest amount of the examined mycotoxins similarly to the formerly published results about the B-type trichotechene production of *F. graminearum* isolates.

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A POLYPHASIC APPROACH TO REVEAL TAXONOMIC AND METABOLIC DIVERSITY OF BACTERIA IN A BIOFILM OF A HYDROCARBON CONTAMINATED GROUNDWATER

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Nowadays culture independent genomic sequencing (metagenomics) offers an unprecedented view of diverse microbial habitats, such as soils, oceans, body sites of life beings, biofilms and many others. Furthermore, metagenomics is an adequate tool to assess with high accuracy the composition, dynamics and interactions of bacteria also of polluted environments. Therefore, besides conventional isolation procedures the purpose of the present study was to uncover the bacterial community composition of a biofilm developed in a Pump & Treat System treating petroleum hydrocarbon contaminated groundwater by using a high throughput sequencing platform (Ion Torrent PGM). At the same time, since the analyzed groundwater contained considerable amounts of toxic, simple aromatic hydrocarbons (e.g. BTEX – benzene, toluene, ethylbenzene and xylenes) our second approach was to explore the diversity of catechol 2,3-dioxygenase genes

(subfamilies I.2.A., I.2.B and I.2.C of C23O gene) which take part in enzymatic meta cleavage of aromatic rings during the biodegradation processes. Metagenomics in accordance with isolation and 16S rRNA gene based identification of isolates revealed the dominance of Betaproteobacteria (52.3%) represented by sequence reads affiliating with genera *Leptothrix* (6.05%), *Aromatoleum* (4.77%), *Acidovorax* (4.1%), *Thauera* (3.59%) etc. The second most dominant class proved to be Gammaproteobacteria (22.3%) mainly represented by sequence reads affiliating with the genera *Xanthomonas* (1.94%) and *Pseudomonas* (1.39%). The isolation procedure resulted in at least two hitherto unknown bacteria with the nearest neighbours *Xanthobacter autotrophicus* 7cT (97.51% almost full 16S rRNA sequence similarity) and *Novosphingobium aromaticivorans* DSM 12444T (97.17%). As regards the metabolic versatility of the investigated biofilm it has turned out that biodegradative genes affiliating with I.2.C subfamily of extradiol C23O dioxygenases (Betaproteobacteria) are more diverse than those pertaining to subfamilies I.2.A (Gammaproteobacteria) and I.2.B (Alphaproteobacteria).

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AZOXYSTROBIN RESISTANCE OF *BOTRYTIS CINEREA* STRAINS ISOLATED FROM HUNGARY

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Fungicides belonging to the family of quinol oxidase inhibitors (QoIs) e.g. strobilurins, like azoxystrobin play an important role in the protection against several plant diseases caused by fungi. These fungicides bind to the cytochrome bc1 complex so they block electron transport between cytochrome b and cytochrome c1. This way these fungicides inhibit the ATP synthesis consequently they inhibit the mitochondrial respiration. However a point mutation of the cytochrome b gene (*cytb*), e.g. the substitution of a glycine by alanine at position 143 results in high-resistance. *Botrytis cinerea* is the causal agent of grey mould on many economically important crops. Pesticides, like azoxystrobin is the most frequently applied approach to reduce grey mould disease, but resistance against this treatment has often been reported. The azoxystrobin resistance and related cytochrome b gene genotypes was studied in Hungarian *B. cinerea* field isolates with allele-specific PCR reaction and PCR-RFLP method for detecting the G143A mutation. The development of the QoI resistance was induced by using mediums with increasing concentrations of azoxystrobin. We also developed a method to track heteroplasmy by using Real Time PCR. Amplified PCR product containing G143A mutation and single copy gene located in the mitochondrial genome (*cox1*) were used as standards. In a few cases, we were able to detect the mutation with allele-specific PCR but not with PCR-RFLP, and these strains were all sensitive to azoxystrobin. This would indicate marginal presence of the resistance-conferring, mutated mtDNA and these strains may well develop resistance rapidly when faced with QoIs in the field. *In vitro* we managed to stimulate the development of the resistance toward azoxystrobin. Using Real Time PCR we provided evidence that in the presence of azoxystrobin the ratio of the resistant/sensitive mitochondrial genome increased. We relate this change to the increased frequency of the G143A point mutation in the mitochondrial genome.

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HXNW, A NEW MEMBER OF THE NICOTINIC ACID DEGRADATION PATHWAY

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In nature nicotinic acid is available for many microorganisms as nitrogen source. Several degradation pathways are known in prokaryotes, but the method of the degradation in eukaryotes is yet unknown. We aim to investigate the nicotinic acid utilization pathway in a eukaryotic model organism, *Aspergillus nidulans*. We discovered three nicotinic acid degradation clusters (NDC1, NDC2 and NDC3). NDC1 contains the common transcription factor (*hxnR*) for the cluster genes and the four degradation-involved genes (*hxnS*, X, V, M) are dispersed between the three clusters. Here we note, that four additional genes with unknown function (*hxnP*, T, Y, Z) clusterize with *hxnS* and one (*hxnN*) with *hxnM*. Transcription of all the cluster genes including those ones, which functions are unknown are regulated by the *hxnR* and can be induced by nicotinic acid. Two genes (*hxnX, hxnV*) have been identified in NDC2 until now and recently we found a small coding sequence (861 nt) between *hxnX* and *hxnV* that was found to transcribe upon nicotinic acid induction and is regulated by the cluster specific HxnR transcription factor. We named it *hxnW*. In order to investigate whether *hxnW* plays role in the nicotinic acid degradation route, we deleted it from the genome by transforming a riboB+ selection marker carrying substitution cassette into a riboB2, pantoB100 *Aspergillus nidulans* strain. Out of 13 transformants 2 showed single-copy integration of the substitution cassette by Southern blot analysis. The deletion mutants do not grow on nicotinic acid as a sole N-source, which revealed that *hxnW* is part of the degradation route. In order to prove the role of *hxnW*, we transformed a his-tagged *hxnW* into a deletion mutant. The his-tagged *hxnW* expressing strain was further used for the purification and characterization of the protein.

Support: Hungarian Scientific Research Fund (OTKA-K101218), and TÁMOP-4.2.2.A-11/1/KONV-2012-0035.

GENETIC DIVERSITY AND ECOLOGICAL TOLERANCE OF BACTERIA ISOLATED FROM THE RHIZOSPHERE OF HALOPHYTON PLANTS LIVING NEARBY KISKUNSÁG SODA PONDS, HUNGARY

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Many halophyton plant species and halophilic microorganisms are adapted to the extremities of different saline habitats. The aim of the present study was to reveal and compare the genetic diversity and ecological tolerance of bacterial strains cultivated from the rhizosphere of four different plant species living in the vicinity of Kiskunság soda ponds. Following a sampling in September 2013, altogether 145 bacterial strains were isolated using two different media. Bacterial strains were identified on the basis of 16S rDNA sequencing after their grouping with ARDRA method. Salt and pH tolerance of the strains were examined by measuring their growth in broths containing 0-15% NaCl (w/V) and characterized with pH 7-12 values.

Among the isolates genera of *Agromyces, Brachybacterium, Gordonia, Microbacterium, Micrococcus, Nocardiopsis, Nesterenkonia, Isoptericola, Pseudoclavibacter* and *Streptomyces* (Actinobacteria), *Anaerobacillus, Bacillus* and *Exiguobacterium* (Firmicutes), *Halomonas,*

Idiomarina, Loktanella, Ochrobactrum, Nitrincola and *Rheinheimera* (Proteobacteria) and *Anditalea* (Bacteroidetes) were identified. The salt tolerance of the bacterial strains was strongly dependent on the sampling location and plant species. According to the growing characteristics, halophilic and halotolerant bacteria from Böddi-szék and Kelemen-szék clearly separated. In contrast, growing of bacterial strains in broths with alkaline pH values was more balanced.
This research was supported by the Hungarian Scientific Research Fund (OTKA K 108572).

BACTERIAL COMMUNITIES ASSOCIATED WITH THE HYPOGENIC KARSTIFICATION IN THE BUDA THERMAL KARST SYSTEM

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The Buda Thermal Karst System (BTKS) located in Budapest is a recently active hypogenic karst system. In the BTKS two distinct discharge areas can be found where karst fluids with different origin, physical and chemical characteristics contribute to diverse karstification processes. In both the Northern and Southern areas biofilm formation can be observed. The aim of the present work was to reveal and compare the morphological structure and genetic diversity of bacterial communities inhabiting the biofilms developed on the karst surfaces of BTKS. The micro-morphological arrange of bacterial filaments and iron hydroxide precipitates developing on cell surfaces as well as element composition of biofilm samples taken from different sampling sites were studied by FIB-SEM technique. Cultivation based and molecular biological methods were applied to uncover the bacterial phylogenetic diversity. Based on the 16S rRNA gene sequences, the five clone libraries showed high genetic diversity. Taxa belonging to Chloroflexi, Nitrospira, Alpha- and Betaproteobacteria proved to be dominant by cloning while Firmicutes and Actinobacteria by cultivation. Considering the metabolic properties of described species related to the molecular clones and isolated strains, it can be assumed that biofilm communities of BTKS may participate in the local sulfur and iron cycles, and may contribute to biogenic cave formation.
This research was supported by the Hungarian Scientific Research Fund (OTKA NK 101356).

MOLECULAR DETECTION AND GENOME ANALYSIS OF CIRCOVIRUSES OF EUROPEAN EEL *(ANGUILLA ANGUILLA)* FROM LAKE BALATON

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Circoviruses are small, non-enveloped viruses with a circular single-stranded (ss) DNA genome, which ranges from about 1.3-2.3 kb in size. The genome contains at least two open reading frames (ORF) arranged on different strands, these encode the replication (Rep) and capsid (Cap) proteins. Before the last decade, circoviruses were known only from birds and swine, but recently, due to the different metagenomic methods and PCR based diagnostic tests, numerous circovirus-related sequences were described from environmental samples, invertebrates and also from lower vertebrates (amphibian and fish). According to the increasing number of the detected piscine

circoviruses, a monitoring program (Bioclimate) is carried out for screening circoviruses in Hungarian freshwater fishes. Samples were taken regularly also from Lake Balaton. In March 2014, fifteen living European eels *(Anguilla anguilla)* and two razorfish *(Pelecus cultratus)* were caught among other species at the Sió-floodgate sampling site. After euthanasia, tissue samples were collected from the gills, liver, spleen, kidney and intestine. The presence of circoviral DNA was detected by a broad-spectrum nested PCR, targeting the Rep gene. In case of positive results, rolling circle amplification and inverse nested PCR reactions were carried out to amplify the remaining part of the circular genomes. Six out of the fifteen eels, and one razorfish sample were found to be positive for circoviral DNA. Analysis of these fragments revealed the presence of three different circovirus-related rep-like sequences. One of them (representing with 3 samples) was identical with the previously described eel circovirus (EeCV). The whole genome amplification of these 3 samples was also successful. Comparing the partial rep-like sequences, two other samples were similar with 96% nucleotide identity to the above mentioned ones, and were identical to the rep-like sequence originating from razorfish. The 6th circovirus positive sample showed only 50% similarity to the EeCV, but it was found to be identical to the rep-like sequences of roaches *(Rutilus rutilus)* caught from Lake Balaton, recently. Circoviruses were described as host-specific or narrow host range microorganisms. To the best of our knowledge, this is the first report about the detection of the same circovirus related sequences in various, distantly related fishes and the occurrence of different rep-like sequences in the same fish species.

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BIOGAS PRODUCTION FROM CHICKEN MANURE

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Biomass based technologies have several benefits: a wide range of substrates are available in large quantities, the generated energy carriers are environmentally friendly, easy to carry and store. Biogas is a renewable energy carrier which is similar to natural gas and can be used in practically all applications to replace natural gas. It is produced by anaerobic fermentation of the organic materials. Poultry waste management has been an increasing concern in many parts of the world due to the huge amount of this waste stream. Pollutants from improperly managed poultry waste can cause serious environmental problems in terms of water, air, and health quality. Chicken manure (CM) contains two main forms of nitrogen: uric acid and undigested proteins, which represent 70% and 30% of the total nitrogen in CM, respectively. Anaerobic decomposition of uric acid and undigested proteins in CM results in high amounts of unionized ammonia (NH_3) and ammonium ions (NH_4^+). Excess NH_3 inhibits the anaerobic microbial community and thus methane production. We tested the possibility of washing CM with water with the aim of decreasing the nitrogen content of this substrate. After two days soaking CM in tap water, the liquid and solid phases were separated and significant nitrogen content was detected in the water phase. Removal of excess nitrogen-contain compounds could make CM more suitable substrate for anaerobic digestion. Biogas fermentation experiments were carried out with washed CM at mesophilic temperature (37 °C) in batch and continuous operational modes.

The results demonstrated that anaerobic fermentation became sustainable when the reactors were fed with washed chicken manure most likely due to non-toxic ammonium concentrations, which appears to be the limiting parameter in the anaerobic digestion of CM.

ANTIOXIDANT AND ANTIFUNGAL EFFECTS OF TERPINEN-4-OL ON *CANDIDA ALBICANS*

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Essential oils have been widely used for centuries for bactericidal, virucidal, fungicidal and antioxidant applications. Terpinen-4-ol is a monocyclic terpene alcohol, which is the main component of *Origanum majorana*, *Melaleuca alternifolia* and terpinen-4-ol chemotype *Thymus vulgaris*. The aim of this study was to examine the antifungal and antioxidant effects of terpinen-4-ol on *Candida albicans* and the effects on plasma membrane. Growth inhibition at various concentrations was measured in shaken cultures, the cell growth was fully blocked at 1 µl ml^{-1} concentration. To determine the survival rates, cultures were treated with 0.125, 0.25 and 0.5 µl ml^{-1} terpinen-4-ol, which resulted in a 95%, 114% and 76% change, respectively, in the colony-forming ability of the cells after 1 hour. Determination of the intracellular peroxides concentrations was performed in the presence of the above stated concentrations supplemented with 1 µl ml^{-1}. Terpinen-4-ol treatment resulted 29% and 22% decrement in the case of 0.125 and 0.25 µl ml^{-1} and 29% and 111% increment of peroxides in the presence of 0.5 and 1 µl ml^{-1}, respectively, after 1h treatment. The antioxidant capacity of terpinen-4-ol is 367.2 mM trolox Eq/L. Effects of terpinen-4-ol on dynamic properties of *C. albicans* plasma membrane were studied by electron paramagnetic resonance (EPR) spectroscopy, using 5-doxylstearic acid (5-SASL) and by spectrofluorimeter using TMA-DPH. The *C. albicans* protoplasts were treated with 0.5 µl ml^{-1} terpinen-4-ol for 15 min in 0.6 M KCl solution, than the plasma membrane of cells was labeled with 5-SASL for 3 min. Statistical analysis of the h+1/h 0 ratio not showed significant alteration in the fluidity of the plasma membrane by EPR spectroscopy, but spectrofluorimetric analysis demonstrated slightly increased rigidity after the treatment. The vegetative cells were treated with 0.125, 0.25, 0.5 and 1 µl ml^{-1} terpinen-4-ol for 180 min, and 1 µl ml^{-1} induced 52% higher loss of intracellular metabolites absorbing at 260 nm than the control, the other concentrations did not show significant differences. We believe that our results will contribute to understand the mode of action of terpinen-4-ol on opportunist human pathogen yeasts.

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VARIABILITY OF THE GRAPEVINE POWDERY MILDEW FUNGUS, *ERYSIPHE NECATOR* BASED ON MICROSATELLITE MARKERS

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Over the past four years a basically new knowledge accumulated in the international literature concerning the biology and genetics of *Erysiphe necator* causing the grapevine powdery mildew disease. The new results mostly referred to the Western European and North American populations of the pathogen. However, Hungarian populations have not been investigated so far. Our research

tends to eliminate this shortage and, thus, aimed at the molecular genetic analysis of a large number of domestic samples of powdery mildew infected grapevine leaves and berries. Samples were collected from various locations of five Hungarian wine regions including Eger, Tokaj, Ászár-Neszmély, Kunság, Szekszárd, and Villány. More than two hundred powdery mildew samples, that were selected randomly, were submitted to microsatellite analysis. To reveal the genetic variability among the grapevine powdery mildew samples selected eleven microsatellite markers were applied in this work. Microsatellite loci could be amplified successfully in all *E. necator* samples included in the analysis. The microsatellite markers showed a significant variability in fragment length and number of alleles detected. All the microsatellite loci tested were found to be polymorphic. Most of the microsatellite alleles exhibited a fragment length identical to those published in previous studies. However, we detected new alleles that have not been identified so far. Moreover, the microsatellites EnMS3 and EnMS10 showed predominantly different patterns from those published previously. These results suggested that the eleven microsatellite markers tested were found to be appropriate tools to detect the genetic variability among the grapevine powdery mildew samples and, thus, will be applied in further studies of the rest of the samples collected in different Hungarian wine regions in order to reveal the structure of the Hungarian populations of *E. necator*.

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HUMAN POLYOMAVIRUSES IN TRANSPLANT PATIENTS AND IN KIDNEY CARCINOMAS

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Prevalence and possible transmission routes of human polyomavirus 9 (HPyV9), WU (WUPyV), KI (KIPyV) and BK (BKPyV) are studied in immunocompromised patients. Clinical samples (respiratory, blood, urine) from kidney and bone marrow transplant patients (respiratory, blood, urine, bone marrow, stool) are collected after the transplantation. Viral DNA detection is performed by nested and quantitative real-time PCR. Amplified PCR products are sequenced. 600-600 samples from renal transplant patients are analysed. The highest frequencies of KIPyV and WUPyV were observed in respiratory samples. KI virus was found in respiratory samples from 14% of the patients, WUPyV in 7% of the patients. KIPyV was also detected in urine and blood samples, and WUPyV in blood samples. Coinfections were observed. HPyV9 were detected in all sample types with low frequencies. Follow up study of bone marrow transplant patients was begun in this year. Up to now 90-90 samples from 25 patients have been analysed. KIPyV in respiratory, HPyV9 in blood and respiratory samples were found. BKPyV viruria and viraemia were also observed in these patients. The same sample types from 73 patients with different types of lymphoma, leukaemia were also collected and analysed. None of the studied viruses were found in these samples, suggesting that immunosuppression due to transplantation might result in higher susceptibility to infections or reactivation of these human polyomaviruses. Since all human polyomaviruses encode oncoproteins, their tumourigenesis potential is suggested. At the same time, Merkel cell polyomavirus is the only proved oncovirus among human polyomaviruses. Despite the extensive study, the role of BKPyV in human cancers is controversial. KIPyV, WUPyV and HPyV9 in human kidney carcinomas has not been studied yet, but their presence were proved in urine by our team suggesting that these viruses –

similarly to BKPyV – can infect the urinary tract. As a result of this study on kidney carcinoma tissues has been started. Up to the present 90 archived kidney carcinoma tissues are analysed. BKPyV was detected in one sample, but KIPyV, WUPyV and HPyV9 were not found.

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IS *CHLAMYDOPHILA PSITTACI* AN UNDERDIAGNOSED INFECTION?

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Chlamydophila psittaci infection is regarded as a serious or often deadly zoonotic infection in humans. The source of the infection may be close contact with birds. The lethality of infection is high without adequate antibiotic treatment. Until 2002, 1-6 cases were detected annually in Hungary. This was followed by a jump in 2003 to 85, and in 2005 to 140, with 29 and 28 cases in 2006 and 2007. In our studies, microimmunofluorescence (MIF) was used to determinate *Chlamydophila pneumoniae, Chlamydia trachomatis* and *Chlamydophila psittaci* specific IgM and IgG. Seropositive subjects were defined as those with IgM titres >1:10 and IgG titres of >1:16, and seronegative subjects as those whose titres were <1:10 and <1:16, respectively. To rule out the presence of cross-reactive antibodies, testing was also performed for *C. trachomatis* and *C. pneumoniae*. Three adult female patients from different parts of Csongrád County were examined with the MIF method in November and December 2013, primarily for *C. pneumoniae* antibody determination because of a clinical and X-ray diagnosis of pneumonia. Two of the three patients were employed at a meat-processing plants; the third patient was a lawyer. The first patient was treated with macrolid and doxycyclin antibiotics as an out-patient. The other two patients were hospitalized after serious pneumonia, and after laboratory diagnosis were treated with doxycyclin antibiotics. Such infections must be registered officially in Hungary.

As this is an occupationally-exposed disease, registration is compulsory toward, the health authorities. Antibiotic treatment of occupationally-exposed subjects is urgent when infection is suspected. Transfer of clinical samples is obligatory to the National Health Center (Budapest), where our laboratory diagnosis was confirmed.

EFFICACY OF CASPOFUNGIN *IN VITRO* AND *IN VIVO* AGAINST *CANDIDA ALBICANS* ISOLATES SHOWING AND NOT SHOWING PARADOXICAL GROWTH

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Killing rates (k) of caspofungin (CAS) was determined in RPMI-1640 and in 50% serum using time-kill methodology against three *C. albicans* isolates showing and three *C. albicans* isolates not showing paradoxical growth (PG) in RPMI-1640. In MIC tests, regardless of isolates we did not observe PG in 50% serum. In RPMI-1640, for isolates showing and not showing PG the mean k values at 1 and 32 mg/L were 0.54 and 0.33 1/h, and 0.36 and 0.13 1/h, respectively. In 50% serum mean k value ranges at 1-32 mg/L for isolates showing PG was significantly higher than for isolates not showing PG (0.60-0.65 1/h versus 0.18-0.21 1/h). In a neutropenic murine model daily 2, 3, 5 and 15 mg/kg CAS significantly decreased the fungal tissue burden in case of all isolates with and

without PG *in vitro*. CAS killing rates at 1-32 mg/L for *C. albicans* isolates showing and not showing PG was concentration independent in 50% serum, however the mean k values were at least two times higher for isolates with PG than without PG.
Despite the observed significant differences among k values with and without PG in 50% serum *in vitro*, CAS proved to be equally efficacious for all isolates tested *in vivo*, regardless of PG. Our results indicate that there is no unequivocal correlation between the *in vitro* and *in vivo* efficacy of CAS. Clinical relevance of PG is probably small.

REVIEW OF GLOBAL ROTAVIRUS STRAIN PREVALENCE DATA FROM SIX YEARS POST VACCINE LICENSURE SURVEILLANCE

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Comprehensive reviews of pre licensure rotavirus strain prevalence data indicated the global importance of six rotavirus genotypes, G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8]. Since 2006, two vaccines, the monovalent Rotarix (RV1) and the pentavalent RotaTeq (RV5) have been available in over 100 countries worldwide. Of these, 60 countries have already introduced either RV1 or RV5 in their national immunization programs. Post licensure vaccine effectiveness is closely monitored worldwide. This review aimed at describing the global changes in rotavirus strain prevalence over time. The genotype distribution of the nearly 47,000 strains that were characterized during 2007-2012 showed similar picture to that seen in the preceding period. An intriguing finding was the transient predominance of heterotypic strains, mainly in countries using RV1. Unusual and novel antigen combinations continue to emerge, including some causing local outbreaks, even in vaccinated populations. In addition, vaccine strains have been found in both vaccinated infants and their contacts and there is evidence for genetic interaction between vaccine and field strains. The observed predominance of the heterotypic G2P[4] strains in some countries following RV1 vaccination is unusual and this issue requires further monitoring.

ROTAVIRUS SURVEILLANCE IN HUNGARY, 2013

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Rotavirus strain monitoring began in mid-1980s in Hungary. The distribution of rotavirus G and P types that have been in circulation in Hungary till 2012 is already reported. This study was designed to monitor and characterize rotavirus infections in children mainly (65%) under 5 years of age suffering from community-acquired acute gastroenteritis and admitted to pediatric emergency units

during 2013 in 10 geographic areas of Hungary. The samples were amplified by RT-PCR assay and then sequenced by new generation whole genome sequencing technique. Sequence data were analyzed by the CLC Bio program. From the total of 248 strains 209 were genotyped. After eliminating non-typeable, partially typeable strains and mixed infections from the sample set, the dominating strain was G9P[8] (47.4%), followed by G1P[8] (28.1%), G2P[4] (20.7%), G3P[9] (1.5%), G3P[8] (0.7%), G4P[8] (0.7%) and G4P[6] (0.7%). The major change compared to 2012's data is the increasing rate of the G2P[4] strains. The changing epidemiology of rotaviruses reinforces the need for continued strain surveillance.

PREVALENCE AND INTEGRON CARRIAGE IN ESBL PRODUCERS ISOLATED FROM FAECAL SAMPLES OF INPATIENTS AND OUTPATIENTS

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The aim of the study was to determine the prevalence of ESBLs, the presence of integrons and characterization of gene cassettes. A total of 2106 faecal specimens (1623 from inpatient, 324 from outpatients and 159 from screening of medical students) were screened for ESBL producers between October 2010 and December 2011 using cefotaxim-supplemented eosin-methylene blue agar. The presence of ESBL-encoding genes and class1 and 2 integrons was detected by PCRs and identified by sequencing. Overall prevalence of faecal carriage of ESBL-producers was 8.6% (181/2106). Occurrence of ESBLs within intensive care units was higher than in non intensive care units (15.4% (44/285) vs. 9.0% (121/1338), p=0.001) while prevalence was 4.0% (13/324) in outpatients, and 1.9% (3/159) in screening group. Species distribution was as follows, *Klebsiella pneumoniae* 54.8% (102/186), *Escherichia coli* 42.5% (79/186) and others 2.7% (5/186). In total, 88.7% (165/186) of the ESBL genes were of *blaCTX-M* type; this proportion was 100% in adult intensive care units and 96.5% in adult non-intensive care units, and 11.3% (21/186) of *blaSHV* type. The *blaCTX-M-15* gene was the most frequent gene (74.2%, 138/186) of *blaCTX-M* producers. Integrons were detected in 145 (78.0%) ESBL-producing isolates. Class 1 integrons were more frequent than class 2 integrons (75.3% vs. 4.8%). Among inpatient groups adults carried more class 1 integrons than children ($p<0.001$). Eight different gene cassette arrays of class 1 integrons were identified (aadA2, aadA1a, dfrA17-aadA5, aac6'(Ib)-cmlA1, dfrA15-aadA1, dfrA12-orfF-aadA2, dfrA1-aadA1, dfrA7). Among them aadA2 (30.0%), aadA1a (29.3%) and dfrA17-aadA5 (27.1%) were the most prevalent gene cassettes. Gene cassette arrays of class 2 integrons were identical and comprising dfrA1-sat2-aadA1 array. The results highlight the high prevalence of faecal carriage of ESBL-producers in both inpatients and outpatients with *blaCTX-M-15* as the predominant ESBL gene, suggesting these carriers are likely sources of infections. High rate of integron carriage indicates that these integrons are widely present in ESBL-producers especially in adults and may be considered as an important factor in development of multidrug resistant strains.

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SPATIOTEMPORAL DYNAMICS IN THE INVASIVE LINEAGES OF *NEISSERIA MENINGITIDIS*

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Neisseria meningitidis is an obligate commensal bacterium of humans, which normally colonizes the mucosa of the upper respiratory tract without causing invasive disease. Studies using molecular methods have demonstrated the extensive genetic diversity of meningococcocci isolated from carriers, in contrast to a limited number of genetic types, known as the hyperinvasive lineages, associated with invasive disease. Notwithstanding the high levels of horizontal genetic exchange, clonal complexes, especially hyperinvasive lineages, are stable over time with life spans of many decades and during global spread. This stability and the association of clonal complexes with particular antigenic repertoires provides some hope that the development of protein-based vaccines may be possible, if the nature and dynamics of this structuring can be properly understood.
This presentation gives an overview of structure, antigen makeup and evolution of meningococcal population both temporally and geographically in aspect epidemiology.

EFFECTS OF ENZYMATIC PRETREATMENT ON ALCOHOLIC FERMENTATION OF WASTE PAPER

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Nowadays, there has been an emerging worldwide interest in lignocellulose-based forestry, agricultural and agro-industrial wastes for producing value added bio-products. Cellulose based materials (paper industry) are inexpensive and abundant “nonfood” feedstock for large-scale production of second-generation bioethanol. But this process still faces a number of technical barriers, thus research and development are important. In this study increase of the efficiency of bioconversion rate using paper waste substrate was focused. Two types of waste papers were used: with and without ink. Both fermentation technologies (separate hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF) were applied. Enzymatic saccharification with Celluclast as well as AMG was performed. Additionally, the mashes were prepared by supplementation of the complex manure (UVAVITAL) and phosphoric acid. The fermentation was done using Levuline type dried *Saccharomyces cerevisiae* at 35 °C for 8 days. Amounts of carbohydrates, degradation products and concentration of ethanol were monitored by the HPLC and Somogyi-Nelson methods. After pretreatment (4 hours) with Celluclast 1.5L enzyme preparation, the glucose and cellobiose concentration increased to about 1.5 g/100ml and 0.2 g/100ml, respectively. The initial amount of reducing sugar was 0.75 g/100 ml, which then increased up to 2.03 g/100 ml at the end of the hydrolysis. During fermentation, the reducing sugar content increased in the first 24 hours and kept constantly. After 8 days of fermentation, the alcohol content of mashes varied in range from 0.1% (v/v) to 0.2% (v/v) meaning low conversion efficiency. Moreover, no significant differences were detected between both fermentation techniques. Generally, in the case of wastepaper with ink, the alcoholic fermentation went slowly and fractionally due to components in printed ink. In the case of use of enzyme cocktail containing both cellulolytic and amylolytic activities, the final ethanol yield increased from 0.2% (v/v) to 2.2% (v/v) in simultaneous process (SSF).

This result may be still preliminary, but quite promising for development of fermentation technology for production of cellulose-based bioethanol.

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SELECTIVE ENRICHMENT OF FE(III)-REDUCING BACTERIA FROM MICROAEROBIC, BTEX-CONTAMINATED GROUNDWATER

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In our recent study, an attempt was made to link the microaerobic degradation of BTEX compounds to specific groups of microorganisms. Consequently, a yet uncultured member of the genus *Rhodoferax* was identified as possible microaerobic BTEX degrader which possibly harbors subfamily I.2.C catechol 2,3-dioxygenase (C23O) gene. *Rhodoferax* species and other Fe(III)-reducing microbes e.g. members of the genus *Geobacter* are usually important members of oxygen-limited, BTEX contaminated subsurface environments. It is well known that *Geobacter* species usually harbor benzylsuccinate synthase (*bssA*), the key gene of anaerobic degradation of toluene, while some *Rhodoferax* species may have a role in the microaerobic degradation of aromatic compounds. To be able to link *bssA* and subfamily I.2.C C23O genotypes unambiguously to *Geobacter* and *Rhodoferax* species of the "Siklós" BTEX-contaminated groundwater, efforts were made to selectively enrich these bacteria. Groundwater microcosm was incubated anaerobically for two weeks to decrease the number of strict aerobes. After incubation phosphate buffer based acetate enrichment mediums were inoculated with groundwater microcosm samples under anaerobic circumstances. Fe(III)NTA was added to the media to provide Fe(III) as sole electron acceptor. Four different kind of enrichments were set up: (i) supplemented with 0.05% (w/v) yeast extract and NH_4Cl; (ii) supplemented with 0.05% (w/v) yeast extract and omitting any fix nitrogen source; (iii) supplemented with NH_4Cl and omitting yeast extract; and finally (iv) omitting both yeast extract and NH_4Cl. It was observed that the diversity of the initial bacterial community considerably decreased during the enrichment culturing, and the lowest diversity was observable in case of enrichment cultures omitting yeast extract. However, selective enrichment of *Geobacter* species was observable also in these latter cultures. Unfortunately, selective enrichment of *Rhodoferax* species was not succeeded and subfamily I.2.C C23O genes were only detected in enrichment culture supplemented with both yeast extract and NH_4Cl, *bssA* genes were detectable in every type of enrichment culture.

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WHOLE GENOME SEQUENCE OF GOOSE HAEMORRHAGIC POLYOMAVIRUS STRAINS DETECTED IN HUNGARY

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Goose haemorrhagic polyomavirus (GHPV) may cause fatal nephritis and enteritis in immunocompetent geese and thus may result in serious economic losses in our goose flocks. The disease was recognized in 1969 in Hungary, but the virus was isolated only 30 years later. GHPV was also detected in ducks, however, diseases could not been connected to the infection in these animals. Whole genomic sequences of GHPV are available in the GenBank from France, Germany and China and some partial sequences from Hungary. Potential viral genes were predicted based on the genome sequence, but the exact role of those is unknown; at present vaccines are not available. In this study our aim was to describe complete genomic sequences of representative Hungarian strains, including the virus of the first known outbreak, to predict the genetic structure of the viral genomes and to compare those to the references.

The complete genome of a Hungarian GHPV strain was analysed. It had a length of 5252 bp and was highly similar to the references encoding the same possible proteins. The genetic material of 20 other GHPV strains originating from geese and ducks are being processed. We plan to determine the diversity and the reservoirs of the GHPV and to investigate the viral transcript to reveal virus-host interactions that are needed in the development of effective vaccines.

CHARACTERIZATION OF GYROVIRUSES IDENTIFIED IN THE FERRET FECAL VIROME

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Beside chicken anaemia virus, new gyroviruses of the Circoviridae virus family were recently described not only in poultry but also in human blood, skin and stool samples. In this study, the viromes of 21 fecal samples of 18 pet ferrets with lymphoid organ enlargement were determined by metagenomics and traces of avian gyrovirus 2 (AGV2), human gyrovirus (HGyV), gyrovirus 3 and 4 (GyV3, GyV4) and chicken anaemia virus (CAV) were detected in 8 animals. PCR assays targeting the VP1 and VP2 region of the virus genomes were used for screening the gyroviruses. In total, 14 of the 20 animals tested positive for gyroviruses. Three HGyV, one AGV2, one GyV3 and one GyV4 genomes were successfully amplified and determined by an altered method using modified nucleotides in the PCR and Sanger sequencing reaction.

The HGyV, AGV2 and GyV4 genomes showed higher similarity (99.5%, 94.2-95.9% and 90.7%, respectively) with the appropriate sequences of the GenBank, while GyV3 was more distantly related (<80%) to the only homotypic reference sequence deposited in the database. Further investigations are required to reveal the origin and role of new type gyroviruses in the ferret feces.

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EXAMINATION OF THE OCCURRENCE OF *FUSOBACTERIUM NUCLEATUM* ON ORAL MALIGNANT SURFACES

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Fusobacterium nucleatum is an opportunistic anaerobe species in the oral cavity playing an important role in periodontitis and it is associated with intrauterine and other extra-oral infections. Recently its association with colorectal cancer (CRC) has also been demonstrated where *F. nucleatum* has been proved to be able to invade the colon's epithelial cells and promote the carcinogenesis via an inflammatory process. The key figure in this process is the *F. nucleatum*'s unique adhesin the FadA, which can bind the epithelial cell's E-cadherin, is able to activate β-catenin signaling, and thus is able to differentially regulate inflammatory and oncogenic processes. In our experiments, we wished to investigate the association of *F. nucleatum* with oral squamosus cell carcinomas (OSCC) in which the role of E-cadherin-mediated signalization is also an important transformational factor. We emphasize that the *F. nucleatum*'s main habitat is the oral cavity where it has the maximal occurrence rates and it also has a chance to interact with E-cadherin despite the histological differences of the epithelia between the intestine and the oral cavity. Biofilm samples were obtained from the central surface from the malignant (OSCC) or pre-malignant (sine morbo, lichen, leukoplakia) lesions and from the contiguous healthy mucosa from the same patients (n=29). Total DNA was extracted, and the rates of carriage of *F. nucleatum* were detected by 16S RNA RT-PCR using serially diluted *F. nucleatum* samples as calibration controls. The detected *F. nucleatum* CFUs in the non-cancerous samples were in the range of $5x10^2$ to $5x10^5$ whereas in the OSCC samples the *F. nucleatum* CFUs were in the range of 10^3 to $3x10^6$. The changes in the prevalences of *F. nucleatum* between the affected and the non-affected (healthy) sides where in the range of 1 and 60 and this increase/difference between the OSCC and the other sites was statistically significant.

PHYLOGENETIC AND FUNCTIONAL ANALYSIS OF SEQUENCE VARIATIONS OF HUMAN PAPILLOMAVIRUS (HPV) TYPE 31 E6 AND E7 ONCOPROTEINS

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Human papillomaviruses (HPVs) are small, double-stranded DNA viruses that infect the anogenital tract. Cervical cancer, caused by infection with high-risk HPV types (HPV 16, 18, 31, 33, 35, etc.) is a major public health problem. In transformed cells the expression of E6 and E7 oncogenes is increased and these oncoproteins contribute to oncogenesis by associating with the p53 tumor suppressor protein and the retinoblastoma (Rb) cell cycle regulatory protein, respectively. In the present study we explored the variability of E6 and E7 of HPV 31 based on DNA and amino acid sequences in clinical samples taken from women who had colposcopical atypia in the cervix. Phylogenetical trees were constructed based on the DNA sequences. To investigate the functional activities of these oncogenes, the E6 and E7 variants were cloned in to pcDNA His tagged expression vector and MCF-7 cells were transiently cotransfected with E6 or E7 expression vectors along with p53 or E2F reporter vectors. The expression level of the different E6 and E7 variants and their effect on p53 and retinoblastoma proteins were examined by Western-blotting in stably transfected MCF-7 cells. Based on the DNA sequences we identified nucleotide changes which

caused conservative and non conservative amino acid alterations. The phylogenetical trees of the E6 and E7 variants showed three major intratypical groups ("A", "B", "C"). In the course of the investigations focusing on functional activities of E6 and E7 variants, we found differences between the effect of E6 variants on p53 activity; the variants belonging to intratype "A" and "C" decreased the activity of p53 similarly to HPV 16 E6 wild type oncogene, which was used as a positive control. E6 variants belonging to intratypical group "B" had no effect on p53 activity. The HPV 31 E7 variants showed the same effect on E2F activity as the HPV 16 E7 variant, which was the positive control; they increased the E2F luciferase activity. In contrast with HPV 16 E6 and E7 oncoproteins, it seems the HPV 31 E6 and E7 oncoproteins are not able to induce the degradation of p53 and retinoblasoma proteins.

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ARBOVIRUSES IN HUNGARY: HISTORY AND RECENT CHALLENGES

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The first Hungarian tick-borne encephalitis (TBE) virus strain (KEM1) was isolated in 1952. Since then TBE became more and more obviously a public health problem. The main diagnostic method became indirect immuno-fluorescence test with IgG, IgM and IgA determination in 1980. Additionally the laboratory performs neutralization. Another *Flavivirus*, West Nile virus (WNV) caused domestic outbreaks of neuroinfections with human and susceptible animal cases first in 2003. Since that time we have diagnosed some infections yearly at the Hungarian Great Plain till 2008, when cases occurred in the Transdanubian area. All of the viruses in the *Flavivirus* genus are more or less cross-reacting with each-other, so the interpretation of serological results is not easy. Nucleic acid detection or isolation of the virus with central neurological symptoms are almost impossible. Very many issues are needed to be answered to decide the pathogenic virus. Traveling occur more and more often, which can cause a lot of difficulties for a human virologist, who beyond diagnosis, should give public health suggestions, which is impossible without truly knowing the pathogens inside and outside of the land. One of the most often requested virus examination is Dengue, which has four serotypes. Infection with the first type usually does not have a severe consequence, only mild symptoms like fever and rash, but the second infection with a different type causes severe disease, which is the antibody dependent enhancement of infectivity (ADE) phenomenon. This phenomenon operates in TBE in spite of vaccination as well as in some of other flaviviral infections with a former flaviviral infection. The ground of this phenomenon is the partly identical and partly different antigenic structure of the Flaviviruses. The lecture will include Flaviviruses by species names, which tests are available for serology and will show, which viruses' nucleic acid detection is possible in our laboratory. Additionally we diagnose the most important other zoonotic viruses that are important from our point of view (e.g. Chikungunya virus, which belongs to the *Alphavirus* genus). Hantaviruses are domestic zoonotic viruses with public health importance in Hungary. They are rodent-borne pathogens. Its diagnosis started in the eighties with different methods. Nucleic acid sequencing detected at least three hantaviruses in three different primary hosts with different spread.

SUSCEPTIBILITY OF *MANNHEIMIA HAEMOLYTICA* STRAINS TO DIFFERENT ANTIBIOTICS

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The antibiotic susceptibility 60 *Mannheimia haemolytica* strains isolated from pathologic cases of animals in Hungary was examined in the case of 18 antibiotics representing different antibiotic classes. The minimum inhibitory concentration (MIC) values of some beta-lactam antibiotics (penicillin, amoxicillin, ampicillin, ceftiofur, cefoperazone), aminoglycosides (gentamicin, spectinomycin), tetracyclines (oxytetracycline, doxycycline), macrolides (erythromycin, tilmicosin), pneuromutilins (tiamulin), phenicols (chloramphenicol, florfenicol), folate pathway inhibitors (sulfamethoxazole, sulfamethoxazole-trimethoprim) and fluoroquinolones (enrofloxacin, marbofloxacin) were measured using the microdilution method. Besides MIC the concentration capable of inhibiting 50% of the strains (MIC_{50}) was also defined. Wide ranges of the MIC values of the different beta-lactam antibiotics were seen (penicillin 0.06->32 μg/ml, a MIC_{50} 0.25 μg/ml; amoxicillin 1->64 μg/ml, MIC_{50} 4 μg/ml; ampicillin <0.03->32 mg/ml, MIC_{50} 0.25 μg/ml; ceftiofur 0.25->32 μg/ml, MIC_{50} 2 μg/ml; cefoperazone <0.06-32 μg/ml, MIC_{50} 4 μg/ml). The aminoglycosides had high MICs (gentamicin 0.5-128 μg/ml, MIC_{50} 8 μg/ml; spectinomycin 16->256 μg/ml, MIC_{50} 64 μg/ml), while good inhibition was shown by the tetracyclines (oxytetracycline 0.125->64 μg/ml, MIC_{50} 1 μg/ml; doxycycline <0.125-64 μg/ml, MIC_{50} 0.5 μg/ml). The inhibitory activity of the macrolides was weaker than expected (erythromycin 1->64 μg/ml, MIC_{50} 8 μg/ml; tilmicosin 2-128 μg/ml, MIC_{50} 4 μg/ml) and 4-64 μg/ml tiamulin could inhibit the growth of the *M. haemolytica* strains (MIC_{50} 16 μg/ml). A wide range of inhibitory concentrations was typical in the case of phenicols (chloramphenicol 1-128 μg/ml, MIC_{50} 4 μg/ml; florfenicol 0.5-32 μg/ml, MIC_{50} 2 μg/ml) and the folate pathway inhibitors (sulfametoxazol 25->1600 μg/ml, MIC_{50} 1600 μg/ml; sulfametoxazol-trimethoprim 0.6/0.03->304/16 μg/ml, MIC_{50} 4.75/0.25 μg/ml). Even low concentrations of the fluoroquinolones had excellent inhibitory effect (enrofloxacin 0.03-0.5 μg/ml, MIC_{50} 0.125 μg/ml; marbofloxacin 0.25-4 μg/ml, MIC_{50} 1 μg/ml).

The increasing range of the MICs of several antibiotics underlines the importance of regular examination of the antibiotic susceptibility of *M. haemolytica* strains.

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BACTERIAL ENDOPHYTES OF SWEET PEPPER: DISTRIBUTION IN THE PLANT AND SELECTION FOR *IN SITU* INVESTIGATIONS

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Endophytic bacteria are known to reside in the internal tissues of every plant without any sign of infection and deleterious effect; rather they provide several benefits to the host plants. The aims of our study were the isolation of endophytic bacteria from different organs of sweet pepper (*Capsicum annuum* var. *grossum*), determination of their diversity at genus or species level and localization to different organs of the plant. Furthermore, based on the influence of the endophytic isolates on the germination of pepper's seeds and phylogenetic relations two bacteria were selected for fluorescence *in situ* hybridization (FISH) experiments. Based on the phenotypic characterisation the Gram-negative isolates (101) were clustered into 81 phenogroups, while the Gram-positive isolates (69) formed 54 different clusters. Molecular typing of the selected bacteria from each phenotypic group

was done by PCR fingerprinting using the M13 mini-satellite primer. Significant differences among the isolates were observed, as 54 and 45 distinct fingerprints were generated in case of the Gram-negative and Gram-positive bacterial isolates, respectively. A genus-specific PCR was used for detection of the *Pseudomonas* isolates. Bacteria from 24 clonal clusters belonged to this genus and were identified at species level by sequencing the *rpoB* gene. Molecular identification of non-*Pseudomonas* isolates was performed by sequence analysis of the 16S rRNA encoding genes. Majority of the Gram-negative isolates belonged to the *Pseudomonas*, *Agrobacterium / Rhizobium* and *Stenotrophomonas* genera, but members of the Enterobacteriaceae family could also be detected in significant number. Gram-positive isolates were identified as members of the *Bacillus, Curtobacterium, Clavibacter, Deinococcus, Kocuria, Leucobacter, Leifsonia, Microbacterium, Micrococcus, Paenibacillus, Rothia* and *Staphylococcus* genera. Root tissues comprised most of the isolates, just a small proportion derived from the above-ground plant tissues. The results of seed germination test showed that some species had strong or slight inhibitory effect, while others were neutral or even enhanced the germination process. Isolates from these last two categories were selected for fluorescent in situ hybridization (FISH) experiments. Fluorescently labeled oligonucleotide probes were designed to detect the isolates belonging to the *Pseudomonas* or *Chryseobacterium / Flavobacterium* genera.

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HAS THE USE OF FLUOROQUINOLONES FACILITATED THE WIDESPREAD DISSEMINATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AND MULTIRESISTANT *KLEBSIELLA PNEUMONIAE* IN THE HEALTHCARE SETTING?

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We recently demonstrated that diverse fitness cost associated with resistance to fluoroquinolones allowed the widespread dissemination of the major international clones of both methicillin-resistant *Staphylococcus aureus* (MRSA) and multiresistant *Klebsiella pneumoniae*. The mechanism described by us was subsequently confirmed by British authors investigating the dynamics of MRSA clones in the UK. Our results imply that the the use of fluoroquinolones should boost the incidence of both MRSA and multiresistant *K. pneumoniae*. The presentation will review the related literature and show that most of the reports support the notion that a rise in the use of fluoroquinolones will result in elevated rates for both pathogens while a decrease will lower the incidence of the bacteria. Though the association seems to be convincing and the mechanism behind it unequivocal the use of fluoroquinolones should not be abandoned; a judicious application can be recommended.

A BRIEF OVERVIEW OF CYSTEINE-RICH ANTIFUNGAL PROTEINS SECRETED BY FILAMENTOUS ASCOMYCETES

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From the second half of the 1990s several extracellular cysteine-rich proteins with remarkable antifungal activity have been isolated and characterized from filamentous Ascomycetes. The main

characteristics of these proteins are a low molecular mass and the presence of six to eight cysteine residues that form three to four intramolecular disulfide bonds which provide a high stability against protease degradation, high temperature and broad pH range. A further common feature is the cationic character due to a high amount of arginine and lysine residues. In spite of the fact that the mature proteins differ in their amino acid sequences; conserved homologous regions can be identified at the flanking regions of cysteines. Based on these they can be divided into two main groups: peptides which contain the *Penicillium chrysogenum* antifungal protein (PAF) cluster in their amino acid sequences, and peptides with *Penicillium brevicompactum* "bubble protein" (BP) cluster. Overall they have similar protein folding patterns: five antiparallel β-strands connected by three loops showing β-barrel topology. Although they have similar tertiary structures their antifungal spectrum and antifungal mechanism show differences. The cysteine rich antifungal proteins from filamentous Ascomycetes are interesting in practical respect. Their features that they have potent antifungal activity against (opportunistic) human, animal, plant and food-borne pathogenic fungal species, show no toxic effects on plant and mammalian cells *in vitro* and *in vivo*, interact synergistically with other antifungal drugs and peptides, are highly stabile, and they have low cost production render them exceptionally suitable compounds of commercial preservatives, bio-pesticides and drugs against filamentous fungi and offer an alternative, safely applicable antifungal strategy. In this lecture the physical and chemical properties, antifungal spectrum, antifungal mechanism, biological role, and potential application of these proteins are discussed briefly.

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CYTOKININ PRODUCTION BY *SCENEDESMUS OBTUSIUSCULUS / RUBESCENS* UNDER DIFFERENT CULTURE CONDITIONS

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Numerous scientific studies have been published about algal cultures or extracts containing different types of plant hormones and their effect on plant growth and stimulation of germination. Five major groups of phytohormones are known, such as auxins, cytokinins, gibberellins, abscisic acid and ethylene. The most of naturally occurring cytokinins in plants are N6-substituted adenine derivatives, generally with isoprenoid or aromatic side chain. In case of plants and bacteria these are normally formed as free base, ribotide, riboside, glucoside and amino acid conjugates and have a purine skeleton. This group includes zeatin, trans-zeatin riboside, and the artificially prepared kinetin, which is rarely found in nature. In higher plants, zeatin and isopentenyl adenine riboside commonly occur. In green micro- and macroalgae – for example *Protococcus, Scenedesmus* and *Chlorella* species – kinetin and zeatin riboside has been already detected, the zeatin riboside has been characterised as the cis-isomer. The kinetin - as the first molecule with cytokinin effect - was isolated by Miller in 1954 and its name was given after its ability to promote cell division. It is present in DNA as a result of spontaneous, oxidatively induced mutation and excision, as a free degradation product. In our study we have measured the cytokinin concentrations in the biomass of the unicellular green algal *Scenedesmus obtusiusculus / rubescens* BEA (Banco Espanol de ALGAS) D01_12 cultures incubated at different nutrient (phosphorus and nitrogen) concentrations and at various temperatures. The solvent extraction was carried out from lyophilized samples. Cytokinins were detected by thin layer chromatography after solvent extraction and vacuum evaporation. The quantitation was performed by HPLC-PDA. The results were evaluated using the Statistica

software. The results show that trans-zeatin riboside and kinetin were also present in various concentrations in relation with nutrient composition and temperature changes.

IDENTIFICATION OF A G3P[3] CANINE ROTAVIRUS A IN HUNGARY

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Group A Rotaviruses (RVA) are the major viral pathogens causing acute dehydrating gastroenteritis among newborn/young animals and humans less than 5 years of age. RVA is not considered a major cause of diarrhea in dogs, although RVA was detected in dogs and puppies several times, and *in vivo* experiments have demonstrated firmly that RVA can induce diarrhea in young dogs. All the detected canine RVAs have been characterized as G3P[3], at the same time the canine-like G3P[3] RVAs have also been identified from human patients hospitalized with gastro-enteritis worldwide, suggesting the existence of zoonotic potential and virulent phenotype of canine RVAs in a heterologous host. In this study the full-length genome of a RVA strain was detected in the stool of a clinically asymptomatic puppy, in Hungary. The virus was detected by random primed RT-PCR coupled with high-throughput sequencing. Overall the Hungarian canine strain displayed the greatest genomic conservation with the Italian human RVA strain (PA260-97). The two strains shared very high sequence identity of all the 11 genes (rage 98.3-99.2% nt). The minimal genetic diversity observed between these two strains is notable and might suggest a direct interspecies-transmission from the canine host to humans. As RVAs in many cases induce mild or asymptomatic infections in dogs, the risk of zoonotic transmission of canine RVAs may be underestimated.

MOLECULAR DIVERSITY OF PHEASANT ROTAVIRUSES DETECTED IN HUNGARY

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Group A rotaviruses (RVA) are a leading cause of viral acute gastroenteritis in human and animals. RVAs are icosahedral, non-enveloped viruses possessing a 11-segmented dsRNA genome. Traditionally, RVAs have been classified into serotypes and genotypes of the neutralization antigens, VP7 and VP4. Recently the genotyping system has been extended to all 11 RVA gene segments and used to describe genetic configurations of individual rotavirus strains. In this study three full genome and two partially sequenced pheasant RVA genomes are reported. The rotaviruses were detected in pheasant stool samples; the viral RNA was amplified by RT-PCR assay with avian rotavirus specific primers, and the PCR products were sequenced by the classical Sanger-sequencing technology. One strain (RVA/Pheasant-wt/HUN/14246/2008/G23P[37]) shared common genome constellation with a pheasant rotavirus from Germany (RVA/Pheasant-tc/DEU/10V0112H5/2010/G23P[37]), with the sequence identity of all the 11 genes between 90.5-

97.4% nt range. The other Hungarian pheasant strains were reassortants between pheasant, turkey and chicken rotavirus strains. The strain RVA/Pheasant-wt/HUN/18769/2011/G22P[35] shared common genotypes with turkey rotavirus strains in genes VP7 and VP4 (G22, P[35]).
The strain RVA/Pheasant-wt/HUN/81626/2008/G23P[30]P[37] proved to be mixed infection with combination of two distinct VP4 genotype, one shared with a German chicken rotavirus strain (RVA/Chicken-tc/DEU/02V0002G3/2002/G19P[30]) and the other shared with the German pheasant rotavirus strain. In these strains the reassortment events caused antigen shift, the strains carried turkey or chicken genotypes instead of common pheasant rotavirus genotypes in genes of the outer capside antigens, VP4 and VP7. Phylogenetic analyses of the nine backbone genes of both Hungarian pheasant RVA revealed they share genotype specificities with pheasant, turkey, and chicken RVA strains (I4-R4-C4-M4-A16-N10-T4-E4-H4), and most of the genes clustered with the German pheasant RVA strain. The partially sequenced pheasant strains shared common genotypes with chicken (G19P[31]), and turkey (G22) rotavirus strains. These Hungarian strains with mosaic genome highlight the close relationship among avian rotavirus, whose primary driving force is gene reassortment involving homologous genes, and showed the common possibility of reassortment events between wild and domesticated avian species.

SCREENING FOR MYCOTOXIN (AFLATOXIN B1, ZEARALENON) BIODEGRADATION FROM ACTINOMYCETE STRAIN COLLECTION

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Fungal species line (*Aspergillus* spp., *Fusarium* sp.) produce hazardous mycotoxins like aflatoxin B1 (AFB1) and zearalenone (ZEA), as secondary metabolites. AFB1 has cytotoxic, mutagenic and carcinogenic effects, whereas ZEA can disrupt the endocrine system as it can mimic the effect of estrogens. Biodegradation by microbes is an effective method for elimination of these hazardous toxins. From aspect of biotechnology Actinomycetes are ones of the most important prokaryotic groups since they are widely used as sources of industrial enzymes and antibiotics. The aim of this work was to screen our Department's Actinomycetes collection consists of 133 strains, especially from the *Streptomyces* genus, of their AFB1 and ZEA biodegradation potential. Two different biotests were used for screening purposes: SOS-Chromotest based on β-galactosidase activity was used to select microorganisms having the best AFB1 degrading potential and being able to degrade AFB1 without genotoxic by-products. Estrogenic effect of ZEA was measured with a yeast based bioluminescent biotest (BLYES). First of all lyophilised strains were recultured and identified with molecular methods, based on their 16S rDNA sequences analyses. Biodegradation experiments were conducted in Erlenmeyer flasks which contained 1 mg/L AFB1 and 1 mg/L ZEA and were incubated for 120 hours. Samples at the end of the incubation period and their estrogenic and genotoxic effect was measured by BLYES and SOS-Chromotest, respectively. On the base of the results ten strains were selected for further analyses, where biodegradation experiment were conducted in 3 parallels and evaluated by ELISA test. According to the results of repeated biotests 5 out of the 10 investigated strains could degrade AFB1 and 4 strains could degrade ZEA in over 85% comparing to the control. The best AFB1 degrader was *Streptomyces baarnensis* K116, whereas the best ZEA degrader was *Streptomyces rimosus* K189. In conclusion, some *Streptomyces* strains might be proper bases for industrial degradations of these two mycotoxins.

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MORPHOLOGIC CHARACTERISATION OF *BOTRYTIS CINEREA* ISOLATES COLLECTED FROM EGER WINE-GROWING REGION

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Two main characteristics of filamentous, ascomycetous fungus *Botrytis cinerea* are considering, the one hand the gray rot can cause big losses in the vineyards and in the agriculture, but on the other hand the appearing of noble rotting. The unique "aszú" berries and the possibility of noble rotting are very rare in the wine growing territories of the world. This economic importance of *B. cinerea* has inspired an extensive research activity into its biology and as well as in disease management. Based on these considerations in the course of this experiment phenotypic properties of *Botrytis cinerea* isolates were analysed from two vineyards of Eger wine-growing region. Morphological characteristics of more than 150 noble rotted isolates were distributed into two main classes (mycelial or sclerotial) by morphological appearance according to the sampling dates. The isolates were collected during the year 2011 and 2013 from two single variety vineyards: Olaszrizling and Turán. The strains were obtained by single-spore isolation. All single-spore strains were stored at 20 °C on potato dextrose agar (PDA). The morphological characteristics and growing rate of every strains were measured at 15 °C, 20 °C, 25 °C on PDA. For each experiment, mycelia plugs (5 mm diameter) were taken and incubated four days at 20 °C in the dark. In 2011 high sclerotium production and low mycelium production was observed during the experiments. There were not differences between the samples from different grape varieties, but between sampling dates significant differences were identified. The sclerotium production possibility and intensity was higher in late collected (noble rotted) samples.

The high proportion of sclerotium demonstrates the good adaption ability of *Botrytis cinerea* to the environmental effects. The morphological evidences of the 2013 year samples shows similar effect. In general, results contrasting with the earlier experiments our latest observations support the statistical identification of the specialty of this local population.

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PHYLOGENETIC ANALYSIS OF HEPATITIS A EPIDEMICS IN HUNGARY IN 2012-2014

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The hepatitis A virus (HAV), a picornavirus, is a common cause of hepatitis worldwide. High endemicity of HAV infection is found in countries with poor sanitary and socioeconomic conditions. Hungary belongs to countries with intermediate endemicity of HAV, where improving housing and hygienic conditions result in lower anti-HAV immunoglobulin G (IgG) prevalence. In these countries the paediatric population may escape HAV infection in early childhood. It means that older children and young adults become susceptible to HAV infection resulting increased disease incidence rates and severity despite the presence of improved conditions. In recent years there was a considerable rise in HAV incidence in Hungary due to several HAV outbreaks. We investigated ninety serum samples in total, of which 83 were PCR positive. A fragment of the HAV genome was

amplified, thirty of the PCR positive samples were sequenced and a phylogenetic tree was constructed. The majority of the sequenced samples were of genotype 1A. These samples formed two distinctive subgroups. In the first group samples showed similarity to the earlier isolated Hungarian genotype 1A strains, while samples in the other group differed from the earlier isolated HAV strains and were totally identical based on the VP1/2A fragment of the HAV genom.
The higher incidence of HAV in the last two years in Hungary underlines the importance of public awareness campaigns about HAV that may lead to understanding the importance of hygiene as far as HAV is concerned and may result in higher vaccination rate in the Hungarian population.

ANTIFUNGAL ACTIVITY OF NON-ANTIFUNGAL DRUGS AGAINST *SCEDOSPORIUM* AND *PSEUDALLESCHERIA* ISOLATES

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The members of the genera *Scedosporium* and *Pseudallescheria* are associated with a wide spectrum of human infections, including deep-seated infections in immunocompromised patients, which are commonly fatal and have a tendency for dissemination and central nervous system involvement. As these species are typically resistant to licensed antifungal drugs, it is especially challenging to find appropriate therapeutic agent(s) for the treatment. For these reasons, there is an urgent need for new, safely applicable antifungal strategies against *Scedosporium* and *Pseudallescheria* infections. Non-antifungal drugs with secondary antifungal activity, as monotherapeutic agents or in combination with conventional antifungal drugs, could be the potential solution to this problem. According to previous studies cysteine derivatives would be appropriate to treat lung infections, suramin for invasive infections, while antipsychotic drugs for cerebral fungal diseases. In the present work the *in vitro* antifungal activities of suramin, six mucolytic compounds (D-, L-cysteine, L-cysteine-methyl-ester (LCME), N-acetyl-cysteine (NAC), N-isobutyryl-D-cysteine and N-isobutyryl-L-cysteine), five antipsychotic drugs (chlorpromazine (CPZ), trifluopherazine (TFP), amantadine hydrochloride, R-(-)-deprenyl hydrochloride (R-DEP) and valproic acid) and their combinations with four conventional antifungal agents (voriconazole, amphotericin B, terbinafine and caspofungin) were investigated in broth microdilution test against nine fungal isolates belonging to the genera *Scedosporium* and *Pseudallescheria*. When used alone, LCME, NAC, CPZ, TFP and R-DEP exerted remarkable antifungal effects. In combinations with antifungal agents, LCME and NAC acted synergistically with all investigated antifungal drugs. Similar interaction was observed when CPZ or TFP was combined with amphotericin B or terbinafine. In all other cases indifferent interactions were revealed.
After *in vivo* studies, these combinations could be bases of effective, less toxic therapies for treatment of different *Scedosporium* and *Pseudallescheria* infections.
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PHLYOGENETIC ANALYSIS OF SEVERAL FISSION YEAST CELL WALL SYNTHASE AND CELL SEPARATION PROTEINS

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Coordinated cell wall synthesis and degradation is essential during cell growth, cell separation and sporulation in Fungi. 1,3-alpha-glucan is one of the main compounds of the fission yeast (*Schizosaccharomyces pombe*) cell wall, and the enzymes involved in its synthesis (alpha-1,3-glucan synthase; Ags1, Mok11, Mok12, Mok13, Mok14) and degradation (endo-(1,3)-alpha-glucanase; Agn1, Agn2) are characterized and well known. Although these proteins are well described in fission yeast, less is known about the homologous proteins in other species, which may carry out the same or similar processes. The aim of this study was to perform an in silico analysis of these proteins, to identify homologs in other species, and to identify the evolution of these proteins. We have performed the phylogenetic analysis of these proteins including numerous homologs from Ascomycota and Basidiomycota species. Homolog sequences were obtained after a Blastp search in several databases (NCBI, JGI, BROAD). Interestingly a lot of species (for example *Aspergillus* species) contain more than 4 Agn1 homologs and 1-3 Ags1 homologs, however no ortholog was found in the Saccharomycotina subphylum. To identify the domain structure of the putative homologous sequences, a search was carried out using the Pfam database. The full protein and the domain sequences were used for the phylogenetic analyses. Multiple alignments were generated using ClustalW, Prank and Mafft methods.

Trees were constructed with MEGA, PHYLIP and MrBayes programs with different methods (Neighbor Joining, Maximum parsimony, Maximum likelihood, Bayesian inference). Notwithstanding different methods result in slightly different topologies, we concluded that according to the phylogenetic trees the homologs in the *Schizosaccharomyces* genus seem to be more closely related to the Basidiomycota homologs than to the Ascomycota ones.

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ANTIBACTERIAL ACTIVITY OF *THYMUS VULGARIS* ESSENTIAL OIL AGAINST *KLEBSIELLA PNEUMONIAE*

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Klebsiella pneumoniae is one member of the Enterobacteriaceae and most important Gram-negative bacterium causing nosocomial infections. Several isolates carries multiresistance against the most important practically relevant antibiotics. For this reason there is an urgent need to find novel or alternative antibacterial agents. Because of their antimicrobial features essential oils (EOs) have the potential to contribute to solve this emerging problem. In this study the antimicrobial activities of 14 different EOs against *K. pneumoniae* were tested and among them it was revealed that thyme (*Thymus vulgaris*) showed the most marked inhibition zone in the case of the all 120 *K. pneumoniae* isolates. Minimal inhibitory and bactericidal concentrations (MIC and MBC, respectively) of this EOs were determined with one representative strain in liquid culture and both values proved to be 0.312 µl/ml. Major components of thyme essential oil were separated by gas chromatography (GC),

and the following percentual composition was revealed: thymol (47%), p-cymene (34%), carvacrol (4.2%), γ-terpinene (2.03%), α-pinene (1.5%) and linalool (1.26%).
Thin layer chromatography – direct bioautography (TLC-DB) was applied in order to reveal components of thyme essential oil possessing antibacterial activities. Based on the applied standards and the Rf values thymol and carvacrol, two major components of thyme EO, were identified to be responsible for the antibacterial activities. Nevertheless a third active component, linalool, with a minor bactericid effect was also detected. *Thymus vulgaris* essential oil may have a potential for use against multidrug resistant organisms such as clinical isolates of *Klebsiella pneumoniae*.

COMPARATIVE SEQUENCE ANALYSES OF PICORNAVIRUSES ISOLATED FROM DIFFERENT TERRESTRIAL TORTOISE SPECIES

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Although exotic animal keeping is becoming more and more popular in the world, our knowledge about viruses of lower vertebrates is scanty. In the absence of sufficient data diagnosis of these viruses has significant difficulties; therefore in most of the cases the pathogenic background of infectious diseases of reptiles remains unclear. Molecular characterization of tortoise PVs is the first step in developing more effective diagnostic systems to identify these frequently isolated viruses. All tortoise PV strains used in this study were isolated in Europe in different years, most of the strains in Germany between 2003 and 2010, and 2013/T4 in Hungary in 2013. Strains 124/10, 144/10 and 5/04 originated from *Testudo graeca*, 9/05, 5/03 and 2013/T4 and 14/04 were detected in *Geochelone sulcata, Pyxis arachnoides* and *T. hermanni*, respectively. Near complete genome sequences of the isolated strains were determined applying a combination of next generation and Sanger sequencing techniques. As observed in most PVs one predicted ORF was identified encoding a single 2218 aa long polyprotein. Comparing the determined genome and predicted polyprotein sequences, high nt (80.3-98.8%) and aa (89.3-98.7%) sequence identity values characterized the seven strains which were most similar to each other. Genome organization of the isolated strains proved to be similar and showed a typical picornavirus layout: VPg+5'UTR[L/1A-1B-1C-1D-2Anpgp/2B-2C/3A-3BVPg-3Cpro-3Dpol]3'UTR-poly(A). The base content of these virus genomes is heavily biased towards A+T compared to other members of the Picornaviridae family. The polyprotein encoding regions of the strains showed low similarity with other PVs (P1 region ≤38.9%; P2 region ≤26.2%; P3 region ≤34.7%). The predicted proteins encoded by the tortoise PV genomes were related to the corresponding genome regions of viruses belonging to distinct genera, implying modular evolution of the genome parts of these novel viruses. Phylogenetic calculations of the different genomic regions supported the results of sequence analyses. Our results suggested that these closely related tortoise PVs belong to a prototype species in a separate proposed genus, tentatively called *Topivirus* (Tortoise picornavirus) of the family Picornaviridae.

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HUMAN CORONAVIRUSES: SARS AND MERS

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Since the first descriptions of AIDS in the early 1980s, much has been written about the causes and consequences of emerging viral diseases. The 'new' viruses (emerging and re-emerging) identified since the rise of AIDS, such as Sin Nombre, Nipah, Hendra, KI, Wu poliomaviruses, SARS coronavirus (SARS-CoV), avian flu (H5N1 and H7N9 subtypes) and most recently MERS-CoV. Defining emerging viruses as those that are newly appeared or have recently increased in prevalence and/or geographical range reveals some important general patterns. First, almost all emerging viruses have RNA rather than DNA genomes. Second, almost all emerging viruses have an animal reservoir, such that the process of viral emergence can usually be categorized as cross-species transmission. For example, HIV type-1, the major cause of AIDS, has its origins in the related SIV found in chimpanzees while SARS-CoV has close relatives in *Himalayan palm* civets. Despite surveying a number of animal species, the ultimate reservoir species for several viruses remains a mystery, although this is more likely to reflect the fact that the wrong species have been surveyed or that the reservoir viruses are too divergent in sequence to be recognized, than the absence of an animal reservoir altogether. A novel human coronavirus (MERS-CoV) was recently identified in the Middle East as the causative agent of a severe acute respiratory syndrome resembling the illness caused by SARS-CoV. Although derived from the CoV family, the two viruses are genetically distinct and do not use the same receptor. On the other hand, both coronaviruses induced a similar activation of pattern recognition receptors and the interleukin 17 pathway, but the MERS-CoV specifically down-regulated the expression of several genes within the antigen presentation pathway, including both type I and II major histocompatibility complex genes. This could have an important impact on the ability of the host to mount an adaptive host response.
However, MERS-CoV and SARS-CoV elicit distinct host gene expression responses, which might impact *in vivo* pathogenesis and could orient therapeutic strategies against these emergent viruses.

QUANTIFICATION OF BACTERIA IN A SIMULATED LARGE INTESTINAL MODEL BY REAL-TIME PCR

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In vitro digestion models are widely used in food industry. Our in vitro digestion model is aimed to study the structural changes, digestibility and bioavailability of food components under simulated gastrointestinal conditions, as well as determine the microbiota modulation properties. The model consists of four phases, imitating the digestion process in mouth, stomach, small intestine and large intestine. In this study, we focused on the changes and quantification of bacterial numbers of the simulated large intestine model. A continuous colon model was set up to represent the human colonic microbiota during 96 hours of fermentation. The large intestinal phase was represented by two probiotic (*Bifidobacterium, Lactobacillus*) and four other common bacterial (*Clostridium, Bacteroides, Enterococcus, Escherichia*) species in the human gut. To predict the effect of some prebiotic and other digested food components it was needed to determine the growth rates of the bacterial strains. Traditionally selective agar plates are used to evaluate the composition of bacterial communities. However, cultivation took too much time to monitor the changes in bacterial numbers in our case; therefore, quantitative real-time PCR technique was applied for enumeration of

bacteria. It has been successfully applied for quantification of bacterial DNA in various environments such as faeces, colonic or gastric tissue. In this study, we report the design and optimization of a new set of 16S rDNA targeted species specific primers for improved detection and quantification of bacteria from *in vitro* colonic microbiota model. To design the new species specific primers 16S rDNA of each strain was sequenced and compared. The potential primer target sites were assessed either manually or using Primer3 online interface. Performance and optimal annealing temperatures of the PCR primer pairs were first determined with gradient PCR. Quantification of target bacterial DNA was carried out using Rotor-Gene 6000 real-time PCR instrument. For construction of standard curves the chromosomal DNA was isolated from 10-fold dilutions of individual bacterial reference strains. The class specific 16S rDNA targeted primer pairs were successfully used to determine each bacterium from mixed culture. The results of real-time PCR quantifications were compared with the bacterial numbers determined by selective plate counts technique and they were similar in most cases.

PROBLEMS OF CONTAMINATION BY *LISTERIA MONOCYTOGENES* IN FOOD INDUSTRY IN SLOVAKIA

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Listeria monocytogenes is a pathogenic bacterium widely distributed in the environment that can cause listeriosis in humans. Cheese and meat products have been implicated in sporadic cases and in outbreaks of listeriosis worldwide, including Slovakia. The pathogen was previously detected in small and medium-sized food processing factories in Slovakia. The aim of this study was to obtain more detailed information on the character of contamination, by means of implementation of a tracing approach based upon advanced sampling and molecular methods for sub-species identification of *L. monocytogenes*. A total of 400 samples were collected and analysed in 2010 - 2014 from the internal environment of three cheese processing plants, and 242 swab samples were collected and analysed from the internal environment of one meat processing plant. Out of these, 60 samples were found to be positive for *L. monocytogenes*. Positive colonies were confirmed by real-time PCR and classified by PCR-serotyping as well as classical serotyping. Serotype 1/2a was found to dominate (80%) in all three cheese processing plants, while serotypes 1/2a and 4b dominated in the meat processing plant (51% and 27%, respectively). Isolates were then subjected to DNA restriction (AscI and ApaI) and pulsed field gel electrophoresis (PFGE). Three pulsotypes, 2 (30%), 9 (27%) and 1 (19%), were dominant in the meat plant. The repeated presence of isolates of certain pulsotypes during a three-year period indicated persistence of *L. monocytogenes* in the meat processing plant despite of regular cleaning and sanitation. The sources of contamination could originate from external environment (slaughter house) and, breaking the hygienic barriers, could cause contamination of the internal environment of the plant. Application of the tracing approach based upon advanced sampling and molecular methods for sub-species typing of *L. monocytogenes* facilitated detection of potentially persistent strains, identification of sources and routes of contamination and, subsequently, will allow to optimize the technical and sanitation measures to ensure hygiene of the food production.

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BIOLOGICAL PRETREATMENT OF AGRICULTURAL BY-PRODUCTS FOR THE ENHANCEMENT OF SUBSEQUENT ANAEROBIC DIGESTION

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Global warming and the increased concentration of pollutants in the atmosphere due to the extended utilization of fossil fuels pose a threat to the whole biosphere. Renewable and CO_2-neutral biomass comprises a base for sustainable development, and contributes to energy independence. Biogas can be generated from almost any kind of organic material therefore it can be a promising source of renewable energy for countries with high agricultural potential – such as Hungary. Corn silage is the conventional substrate for industrial-scale biogas fomenters, but considering the debate over using agricultural land for food or fuel production, alternative substrates are desired. Corn stover, which is the by-product of corn starch production, is a biomass generated in vast amounts every year. The decomposition of this cellulose-rich substrate however, is not an easy task for the hydrolyzing microorganisms. In addition, due to its insolubility in water, corn stover forms a solid crust, which causes mixing difficulties, blocks the release of biogas from the aqueous phase and thereby decreases the overall efficiency of the anaerobic digestion. Various pretreatment methods have been tested to improve the degradability of agricultural wastes and corn stover in particular. These are predominantly chemical or physico-chemical approaches that have the disadvantages of high energy consumption and subsequent microbiological process inhibitions. Biological pretreatment methods require lower energy input and milder conditions, but the incubation time is usually long compared with the chemical pretreatments and may involve the use of expensive enzyme preparations. In our study we investigated a biological pretreatment method of corn stover, which uses microorganisms to partially destroy the lignocellulosic structure. Several microorganisms were tested, including the cellulose degrading *Clostridium cellulolyticum, Isoptericola variabilis*, and the lignin degrading *Phanerochaete chrysosporium*. The biogas potential of the pretreated biomass was determined in subsequent batch anaerobic fermentations. In some cases an impressive 55% increase in the methane yields were observed. The results established that following appropriate pretreatment, corn stover may become a fairly good substrate for biogas generation. The scaling-up of the procedure for industrial applications and the optimization of the pretreatment need further research however.

TAXONOMIC EXAMINATION OF TOKAJ WINE YEASTS ISOLATED AT THE BEGINNING OF THE 1900'S

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The main objective of wineries is producing excellent quality wine. One of the technology processes to achieve this goal is to inoculate the must with a starter culture. In this way the speed of fermentation can be increased and the fermentation process becomes easier to direct and control. Through this method we can lower the chances of stuck and sluggish fermentation. For obtaining starter cultures, yeasts were isolated in well-known foreign wine regions based on their excellent winery behavior. A remarkable disadvantage of their use is that the fermentative flavours they

produce oppress the local aromas. Hungarian researchers were aware of both the technological advantages and the above mentioned problems associated with the applications of starter cultures as early as the beginning of the 20th century. In 1901, the former Ampelology Institute (Winery Research Institute) started collecting and growing cell cultures of wine yeasts coming from Hungarian wine regions. Parts of these strains have been preserved in the National Collection of Agricultural and Industrial Microorganisms in Budapest. Our aim was to examine the oenological properties of these isolates after a century-long maintenance in laboratory conditions. Collecting test results from previous examinations on these yeast strains. Their taxonomic identification (it was not possible at the time of collecting). Their genetic characterization (kariotypization, DNA-fingerprinting, and mt-DNA RFLP). Examination of the killer activity of the strains.
Performing test fermentation on strains, which showed the best performance in the experiments carried out by the researcher, who isolated the strains. We evaluated the final products using sensorial analysis and chemical tests. Based on the results, we have found out the taxonomic affiliation of yeasts fermenting Tokaj wines 100 years ago, and what genetical or physiological features these selected yeasts have. We have also clarified the effects of long-term laboratory treatment on the strains, and how they react once they are back in the must, which is their natural medium. Through the test fermentations it is now proven that by using accurate winery criteria researchers of the past selected types of yeasts which, even in modern times can be suitable for substituting commercial starter cultures.

LIPID RAFTS OF HIV INFECTED CELLS AS TARGET FOR THYOLATED PYRIMIDINE NUCLEOTIDES

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Upon HIV infection, cells become activated and cellular components express increased number of –SH groups. Earlier we demonstrated significant *in vitro* anti-HIV effect of our new compounds: thyolated pyrimidine nucleotides termed UD, and derivatives which modify –SH groups. The aim of present study was to determine molecular events resulting selective cytotoxicity of these compounds on monolayer and suspension cells, non-infected and infected acutely by HIV-1. Six chemically modified derivatives of thyolated pyrimidine nucleotides (UD29, UD29-new, UD30 ,UD31, MOD-94 and MOD-2012) were used in a concentration ranged 5 -100 micromole. Compounds were added for 24h or 48h to H9 human lymphoid cells, and on HeLaCD4+β-gal monolayer cells non-infected, or infected with HIV-1IIIB (m.o.i.: 0.5,1 and 2 respectively) 30 min after adding compounds. Viral infectivity was demonstrated by an *in vitro* MAGI (multinuclear activation galactosidase indicator) assay on HeLaCD4+β-gal cells. Cytotoxic effect of compounds have been determined quantitatively in vitro by XTT based Toxicology Assay Kit (Sigma-Aldrich), which is a spectrophotometric measurement of cell viability based on mitochodrial dehydrogenase activity in living cell. With the exception of UD29, all compounds exhibited a certain degree of cytotoxicity. In noninfected cells cytotoxicity ranged from 10% (UD30) to 20% (UD29-new) on H9 cells and 10% (Tox-2) to 12% (UD29-new) on HeLa cells. On HIV infected cells however cytotoxicity were much more pronounced: on H9 cells 23% (UD31) to 50% (UD29-new), on HeLa cells 47% (UD29) to 72% (Tox-2). Cytotoxic effect was dose-dependent with UD30 and Tox-2 only. Thyolated pyrimidine nucleotides inhibit the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), suggesting that these compounds may interfere with the function of the essential –SH groups of CD4 molecule (the primary receptor of HIV) as well as –SH groups in HIV viral envelope. Our recent study further

support this possibility, as compounds used, induced a more pronounced selective cytotoxic effect on HIV infected cells as compared to that of uninfected cells.
It is known, that cells become activated upon HIV infection, and cellular components – including cell membrane lipid rafts – expressing increased number of – SH groups. Our compounds, especially MOD-2012 may function as an effective, new generation entry inhibitor for HIV.
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GENETIC DIVERSITY AND TETRACYCLINE RESISTANCE MECHANISMS AMONG HUNGARIAN *ACTINOBACILLUS PLEUROPNEUMONIAE* ISOLATES

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Actinobacillus pleuropneumoniae causes severe pleuropneumonia in swine, which is associated with significant economic losses; biovar 1 strains need nicotineamide adenine dinucleotide (NAD) and are associated with outbreaks, while biovar 2 strains do not need NAD and cause sporadic cases. The aim of the study was to investigate the genetic diversity, occurrence of tetracycline resistance genes and resistance integrons in a collection of Hungarian isolates. Seventy-two Hungarian isolates (44 biovar 1 and 28 biovar 2) were examined. Biotyping was based on biochemical and cultural characters and the strains were serotyped with the indirect haemagglutination test using hyperimmune sera produced against the 15 type strains of *A. pleuropneumoniae*. Species identification was confirmed by species-specific PCR; tetracycline resistance genes *tetB*, *tetH*, *tetL*, *tetO* and class 1, 2 and 3 integrons were sought for by PCR; genetic diversity was assessed by pulsed-field gene electrophoresis using ApaI and SpeI macrorestriction. Macrorestriction analysis showed relatively high diversity among the 44 biovar 1 isolates; three clusters were identified; one with 19 isolates showed country-wide distribution, the other two with seven and seven isolates were rather regionally distributed. Two related isolates carried a *tetB* gene, a single isolate harboured a *tetH* gene. Integrons were not detected. In contrast, the 25 of the 28 biovar 2 isolates belonged to a single cluster showing a country-wide distribution. Tetracycline resistance was relatively frequent in this cluster; nine *tetL* genes and one *tetB* gene were found. The remaining three biovar 2 isolates were also related. Integrons were not carried by biovar 2 isolates either. Though the clonality of biovar 2 isolates is not unexpected, the higher frequency of tetracycline resistance genes warrants further investigation.
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MONITORING OF AGRICULTURAL SOIL INOCULATIONS BY MOLECULAR FINGERPRINT METHOD

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Plant growth promoting rhizobacteria (PGPR) are used for fertilization agricultural soils which has beneficial effect on plant productivity and crop yield. PGPR microorganisms facilitate the plant growth by favoring nutrient uptake (phosphorus, nitrogen, and essential minerals), phytohormone production and decreasing inhibitory effects of various pathogens. Environmental parameters of the different soils could have effect on the viability and growth of bacterium strain used for soil inoculation. Fifteen different bacterium strains were used for inoculating alkaline and acidic soils to enhance plant growth and crop yield. The aim of the study was to detect the presence and estimate the relative abundance of the applied bacterium strains in treated soils from inoculation time till harvesting. Further, the total bacterial community structures of the soils were characterized during the treatment. For detecting the inoculated strains and the total bacterium community structure Terminal Restriction Fragment Length Polymorphism (T-RFLP) was applied. Before soil inoculation a model system was created in order to distinguish the fifteen bacterium strains. The aim was to find adequate primer pair and restriction endonuclease enzyme combinations generating unique terminal fragments (T-RF) from each strain. The complete 16S rDNA base sequence was determined in order to find in silico the most suitable primer pair combination to amplify the most variable 500 base pair length partial 16S rDNA region. In this region numerous restriction endonucleases were tested for detecting terminal fragments (T-RF). One primer pair and 3 enzymes proved to be appropriate for monitoring the presence and relative abundance of the inoculated bacterium strains during the crop season.

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GC-FID MEASUREMENT METHOD FOR ESTERIFICATION ACTIVITY OF FUNGAL LIPASE ENZYMES

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The extracellular lipase enzymes of fungi are frequently used in various industrial applications processing different lipids such as tryacylglicerols, fats and oils. However, besides the well known hydrolyzing activity, these enzymes are able to catalyze esterification- and transesterification reactions of different fatty acids under proper conditions. The lipase enzymes of strains belonging to the Zygomycetes is an intensively studied research objects because some of them are already utilized in the industry and many enzymes have been isolated and characterized in detail. The hydrolytic activity of some enzymes is well characterized according to the literature, but there is little information about their esterification properties. In our study, we developed a method for the investigation of lipase esterification activity originated from different zygomycetes fungi. The enzyme-reactions were set up in non-aqueous condition using methanol and short-, medium and long chain fatty acids. The reaction mixtures were incubated for 30 min at different temperatures and the synthesized methyl-esters were analyzed based on the gas chromatographic measurements. The analyses were carried out on 60 m capillary column containing bonded PEG phase using proper temperature program and flame ionization detection. The chromatographic method was validated, the system suitability test, specificity, limit of detection and limit of quantitation, linearity, system

precision and method precision were determined. Calibrations of fatty acid methyl esters were made internal standard method with injection of heptadecanoid acid methyl ester.

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MOLECULAR SURVEY OF BAT-TRANSMITTED RNA VIRUSES: DISCOVERING NOVEL ASTROVIRUSES, CORONAVIRUSES AND CALICIVIRUSES

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With over 1250 species, bats represent the most widespread mammalian order worldwide. Emerging infectious diseases pose a significant threat to human and animal welfare. Moreover, anthropogenic activities, such as urbanization and destruction of natural bat habitats are increasing interactions between bats, humans, and livestock. We investigated the occurrence and genetic diversity of bat RNA viruses in Hungary. Bat faecal samples were collected from different geographic areas of Hungary and screened for RNA viruses of six distinct virus families (Astroviruses – AstV, Coronaviruses – CoV, Caliciviruses – CalV, Lyssaviruses – LysV, Orthoreoviruses – OrthV, Paramyxoviruses – ParmV, Rotaviruses – RotV) with different sets of consensus primer pairs. A total of 447 bat faecal samples were collected in 2012 and 2013 from 45 sampling sites across Hungary. Twenty-four out of the 28 known Hungarian bat species were sampled and tested in this study. Novel strains of AstVs, CoVs and novel CalVs were detected, while LyssV-, OrthV-, ParmV- and RotV were not identified in the samples. The overall prevalence of AstVs in bats was 6.93% (95% confidence interval [CI] 4.854, 9.571).

Out of the 24 bat species tested, AstVs were identified in the following eight species: *Miniopterus schreibersii, Myotis bechsteinii, Myotis daubentonii, Myotis emarginatus, Myotis nattereri, Nyctalus noctula, Pipistrellus pygmaeus* and *Plecotus auritus*. CoV RNA was detected in seven bat species: *Myotis daubentonii, Myotis myotis, Myotis nattereri, Pipistrellus pygmaeus, Rhinolophus euryale, Rhinolophus ferrumequinum* and *Rhinolophus hipposideros*. The prevalence of bat CoV among the sampled bats was 1.79% (95%, CI 0.849, 3.348). Novel bat CalVs were detected in three bat species, namely *Myotis daubentonii, Myotis alcathoe* and *Eptesicus serotinus*. The prevalence of BtCalV among the sampled bats was 0.67% (95%, CI 0.189, 1.780). As the main results of this study, i) we have successfully confirmed SARS-related CoVs in bats, ii) we have described new bat species harbouring AstVs in Europe and iii) first time we found new species of CalVs in bats.

WEST NILE VIRUS SURVEILLANCE IN MOSQUITOES, APRIL TO OCTOBER 2013, VOJVODINA PROVINCE, SERBIA

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WNV has been documented in Serbia since the 1970s by seroepidemiological surveys and was detected in *Culex pipiens* mosquitoes during a mosquito surveillance programme conducted in 2010. After the 2012 epidemic, which was associated with WNV lineage 2 and caused 58 human cases in Serbia, the virus was successfully detected and characterised in migratory bird samples. The 2012 season was followed by a more severe epidemic season in 2013 with over 300 human cases. In the absence of information about strains causing the WNV outbreak in Serbia during 2013, it was not possible to elucidate whether the 2012 strains circulated and overwintered to cause epidemics in the consecutive year. The aim of the present study was to detect and characterise WNV strains circulating in the 2013 mosquito activity season in Vojvodina. Center for Disease Control and Prevention (CDC) light traps baited with dry ice were placed at 13 sampling sites in Vojvodina province, Serbia, between April and October, 2013. A total of 6.369 female mosquitoes (combined in 180 pools) representing 11 species were tested in this study. Ten (5.5%) of 180 pools (minimal infection rate (MIR) value: 1.57) sampled were positive for WNV RNA. WNV was detected in nine pools of *C. pipiens* (MIR value: 1.61; 95% confidence interval: 0.70–3.10) and a single pool of *Anopheles maculipennis* (MIR value: 45; 95% confidence interval: 1.20–228.40), suggesting that these two mosquito species may play an important role of WNV transmission in the study area. Phylogenetic analysis showed that Serbian WNV strains from 2013 were most closely related to Italian and Greek WNV strains isolated in 2012 and 2010 and were more diverse than strains identified in 2010 and 2012 in Serbia. Previous studies found at least two different genetic clusters of lineage 2 WNV that circulated simultaneously in Serbia in 2012, suggesting that WNVs were introduced to the country by at least two different events. Phylogenetic analyses with the WNV strains detected from mosquitoes in the present study point to a potential third independent introduction event of the virus. Here we report a greater than expected prevalence of WNV in *C. pipiens* and *A. maculipennis* mosquitoes collected during 2013 in parts of Serbia.
Our findings were consistent with the great number of human cases in Serbia during 2013 and provide a possible explanation for the explosive spread of WNV in the affected area.

PUTATIVE NEW LINEAGE OF WEST NILE VIRUS IN THE MOSQUITO *URANOTAENIA UNGUICULATA*

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Mosquito-borne viruses (MBV) represent a considerable healthcare problem worldwide, causing numerous infections both in humans and animals. MBVs belong to a variety of virus families; most prominent members of MBVs are those within the Flaviviridae (eg. West Nile virus, Dengue virus, Usutu virus), Bunyaviridae (eg. Tahyna virus), and Togaviridae (eg. Sindbis virus). In many parts of Europe, the greatest medical and veterinary burden is attributed to flaviviruses, and particularly West Nile virus (WNV). WNV has been subdivided into at least eight distinct lineages (WNV-1 to WNV-8): WNV-1 and WNV-2 strains have been identified most frequently in human and animal

diseases, while strains within WNV-3 to WNV-8 have been detected sporadically during MBV surveillance in parts of Europe, Asia, and Africa. We report the description of a novel putative lineage of WNV (WNV-9) which was identified during a 3-year surveillance of MBVs conducted in Southwest Hungary. A total of 23,029 female mosquitoes captured in a range of habitats in Hungary were tested for flaviviruses and orthobunyaviruses. WNV was detected in a single pool of *Uranotaenia unguiculata*. Sequence- and phylogenetic analyses for two different regions (NS5 and E) of the viral genome indicated the virus was different from other previously described WNV lineages. In the phylogenetic tree, the Hungarian WNV sequence clustered with other WNV strains, but unambiguously formed a separate monophyletic branch. The NS5 gene-based analyses showed that the Hungarian novel WNV strain shared the greatest similarity with representatives of WNV-4 (up to 82%, phylogenetic distance: 0.413) and the putative lineage 8 strain (83%, phylogenetic distance: 0.360), originating from Russia and Spain, respectively. Similarly, the E gene-based analyses indicated that the novel Hungarian WNV strain was different from the WNV strain that derived from the same mosquito species in Volgograd, Russia, which rather clustered together with WNV-4. These findings may indicate the presence of a novel lineage of WNV (WNV-9) in Europe.

GW/WG DOMAINS AND A ZINC-FINGER FOLD ARE ABSOLUTELY REQUIRED FOR SILENCING SUPPRESSOR ACTIVITY OF THE P1 PROTEIN OF THE SWEET POTATO MILD MOTTLE VIRUS

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RNA silencing is a post-transcriptional gene regulation mechanism conserved in almost all eukaryotes and involved in many essential biological processes. The trigger of RNA silencing can be pri-miRNAs for miRNAs or double stranded (ds) RNAs as replicative forms of plant viruses. Trigger RNAs are processed into si- and miRNAs by the RNAse III type enzymes Dicers, then small RNAs are loaded into the Argonaute (AGO) protein containing protein complexes called RNA induced silencing complex (RISC). In the last few years, several AGO binding proteins were identified. Most of them contains WG/GW domains that mediates the interaction with AGO. This group of proteins was named WG/GW proteins after the founding member GW182 protein of human. The P1 protein of the Sweet potato mild mottle virus is a WG/GW protein hampering target cleavage and miRNA driven translational inhibition by active RISC complexes. Our results showed that three WG/GW motifs resembling the AGO binding platform conserved in plants and metazoans at the N-terminal part of P1 are required for AGO binding and silencing suppressor activity.
Recently, we found that 4 cysteine residues surrounding the second GW domain on the SPMMV P1 protein might form a zinc finger (Zfn) motif and essential for activity. Moreover, our bioinformatics analysis revealed that no other known WG/GW domain protein has any Zfn domain indicating the novelty of our finding. We studied the significance of the WG/GW domains and the role of the zinc finger motif in the activity of P1 protein, which will be discussed.

ANTIFUNGAL ACTIVITY OF SELECTED ESSENTIAL OILS ON FOOD-SPOILAGE *ASPERGILLUS* SPECIES

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Mold contamination of stored food commodities and products represent a serious problem for food safety and food security because of the loss in crop yield and production of mycotoxins. The losses were more severe in tropical and subtropical countries but global warming may influence the occurrence and distribution of mycotoxin producing fungi in regions with temperate climate, including Central Europe. To reduce the problem of food contamination, there is a need to adopt new strategies which are simple in application and have a broad-spectrum fungitoxicity. In the last two decades scientists tried to minimize the use of synthetic chemicals for the management of plant pathogens to avoid environmental pollution and turned their attention to the search for natural substances able to act as antimicrobials or decontaminants. Essential oils have gained widespread interest in the search to identify alternatives for microbial control because of their good antimicrobial qualities. The advantage of EOs is their bioactivity in the vapour-phase, a characteristic that makes them attractive as feasible fumigants for stored product protection. In the present study the antifungal effect of cinnamon, clary sage, juniper, lemon and marjoram essential oils (EOs) on food-spoilage fungi of the *Aspergillus* genus such as *Aspergillus awamori, A. parasiticus* var. *globosus* and *A. westerdijkiae* was tested. Using different amounts of EOs (5 mg and 10 mg/Petri dish) the growth rate (mm/day) of the colonies and the antifungal index (%) were determined using reversed Petri-dish method. Lemon and juniper EOs showed no antifungal effect on the investigated molds. On the other hand cinnamon, clary sage and marjoram had good inhibitory effect on *Aspergillus* species even at lower concentrations. Best results were achieved by cinnamon EO leading to almost total inhibition of growth of all three *Aspergillus* species.

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INVESTIGATION OF ACTINOMYCETES ISOLATED FROM HYDROCARBON-POLLUTED SOIL

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The aim of the work was to set a method to measure the hexadecane consumption of strains collected from hydrocarbon-polluted soil and water and to evaluate the hexadecane consumption of strains previously characterized as promising hydrocarbon-decomposers. The method had to be feasible with the available equipments so extraction with hexane was chosen. During setting the method the volume of the hexane (one- or two-fold volume of the sample), the number of times of extraction (one or two times), the number of vortexing (1× or 2× 1 minute) were changed and 250 rpm shaking, as well as 15 min 6000 rpm centrifugation (Hettich Mikro 22R centrifuge) were applied. After the extraction the hexadecane concentration was measured by gas chromatography with flame ionization detector (GC-FID). Also the growth of strains were investigated and after 10

days of culturing the weight of cells growing in a medium containing 0.1% peptone plus 1% hexadecane was compared with the weight of cells growing in an only 0.1% peptone-containing medium. Because of the hydrophobicity of hexadecane even sample taking was not possible to do precisely, so the total culture (2 ml) was handled as a single sample. We set up three controls: one for the evaporation of hexadecane; another for the evaporation of the culture; and the third to evaluate the efficiency of the extraction. We applied triplicates for each sample and control. The finally chosen method was the extraction of the sample with the same volume of hexane, 1×, application of 1 hour 250 rpm shaking, 1 minute vortexing before and after the shaking, and 15 minutes 6000 rpm centrifuging. The time of vortexing was the only parameter of the method that proved to affect significantly the efficiency of the extraction: it was elevated from ~70% to ~100% using 2×1 minute vortexing instead of 1×1 minute. All the investigated strains proved to be hexadecane decomposers: 4-38% of hexadecane was eliminated by different strains during the 10 days of culturing. It has occurred that the injector of the gas chromatography equipment delivered not totally even volumes of samples to the detector.
This effect could be handled to some extent with calibration curves, working in triplicates and with the help of comparing the areas belonging to our samples with the areas belonging to 1% hexadecane, but the use of internal standards suggested to be preferred.

ANTIBIOTIC SUSCEPTIBILITY OF HUNGARIAN *BORDETELLA BRONCHISEPTICA* STRAINS ISOLATED FROM PIGS

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Bordetella bronchiseptica is a widely distributed bacterial pathogen causing various respiratory diseases in a variety of mammals like kennel cough in dogs, snuffles in rabbits, bronchopneumonia in cats and laboratory animals, and human illnesses have also been reported. In economic and veterinary aspects, the most important disease is atrophic rhinitis of pigs. Antibiotic treatment is still the most effective tools against bacterial infections, nevertheless little is known about the antibiotic resistance of the Hungarian *B. bronchiseptica* strains. Our aim was to fill this gap by a retrospective analysis of strains originating from pigs with atrophic rhinitis. Fifteen strains were selected with various geographical origins and year of isolation. They were cultured on Columbia agar containing 5% sheep blood. Antibiotic susceptibility of the strains was determined by Kirby-Bauer disk diffusion method on Müller-Hinton agar with eighteen different antibiotic discs. The resulted inhibition zones were compared with standard values of the Veterinary Diagnostic Directorate of National Food Chain Safety Office. Plasmid isolation was performed with QIAprep Spin Miniprep Kit and plasmids were analysed on a 0.6% TopVision agarose gel and visualised by ethidium bromide after electrophoresis. The results showed that all strains were susceptible to colistin and amphenicols, but a high level of resistance was detected to a number of other antibiotics as well (penicillin, vancomycin, lincomycin and ceftiofur). The resistance to ampicillin, neomycin, tilmicosin and flumequine was varied widely among the strains. All but five of the strains were susceptible to sulphonamides, while only one isolate was resistant against tetracycline and nalidixic acid, respectively. Four out of the 15 strains presented plasmids. These four strains showed sulphonamide resistance, but not any plasmid was isolated from the fifth sulphonamide-resistant strain. It suggests that sulphonamide resistance encoded on plasmids or on the bacterial chromosome, too. The practice of antibiotic therapy in livestock farms might affects the rate of

resistance against antibiotics. However, our study revealed no correlation between the antibiotic resistance pattern and the origins of the strains.
The signs of the general antibiotic resistance spreading were not detected.
This study was supported by grant OTKA K83332.

IDENTIFICATION OF *PNEUMOCYSTIS JIROVECI* - FROM MICROSCOPIC IDENTIFICATION TO MOLECULAR DIAGNOSIS

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Pneumocystis pneumonia is a form of pneumonia in patients with impaired immunity (e.g. people with HIV/AIDS, or people with cancer undergoing chemotherapy etc.), caused by the yeast-like fungus *Pneumocystis jiroveci*. *Pneumocystis jiroveci* (previously named as *Pneumocystis carinii*) was formerly classified as a protozoa. Currently it is considered as a fungus based on nucleic acid and biochemical analysis. Because *Pneumocystis* cannot be cultured ex-vivo, direct observation of living Pneumocystis and identification are difficult. The specific diagnosis is based on identification of *P. jiroveci* in bronchopulmonary secretions obtained as induced sputum or bronchoalveolar lavage (BAL) material. Microscopic identification of *P. jiroveci* trophozoites and cysts is performed with stains that demonstrate either the nuclei of trophozoites and intracystic stages (such as Giemsa) or the cyst walls (such as the silver stains). Molecular methods for detection of *P. jiroveci* have shown very high sensitivity and specificity and constitute the gold standard for detection of this pathogen. We started the identification of *Pneumocystis jiroveci* in 2004. The microscopic method Gömöri-Methenamine-Silver stain of sputum or BAL has been used since then. From 2004 to June 2014 170 specimen were tested. Only six were positive. In 153 cases routine mycological culturing were also made. 90 of the specimens were negative and 53 specimens were positive for yeast. As the sensitivity and specificity of the microscopic detection are not sufficient in 2012 together with the Department of Parasitology we started to introduce molecular methods: PCR detection with the LightMix ® Kit *Pneumocystis jiroveci*.
Up to now 27 clinical samples were tested, two of them were positive. In five cases both microscopic observation and molecular detection were made. All of them were negative. We continue this PCR technique and microscopic examinations for getting more data to compare.

NOVEL TAXA OF DARK SEPTATE ENDOPHYTES ORIGINATING FROM SEMIARID GRASSLANDS OF THE GREAT HUNGARIAN PLAIN

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Dark septate endophytic (DSE) fungi represent a frequent root-colonizing form-group common in environments with strong abiotic stress – such as (semi)arid ecosystems – and might play important role in those areas. Since DSE are not a phylogenetic group, but a form group DSEs are present in numerous fungal taxa mainly belonging to several ascomyceteous orders (e.g. Helotiales, Pleosporales, Hypocreales). During our study of the diversity of DSE fungi in semiarid sandy

grasslands in Hungary several unidentified DSE lineages were found. Our main aim was to carry out the polyphasic taxonomic study of three unidentified DSE clades (DSE-7, DSE-4, DSE-8). Our objectives were to (i) collect more isolates belonging to DSE-7 clade (ii) clarify taxonomic affiliation of the three DSE groups by molecular and morphological methods, (iii) to describe new taxa if needed. For molecular phylogenetic study seven loci were amplified and sequenced. Sequences were gained from the ITS regions and partial 18S nrRNA, 28S nrRNA, actin, calmodulin genes. In case of DSE-7 group partial TEF 1-alpha and beta-tubulin genes were also sequenced. For morphological survey and description, strains were transferred onto several media to induce sporulation. Molecular data revealed that two DSE clades belong to Morosphaeriaceae (DSE-4) and to Massarinaceae families (DSE-8) in order Pleosporales representing new genera.
The third lineage (DSE-7) comprising the largest number of isolates represents a new genus and six new species in Lentitheciaceae in Pleosporales.
Supported by Hungarian Scientific Research Fund (OTKA K109102). DGK is supported by the FEMS Research Fellowship.GMK is supported by a János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

APPLICABILITY OF PARTIAL CALMODULIN, 2-TUBULIN AND ITS SEQUENCES IN SPECIES IDENTIFICATION OF MEMBERS OF THE GENUS *ASPERGILLUS*

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The genus *Aspergillus* is one of the more difficult groups concerning classification and identification, and several taxonomic schemes have been proposed. New molecular approaches have shown that there is a high biodiversity among the members of this genus but that taxa are difficult to be recognized based solely on their phenotypic characters. Until now about 300-350 species have been assigned to this genus. Previously the intergenic transcribed spacer region of the rRNA gene cluster (ITS) was chosen as the primary barcoding region for fungi, however it is well-known that this region is not suitable to distinguish all fungi at the species level, including *Aspergillus* species. In the case of Aspergilli the ITS region is useful to assign species to different sections, while sequences of other genes are needed for lower taxonomic levels. We examined the phylogenetic informativeness of the ITS, partial calmodulin and 2-tubulin sequences. Our aim was to conduct a robust phylogenetic analysis including all the available sequences of the members of the genus *Aspergillus* and to find the most suitable gene for species identification. Our studies revealed that part of the calmodulin gene could be the choice as a secondary barcoding region for Aspergilli.
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PURIFICATION AND CHARACTERIZATION OF AN EXTRACELLULAR LIPASE FROM *MUCOR CORTICOLUS*

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Lipase enzymes (E.C. 3.1.1.3) have important roles in the hydrolysis of triacylglycerols and the synthesis of ester molecules. Due to these properties, lipases are applied in biotechnological and industrial processes such as synthesis of aroma or flavour esters in food industry, biodiesel production and development of pharmaceuticals. Because of the increasing use in the industry, identification of new lipases with promising hydrolytic and/or synthetic activity has become very important recently. In our previous works, *Mucor corticolus* showed high lipase yield in wheat bran based submerged and solid-state fermentations; moreover, it exhibited promising transesterification activity in organic solvents. For high-yield production, isolate was grown on inductive liquid media contained wheat bran as carbon source and Tween-80 as inductor. Fermentation was carried out at 25 °C for six days. To purify the enzyme, after filtration and centrifugation, ammonium sulphate precipitation was performed firstly. Then, size-exclusion and ion-exchange chromatographic separations were applied. Biochemical characterization studies were performed with the fractions exhibited the highest lipase activity. The optimal temperature and pH for the activity were 30 °C and pH 7.0, respectively. The enzyme retained its activity during 4-h incubation at 60 °C. In the presence of 5 mM Hg^{2+}, 10 mM N-bromosuccinimide or sodium dodecyl sulphate, remarkable decreasing in the activity could be observed; however, it had slightly stimulated by 5 mM NaCl and KCl. Propanol, hexanol and butanol also inhibited the enzyme, while 5-20% of hexane, cyclohexane, heptane and isooctane had no effect on activity.

The enzyme proved to be fairly stable in ethanol and methanol. The enzyme showed substrate specificity towards the aryl esters with medium-length acyl chain (C8-C12), such as p-nitrophenyl-caprylate, p-nitrophenyl-decanoate and p-nitrophenyl-dodecanoate. The remarkable hydrolytic and synthetic properties and its great stability in organic solvents make the *M. corticolus* lipase very promising biocatalyst for further industrial application.

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MYCELIAL GROWTH RATE AND MYCOPARASITIC POTENTIAL OF *TRICHODERMA HARZIANUM* ISOLATES AGAINST *FUSARIUM OXYSPORUM* FROM GRAPEVINE

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Trichoderma harzianum is mycoparasite with antagonistic and mycotoxic metabolite production properties. *Trichoderma* species can be used as biopesticides against pathogenic fungi. Currently there is no effective fungicide against *Fusarium oxysporum* in grapevine trunk; therefore our aim was to find potential biopesticide against it. Eight mycoparasitic *Tichoderma harzianum* isolates (TR01-TR05; TR07; TR09; TR10) were tested *in vitro* to examine their mycelial growth rate. Those were isolated from grapevine trunk disease (GTD) asymptomatic grapevine trunks from the Tokaj wine region, Hungary. The average linear growth rate (ALG) of the isolates was tested at five different temperatures (18.5-37°C). The mycoparasitic ability of the *Trichoderma* isolates against *Fusarium oxysporum* isolated from GTD symptomatic plants was also determined. 5 mm mycelial disks from the margin of seven days old colonies were cut, and put in 3 cm distance on PDA plates, than incubated at room temperature for 6 days. Finally growth inhibition value was calculated to express the mycoparasitic potential. Significant difference was found between the mycelial growth

of the isolates. TR03 and TR08 isolates had significant lower growth rate, than the other *Trichoderma* isolates. The TR07, TR09 and TR10 isolates grew rapidly in all examined temperatures. The interaction between *Trichoderma harzianum* and *Fusarium oxysporum* f. sp. *ciceris* showed different inhibition ratio on the fourth and sixth day.
The TR08 - *F. oxysporum* had the highest inhibition zone (4. day: 63.6%; 6. day: 82.6%), while TR10 - *Fusarium oxysporum* had the lowest one (4. day: 40.9%; 6. day: 47.3%). The TR09 isolate showed low (<50%) inhibition zone at the examined days, while TR07, the most intensively growing isolate also had high (> 50%) inhibition rate.
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EXPERIMENTAL APPROACHES USED TO IDENTIFY ANTIBACTERIAL MODE OF ACTIONS OF ESSENTIAL OILS

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Emergence of antibiotic resistance among bacteria is one of the most serious healthcare problems in our days. Fast spread of multiresistance can make a formerly effective drug obsolete and in several cases contributes to a failure in therapy. For this reason, there is an urgent need to find novel strategies and alternative agents both in prevention and therapy. Because of their antimicrobial features, certain essential oils (EOs) can be good candidates for future applications. EOs are mixtures of different chemicals produced by plants and some of them play an important role in the defense. Although their antimicrobial effects are known for a long time, their exact mode of action on target bacterial cell is not well understood. In this presentation we will give a brief overview about those classical microbiological, chromatographical, proteomic and transcriptomic methods by which the possible routes of an antimicrobially active EO can be elucidated. Based on our recent findings, we will discuss the applicability of scanning electron microscopy (SEM), EMA-PCR, *in vivo* assays, Quantitative Reverse Transcription PCR and proteomic approaches in the visualization of changes on cellular and molecular levels. Last but not least, the role of bioautography will also be demonstrated in active volatile component identification.

IN VITRO PHARMACODYNAMICS OF CASPOFUNGIN IN THE PRESENCE AND ABSENCE OF 50% SERUM AGAINST *CANDIDA ALBICANS* ISOLATES

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In our experiments *in vitro* efficacy of caspofungin was investigated against four echinocandin susceptible *C. albicans* bloodstream isolates, ATCC 10231 reference strain and an echinocandin resistant isolate (DPL20, FKS F645P). Caspofungin MICs, time-kill and postantifungal effect (PAFE) experiments were performed both in RPMI-1640 and in the presence of 50% serum. Killing rates (k values) of caspofungin were determined after continuous (24 hour) and short (1 hour) drug exposure against *Candida albicans* in RPMI-1640 with and without 50% human serum. The k

values in time-kill and PAFE experiments were determined for each strain and concentration in both media. The killing kinetics of caspofungin was analyzed by fitting the mean data at each time point to an exponential equation: Nt=N0xe-kt (Nt is the number of viable yeasts at time t, N0 is the number of viable yeasts at the beginning of the experiment, k is the killing rate and t is the incubation time). One-way ANOVA with Tukey post-testing and T test were used to compare killing kinetics in different concentrations and different media, respectively. In case of time-kill investigations, colony count decreases were isolate- and concentration dependent (0.25, 1, 4, 8, 16 and 32 mg/L) in RPMI-1640, however concentration independent (1, 4, 8, 16 and 32 mg/L) in 50% human serum. During PAFE experiments in the absence of serum at 4, 16 and 32 mg/L caspofungin resulted in CFU decreases similar to with the findings observed in time-kill investigations. However all isolates demonstrated growth at 4, 16 and 32 mg/L in 50% serum (k values were negative) (P<0,05-0,001). PAFE in 50% serum decreased remarkably at 4, 16 and 32 mg/L concentrations.
Since only a short growth inhibition without killing was evaluated in 50% serum, clinical relevance of caspofungin PAFE *in vivo* is questionable.
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SELECTION OF SOIL BACTERIA ADAPTED TO DETERIORATED SOILS AND ESTABLISHING A STRAIN COLLECTION

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The project aims at the isolation of stress-tolerant soil bacteria from low fertility soils – mostly due to unfavourable soil pH, high salt concentration and low water retention capacity – and the screening of the isolated strains for plant growth promoting, soil ameliorating effects, soil structure improving properties, to create a strain collection of the positive strains. 10-12 different types of deteriorated soils – acidic and alkaline sandy soils, strongly acidic brown forest soils, calcareous-saline and sodic soils, leached soils, strongly acidic meadow soil and acidic silty soil – were targeted throughout Hungary and samples were taken accordingly. Over a thousand different bacterial strains – able to grow in extreme soil conditions – were isolated from the soil samples. Hundreds of strains from each soil sample were selectively cultured to screen those that show a wide range of tolerance regarding the pH, salt concentration and culturing temperature. Strains that are not human pathogen were selected by using the most appropriate selective and culture media. Altogether 600 strains were involved in the next phase of selection, aimed at plant growth promoting properties: mineral phosphate solubilization and nitrogen fixation, indole-3-acetic-acid production, ACC deaminase (inhibiting ethylene synthesis) production and siderophore production (inhibiting the growth of plant pathogens). Strains that produce exopolysaccharides – potentially promoting soil aggregation and biofilm formation – were also selected. Approximately 100 selected strains were identified by 16S rRNA gene sequencing. The Soil Bacteria Screening System (SBSS) provided a strain collection that enumerates strains that are native of the domestic soil types, able to promote plant nutrient supply and growth and to improve soil structure. The strains are able to exert such properties at extreme soil pH and high salt concentrations in the domestic climatic conditions.
Supported by the Research and Technology Innovation Fund project “Development of an effective soil bacteria screening system and production of an instant powder soil inoculant formula” (KMR_12-1-2012-0200).

DEVELOPMENT OF A FOUR-COMPONENT LIQUID SOIL BIOFERTILIZER: BIOEGO

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BioeGO is an environment-friendly soil inoculant consisting of the mixture of two bacteria and two fungi, which have favorable effects on the growth and development of crop plants. It ensures increased nitrogen fixation, phosphorous mobilization, stem degradation and humus production on the treated field and provides protective effects against soil-borne plant pathogenic fungi. The microbial components of the BioeGO soil inoculant do not have negative effects on each other, thereby all the beneficial effects of the components can occur simultaneously, complementing each other. One of the bacterial components is a strain of *Azotobacter vinelandii*, which has nitrogen-fixing abilities proven under laboratory conditions: it is capable of efficient growth even in a culture medium lacking nitrogen. During its application, this free living bacterium is able to fix nitrogen from the air, thereby providing excess nitrogen source for the crop plants in an available form. The phosphorous mobilization and stem degradation abilities of BioeGO are ensured by a strain of the fungus *Trichoderma harzianum*, which has been selected out of 45 *Trichoderma* strains. This fungus is capable of producing cellulose-degrading enzymes even in the absence of stem residues, while this ability is increased 10-15 fold in the presence of grinded maize stem. The fungus is also producing large amounts of enzymes capable of liberating organically bound phosphorous. The humus-producing component is a strain of the beneficial bacterium *Streptomyces albus*, which was selected out of 46 strains of bacteria. The humus-producing abilities of *Streptomyces* bacteria can be primarily attributed to the production of peroxidase enzymes. The selected bacterium has been proven to possess excellent peroxidase-producing abilities. The protective effects against plant pathogenic fungi is ensured by another fungal component, a strain of *Trichoderma asperellum* selected out of 45 *Trichoderma* strains. Laboratory examinations proved that this fungus has outstanding abilities in competing and destroying several soil-borne fungal plant pathogens (e.g. *Fusarium solani, Fusarium oxysporum, Phoma cucurbitacearum, Alternaria alternata, Botrytis cinerea, Rhizoctonia solani*), thereby providing protective effects for the developing crop plants.

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DETECTION OF *BLASTOCYSTIS* SPP. IN HUMAN FAECES USING THREE METHODS

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Blastocystis is an enteric protozoan parasite highly prevalent in humans and animals. It is associated worldwide with aspecific symptoms, like diarrhoea, abdominal pain, anal itching, excess gas, and irritable bowel disease. Therefore the *Blastocystis* infected patients often remained non-diagnosed. Detection of *Blastocystis* is routinely performed by microscopy, culture, and sedimentation concentration technique. These methods are time consuming, laborious and require special skilled personnel. Microscopy is difficult since *Blastocystis* has several morphological forms (vacuolar, cyst, amoeboid, granular, multivacuolar, and avacuolar). Concentration techniques destroy some of

the forms during stool processing, therefore are unreliable. Culture requires 2-4 days for diagnosis and may allow preferential growth of specific strains while eliminating others. ELISA-based test for detection of *Blastocystis* antigens in stool could be a proper alternative to currently used methods, especially the microscopy. This work compares results of stool examination by microscopy, cultivation and antigen detection test (CoproELISA *Blastocystis*, Savyon, Israel). 78 stool samples routinely sent to the laboratory have been tested microscopically and by CoproELISA *Blastocystis*. 67 samples of them were tested with cultivation. For microscopic examination direct wet mount and Modified Merthiolate-Iodine -Formalin (MIF) preparation, for cultivation modified Boeck and Drbohlav's diphasic medium and for *Blastocystis* antigen detection CoproELISA *Blastocystis* (Savyon, Israel) have been used. Microscopically in 20.5% (16/78) of samples *Blastocystis* was detected, by cultivation in 38.8% (26/67) and by ELISA 39.7% (31/78). Comparing cultivation and ELISA: 26 by cultivation positive samples were positive in 96.2% (25/26) by ELISA. Testing 41 culture-negative samples by ELISA further 4 positive have been detected (9.7%, 4/41). Based on this comparison the calculated parameters of the ELISA test are: Sensitivity: 96.2% and Specificity: 90.2%. The ELISA results showed high correlation with results obtained by cultivation, as a standard method. ELISA is expeditious in providing reliable results. Considering this, the ELISA is expected to be the method of choice for diagnosis of *Blastocystis* in the common laboratory.

PHYLOGENETIC AND SEQUENCE ANALYSES OF REPTILIAN ORTHOREOVIRUS STRAINS ISOLATED FROM DIFFERENT EXOTIC SPECIES

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Reptilian orthoreoviruses are frequently isolated pathogens with characteristic fusogenic cytopathogenic effect in cell cultures. Complete genome sequence is available in the case of a single prototype reptilian reovirus strain (47/02) isolated from a green bush viper (*Atheris squamigera*). Only partial sequence data from the RNA-dependent RNA-polymerase gene of other isolates can be found in GenBank. In our project we performed a survey for detection and genetic characterization of orthoreoviruses infecting exotic reptiles in order to explore their genetic diversity and evolution. The organ samples of succumbed animals were processed by traditional and modern molecular virological methods. A consensus nested polymerase chain reaction targeting a conservative portion of the orthoreoviral RNA-dependent RNA-polymerase gene was used for detection of reptilian orthoreoviruses. Near complete genome sequences of all isolates were determined applying next generation sequencing. Additionally reovirus strains isolated in Germany were also processed in our study. Reoviruses were isolated from ball pythons (*Python regius*), an iguana (*Iguana iguana*), a Schneider's skink (*Eumeces schneideri*) and an unknown snake species. German strains originated from a chameleon species (2702/97), a spur-thighed tortoise (97/96; *Testudo graeca*), a carpet python (55/02; *Morelia spilota*), a boa constrictor (IBD 26/00) and an iguana (111/99). Nucleotide (nt) sequences of each genome segments were analysed separately. Based on the sequence and phylogenetic analysis of the genome segments reptilian orthoreoviruses can be divided at least into three well separated groups. Among others species demarcation in the genus *Orthoreovirus* utilizes sequence identity cut-off values defined by the International Committee on Taxonomy of Viruses. Nt sequence identities >75% between homologous genes indicate any two viruses belong to the same

species, whereas nucleotide sequence identity <60% is considered the cut-off value to demarcate viruses into different virus species. Interestingly, analyzing the nt sequence of the tortoise reovirus 97/96 sequence identity values near the cut-off values were determined indicating the pronounced genetic distance between reptilian orthoreoviruses.

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MODELING OF LOW-ALCOHOLIC BEER PRODUCTION WITH IMMOBILIZED CELL TECHNIQUE

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Due to ongoing efforts, immobilized cell systems have been applied in brewing on an industrial scale since the 1990. Immobilized brewer's yeast is mostly used with the aim of making the process of fermentation continuous. The first bioreactor applying immobilized yeast cells, called Bio-Brew was created in 1971: cells were combined with kiesel guhr and then applied on the vertical elements of a kiesel guhr filter. Development of various immobilized reactors followed the first one, while their field of application within brewing widened further to produce alcohol free and low-alcoholic beers. In the latter case the goal is to exploit altered metabolism of yeast cells in an immobilized state, and make them produce aroma compounds that characterize beer without synthesizing alcohol. Aim of our work was to immobilize brewer's yeast cells on different carriers – Ca-alginate beads, spent grain and Siran glass beads – in order to study and compare metabolic activity under circumstances that favour the production of low-alcoholic beer. In all cases the *Saccharomyces cerevisiae* WS 34/70 brewer's yeast strain was applied. Targeted cell concentration was 10^8 cells/ml in case of all three immobilization techniques. Diameter of Ca-alginate beads were 1.5-2 mm, and were created by the standard dripping procedure. Brewer's spent grain was treated with acid and alkali in order to delignify. During the continuous fermentation flow rate of wort was changed between 70 and 150 ml/h. Carbohydrate utilization, extract change and alcohol formation were followed. With this method a low-alcoholic product (less than 2.5 V/V%) was made.

DEVELOPMENT OF A BIOINSECTICIDE MICROBIOLOGICAL INOCULANT FOR BIOLOGICAL PEST CONTROL

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Several species of naturally occurring entomopathogenic soil bacteria are valuable sources in the development of biopesticides. They have been used for over 30 years in biological pest control. Members of the *Bacillus* genus produce a variety of toxins, each of them is specific to a certain group of insect species. The toxins are innocuous to vertebrates (including humans) and plants, and are biodegradable. The aim of the development was to produce a soil inoculant that provides microbiological protection against soil-dwelling insect pests, primarily corn and fruit damaging

insect larvae. The *cry3Ba1* gene encoding the Bt endotoxin has already been successfully transformed into corn, potato and other field cultivars through recombination. Several Bt endotoxin encoding genes have been successfully inserted in the genome of field crops, e.g. the *cry3Bb1* gene into maize. Recent research suggests that the concentration of the toxin produced in genetically modified plants highly exceeds the effective dose and that slow degradation imposes a high environmental risk. However, others have found that toxin concentration in the commercially grown Bt corn is considerably lower than the guaranteed value. Our aim was to develop a plant protection measure for agricultural use against soil-dwelling plant pests that operates with solely environmentally friendly mechanisms compatible with the conditions of organic farming. Main insect pests of corn are the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Coleoptera: Chrysomelidae) and other related *Diabrotica* species. Traditionally, two methods of protection against corn rootworm damage are common: crop rotation (alternating corn and soybean or alfalfa) and chemical insecticides against the larvae. Among soil-living pests, chafer grubs can also cause considerable damage to the roots of both herbaceous and woody plants. Extensive root damage can eventually lead to the death of trees. Cockchafer grubs preferably feed on the roots of raspberry, strawberry, grapes and fruit trees. *B. popilliae* Dutky and *B. thuringiensis* Berliner strains infect and kill the larvae of many cockchafer species. We have isolated soil bacterial strains potentially toxic to root damaging insect larvae and assessed the larvicidal spectrum of isolates by biological, biochemical and molecular methods and have characterised the strains. We have elaborated the production and preservation technology of bacterial strains and their insecticide toxins in semi-industrial scales. Entomopathogen efficiency of the experimental product was assessed by laboratory and field studies.

The three-year project was implemented within the framework of the Central Hungarian Operative Programme (KMOP-1.1.1.-08/1-2008-0042) co-financed by European Union funding.

COMPARATIVE TRANSCRIPTOME ANALYSIS OF THE OIL BIODEGRADING STRAIN, *RHODOCOCCUS ERYTHROPOLIS* PR4

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R. erythropolis PR4, a hydrocarboclastic marine bacterium is able to utilize various hydrocarbons. The complete DNA content of the strain had been sequenced. Apart from the 6.5 Mbp chromosome, two large plasmids provide genes encoding enzyme sets involved in oil degradation and other versatile metabolic pathways. Four alkane-1-monooxygenases and 14 cytochrome P450 genes could be identified in the genome. The large linear plasmid contains two additional cytochrome P450 genes. These genes potentially catalyze the oxidation of hydrocarbons, however, their exact role, their substrate specificity should be still clarified. In order to identify specific elements involved in biodegradation of various hydrocarbons, we performed a comparative whole transcriptome analysis of cells grown in the presence of hexadecane, diesel oil and acetate. The cultures were grown in 5L bioreactors and all growth parameters were controlled. Samples for whole transcriptome analysis were taken in late exponential phase. The mRNA profiles of the cultures were determined by RNA-Seq using a SOLiD^{TM} Next Generation Sequencing system. The expression of two genes coding for alkane-1-monooxygenase enzymes was highly up-regulated in the presence of both hydrocarbon substrates. The transcription 8 out of 16 cytochrome P450 genes was apparently induced in the presence of diesel oil, while the presence of hexadecane resulted in higher expression of only one

cyp gene. Three multicopper oxidase genes were also discovered, among these, one was up-regulated in samples cultivated on either hexadecane or diesel oil. Moreover, it had much higher expression level in diesel oil grown samples as compared to those propagated on hexadecane. The expression of fatty acid synthase I genes were down-regulated by both types of hydrophobic substrates indicating the utilization of the external alkanes in fatty acid synthesis. We also detected an induction of a gene cluster responsible for exopolysacchride biosynthesis in the presence of hexadecane and diesel oil. Exopolysaccharides can play dual role in hydrocarbon assimilation; they can serve as bioemulsifiers and protect the cells from harmful effect of toxic compounds. Moreover, transcription of a large gene cluster involved in siderophore biosynthesis and iron transport alongside other iron transport genes were also up-regulated indicating their important role in hydrocarbon assimilation processes.

TRANSCRIPTIONAL PROFILING OF *CANDIDA ALBICANS* UPON INTERACTION WITH VAGINAL EPITHELIAL CELLS REVEALS SPECIFIC INDUCTION OF THE N-ACETYL-D-GLUCOSAMINE AND OXIDATIVE STRESS PATHWAYS

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Candida albicans is dimorphic yeast that is a ubiquitous member of the human flora capable of causing severe symptoms. One of the most prevalent diseases caused by *C. albicans* is vulvovaginal candidiasis (VVC): a great number of women suffer recurrent VVC highlighting the importance of this pathological condition. To better understand the molecular events underlying vulvovaginal candidiasis, we established an *in vitro* system in which immortalized vaginal epithelial cells are infected with live, yeast form *C. albicans*. As control, a serum-free, low glucose cell culture medium was used, which robustly induced yeast to hyphae transition. Whole transcriptome sequencing approach was used to identify major and significant gene expression changes upon yeast to hyphae transition. We found that signal transduction pathways leading to yeast to hyphae transition and hyphae specific genes are upregulated in both the control hyphae and the hyphae in response to vaginal epithelial cells. However, the response of *C. albicans* to oxidative and nitrosative stresses induced mainly in response to epithelial cells. Moreover, the GlcNAc pathway of *C. albicans* was exclusively triggered by vaginal epithelial cell. Under our conditions, we were able to dissect the main responses of *C. albicans* to vaginal epithelial cells. Our results suggest that the microenvironment determines the morphological transition, since starvation to glucose is solely able to induce yeast to hyphae transition. In contrast, the defense system of *C. albicans* is activated in the presence of epithelial cells, while the induction of the GlcNAc responsive genes in *C. albicans* indicates the importance of this pathway in virulence in vulvovaginal candidiasis.

CHLAMYDOPHILA PNEUMONIAE INFECTION ACCELERATES ATHEROSCLEROSIS DEVELOPMENT IN "APOB100ONLY/LDLR-/-" AND APOE-/- MOUSE STRAINS IN DIFFERENT MANNER

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Atherosclerosis (AT) is a leading cause of death worldwide. Several studies have implicated *Chlamydophila pneumoniae* (Cpn) in atherogenesis. Cpn is a common respiratory pathogen of humans. It is able to enter the circulation and reach the arteries. The contribution of Cpn to AT is not completely understood. Among animal models of AT, the most common mouse models are ApoE-/- (Strain1) mice. ApoB100 only/LDLR-/- (Strain2) mice with serum lipid composition similar to that in hypercholesterolemic humans has not been investigated in respect of Cpn and AT relation. Our aim was to test and compare AT development associated with Cpn infection in these two mouse models. Mice were infected with Cpn three times by intranasal route and kept on normal diet for 12 weeks. Lungs were tested for viable Cpn. Sera were assayed by ELISA for Cpn-specific antibodies. Serum samples were subjected to a cytokine array detecting 22 AT-related cytokines, chemokines and cell adhesion molecules. AT development was evaluated with histological examination of aorta sinus and with in situ microscopic observation of descending aorta. Cpn cultured from the lungs after first infection and Cpn-specific antibodies in the sera of mice confirmed successful infection. Two months after the third infection no viable Cpn was detected in the lungs. Hematoxylin and eosin stained cross-sections of aortic sinus showed early-stage plaques in Strain 2, but in Strain1 advanced plaques were seen. In response to repeated Cpn infection significant increase was detected in the size of plaques in both strains. This accelerated AT development was also visible in the descending aorta. Cytokine array was performed with serum samples one week after first and after third infection. Different pattern of serum content of bFGF, CD40, GCSF, IL-1a, IL-1b, IL-2, IL-6, IL-13, MCP-1, MIP-3a, P-selectin, RANTES and VEGF was detected in the two mouse strains. During the experiment certain cytokines showed significant increase in the serum level irrespective of infection, the amount of others increased in both mouse strains upon infection. After the first or third infection the cytokine composition differed in the two mouse strains. In conclusion, we disclosed the infection-induced augmentation of AT development in Strain2. Differences in lipid metabolism and marked atherogenesis in Strain1 mice might serve as a basis for distinct mechanisms in the two mouse strains.

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LONG CONTROL REGION (LCR) CPG METHYLATION IN HUMAN PAPILLOMAVIRUS (HPV) TYPE 31 INFECTIONS

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Persistent infections with high risk genital human papillomaviruses (HPVs) are the most common cause of cervical cancers. Replication and transcription of the virus is modulated by a large number of regulatory elements in the viral genome, especially in the long control region. Numerous studies have demonstrated that epigenetic regulation of viral gene expression is an important factor in HPV-associated carcinogenesis and the methylation status of the viral genome changes during the viral life cycle and disease progression. Most of the studies have focused on evaluating the methylation pattern of various regions of the most carcinogenic HPV16 genome, while other important high risk types, including HPV31 are underrepresented in the literature. Therefore, we studied the LCR methylation of the fourth most important carcinogenic type HPV31. DNA was extracted from

cervical cytology samples from women infected with HPV31. After bisulfite modification, segments of full-length LCR were amplified by using primers constructed for modified sequence. The measure of CpG methylation of viral LCR was determined by next generation sequencing. We examined all of the 16 potential methylation sites of HPV31 LCR. Our results show, that the level of CpG methylation in LCR was similarly low (<20%) and homogeneous in all of the samples indicating, that genomic region involved in regulation of HPV oncogene expression is largely unmethylated.
This study was supported by a grant from the Hungarian Scientific Research Fund (OTKA K 81422).

EFFECTS OF EXTERNAL PH AND BICARBONATE ON THE BACTERIAL GROWTH AND MIC OF ERYTHROMYCIN

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In cystic fibrosis (CF), defective transepithelial anion transport impairs mucociliary clearance, leading to retention of thick and aggregated mucus in the airways. The acidic pH of airway surface liquid reduces bacterial killing. Aerosolizing bicarbonate in the airways of CF pigs increased bacterial killing *in vivo*. Thus, our aim was to investigate the effects of external pH and bicarbonate on the growth of both Gram-positive and Gram-negative bacteria, as well as on the efficacy of erythromycin against *S. aureus*. The growth of different Gram-positive (*S. aureus, S. agalactiae, E. faecalis*) and Gram-negative (*P. aeruginosa, E. coli, H. influenzae*) bacteria was compared in the presence and absence of 100 mM $NaHCO_3$. Bacterial suspensions were incubated in 96-well plates, and the growth capability of the bacteria was followed by the measurement of the optical density (OD) at 595 nm over a 330-minute period. As an osmolality control, 100 mM NaCl was used. The pH of the bicarbonate solutions was set by equilibration in different CO_2 concentrations, calculated by the Henderson-Hasselbalch equation. The erythromycin MICs of 19 *S. aureus* strains were determined by agar dilution in the presence and absence of $NaHCO_3$, in ambient air versus 5%/20% CO_2, as well as at pH 7.1, 7.5, 8.0, 8.5 and 9.0. Our results show that $NaHCO_3$ (100 mM) significantly inhibited bacterial growth in all strains, whereas neither NaCl (100 mM) or pH ≤8,5 did not influence growth. After incubating the bacteria in a watery solution containing 100 mM $NaHCO_3$ for 0-6 hours, they all retained their full growing capacity (subcultured onto blood agar), suggesting that bicarbonate was bacteriostatic rather than bactericidal. On the other hand, bicarbonate itself did not change erythromycin MICs when both external pH and CO_2 concentrations were kept at constant values, whereas alkalinizing external pH reduced the MIC values of erythromycin significantly. Our data suggest that bacterial growth was inhibited by HCO_3^- rather than by bicarbonate-induced alkaline pH or hyperosmolarity.
On the other hand, increased efficacy of erythromycin was enhanced by alkaline pH, but not by bicarbonate *per se*. We propose that administration of bicarbonate containing aerosols might inhibit bacterial growth and could improve the efficacy of antibiotic treatment in patients suffering from CF or other chronic respiratory infections.
This project was supported by the Hungarian Scientific Research Fund (OTKA K-108631).

A MATHEMATICAL MODEL FOR THE G1 AND G2 SIZE CONTROL MECHANISMS VIA INHIBITOR DILUTION IN FISSION YEAST

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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. 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. In wild-type fission yeast cells, G2 phase is long and size-dependent, meanwhile G1 is short and constant, therefore, size control seems to operate in G2. 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. Recent experimental data seems to give a new clue how this size control mechanism might operate. Namely, a spatial gradient of a mitotic inhibitor (pom1, indirectly acting positively 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. In *wee1* mutants, the G2 size control is abolished. By contrast, these cells are viable (although small, hence the Scottish name wee) and their population proliferate with a generation time similar to that of wild-type. However, in a typical *wee1* mutant cell of a steady-state exponential phase culture, G2 is much reduced and G1 is much extended, indicating that size control acts in G1 rather than in G2. There is unfortunately no known experimental data suggesting any idea on how this G1 size control might operate. We hypothesize that an inhibitor of the G1/S transition might be diluted in the cell centre as a consequence of the increase in cell size; i.e., spatial gradients may cause both size controls in the cylindrical cells of fission yeast. In the case of the G1 mechanism, our candidate to have this role is the ste9 protein, which is responsible for ubiquitination of cyclins in G1, thereby keeping cyclin-dependent kinase activity nearly at 0.

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STUDY OF THE EFFECT OF ARBUSCULAR MYCORRHIZA (AMF) AND DARK SEPTATE ENDOPHYTES (DSE) ON VEGETABLE CROPS

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Majority of terrestrial plants establish a mutualistic symbiosis with root-colonizing fungi, like AM or DSE. In AM fungi improve the nutrient and water uptake capacity of the plants and increase drought-tolerance. Majority of vegetable crops, e.g. pepper, tomato establish AM. Dark septate endophytes (DSE) represent a form-group of fungi colonizing plant roots. These endophytes are

common and frequent members of root associated fungal communities of environments with strong abiotic stress. DSE–plant interactions are not as well understood as the mycorrhizal interaction, however there are several results showing the positive effect of DSE on host plants. This potential could have especial importance when non-mycorrhizal vegetable crops (e.g. member of the family Brassicaceae) are inoculated. Our aim is to study how AMF and DSE inoculation could affect, beside other parameters, the water demand and how this might effect the irrigation of the crops. We aimed to establish efficient inoculums and to test different inoculation technologies to find the most effective way to involve those fungi to farming.
The main aim of the study presented here was to study the influence of DSE, AMF and simultaneous DSE-AMF inoculation on different vegetables under reduced irrigation.
The work is supported by The Hungarian Research Institute of Organic Agriculture (ÖMKI).

STUDY OF THE ROOT AND SOIL COMMUNITY OF ARBUSCULAR MYCORRHIZAL FUNGI IN FOUR DIFFERENT CROP ROTATIONS

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In the last decades the management of arbuscular mycorrhizal fungi (AMF) has drawn increasing attention as biological alternative to chemicals. For a profitable capitalization of these symbiota, a better understanding of which mechanisms could affect the dynamics inside an AMF community is required. In the present study we evaluated the influence of different crop rotations on the AMF community, after a 50 y long-term field experiment established at Martonvásár, Hungary. Four types of crop rotation were chosen for sampling: corn monocropping as control, corn-alfalfa, corn-wheat, corn-spring barley-peas-wheat. Community composition of AMF in the root and in the soil compartment was analyzed with a molecular approach amplifying a portion of 28S rDNA. In the soil AMF community we found a similar number of phylotypes among the different rotations (13-14). In the root compartment the highest number of phylotypes was detected in the corn monoculture (14) and the lowest in the four-crop rotation (9) where generalist taxa resulted to be the dominant. Comparing the different crop rotation types we highlighted differences in the composition and structure of the AMF assemblages showing that the nature of crop sequence has, in the long term, an important role in modeling the resident AMF community.

INVESTIGATION OF BACTERIAL AMYLOID STRUCTURES BY FLUORESCENT TOPO-OPTICAL TECHNIQUES AND CONFOCAL LASER SCANNING MICROSCOPY

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Bacterial amyloid was first detected on *Escherichia coli* strains of bovine origin, and named curli fimbria upon its morphology. Soon after we identified similar fimbriae on human isolates of *E. coli* and *S. enteritidis*, and proposed the name “GVVPQ fimbria” referring to their N-terminal amino

acid sequences. Curli producing strains bind to epithelial cells and several types of matrix proteins, hence a function in host tissue colonisation was proposed. Strains producing this type of fimbria vigorously bind Congo red, and produce colonies with intensive red colour on plates supplemented with this dye. Later on this structure has been identified as bacterial amyloid, demonstrated on the representatives of several further bacterial taxa, and analysed by fluorescence microscopy. Polarisation microscopy with its capacity to display topo-optical reactions is suitable for the investigation of molecular furnishment of biological structures like membranes, polysaccharides, amyloid deposits, and various surface structures of bacteria and fungi. We utilized these methods and confocal laser scanning microscopy in the structural analysis of bacterial amyloid. Unstained bacterial amyloid presented with linear positive birefringence to the fibrillum length when taken up in distilled water or gum arabicum. Imbibition analysis pointed to identical phenomena observed for human amyloid: isotropy in aniline but anisotropy in terpineol, glycerin, butanol, gum arabicum or distilled water. The fibrillar structure stains intensively with Congo red, thiazine red, N,N'-diethyl-pseudoisocyanine chloride and pinacyanol. The sign of birefringence is linear positive indicating a parallel orientation of the bound dye molecules to the fibrillar length. On the contrary rivanol and toluidine blue dye molecules bind perpendicularly to the fibrillar surface inducing a linear negative birefringence. The former type is called additive reaction while the latter one is named inversive type reaction. By laser fluorescent scanning microscopy bacterial amyloid aggregates presented with a robust anisotropy in a manner similar to human amyloid structures.
We have demonstrated the three dimensional structure with Congo red and thiazine red staining. This makes the method promising to investigate bacterial amyloid production during the process of *in vitro* phagocytosis, and also for histopathological evaluation of animal infection assays. We plan to extend our investigations to these directions.

TRICHORZIANINS AND RELATED COMPOUNDS IN *TRICHODERMA PLEUROTICOLA* CULTURES

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Trichorzianins are 19 residue peptides with special components, like α-aminoisobutyric acid (Aib) and isovaline (Iva), an N-terminus protected by an acetyl group and a C-terminal amino alcohol. They are belonging to the group of peptaibols like alamethicin (ALM), the firstly described and the most characterized peptaibol in the literature. The peptaibols are belonging to the family of fungal peptide antibiotics, and they are produced mostly by *Trichoderma* species and some other, related microorganisms. Trichorzianins have characteristic endings after the Aib-Pro bounds with only two variable regions, which can be Val/Iva or Leu/Ile in the positions 14 and 16, and tryptophanol (Trpol) for phenylalaninol (Pheol) replacements at the C-terminus end. In our present work, the mass spectrometric analysis of unknown peptaibols produced by *T. pleuroticola* strain SZMC 12727 have been investigated by on-line reversed-phase high performance liquid chromatography (HPLC) coupled to electrospray ionization ion trap mass spectrometry (ESI-IT-MS) after the solid phase clean-up of culture extracts. For the proper analysis, the parameters of the MS were optimized using an ALM standard solution directly into the ESI source. Then, the component peaks of ALM F50 containing the standard material were separated from each other, which were identified based on their mass spectra. Applying the developed chromatographic separation, the unknown components produced by *T. pleuroticola* were investigated and determined. The produced peptaibols showed the

characteristic mass spectral components of trichorzianins including trichorzianin TA IIIb, trichorzianin TB IVb, trichorzianin TAP-14a and trichorzianin TA VII. Furthermore, seven novel trichorzianin-related compounds were also detected, containing some amino acid changes in the base trichorzianin sequence. Comparing the amount of trichorzianin components, one of the unknown compounds with a molecular mass of 1909 amu was in the highest amount, while the four known trichorzianins and the other six unknown components were present at lower levels.

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ALAMETHICIN F50 IN FERMENT BROTHS OF DIFFERENT *TRICHODERMA* CULTURES

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Alamethicin (ALM) is a type of peptaibols belonging to the family of fungal peptide antibiotics. Peptaibols and related peptaibiotics are produced mostly by *Trichoderma* species and some other, related microorganisms. They regularly comprise 18-20 amino acid residues and are synthesized through non-ribosomal peptide biosynthesis. Alamethicin (ALM) is the firstly described and the most characterized peptaibol in the literature. It was reported to be produced by *Trichoderma viride*, however, the original producer strain was later reidentified as *T. arundinaceum*. Based on their retention factor values in thin layer chromatography (TLC), ALM is composed of two major classes, an acidic and a neutral group distinguished by Glu/Gln18 exchange and named ALM F30 (85%) and ALM F50 (12%), respectively, and an additional minor component (ALM F20). In our present work, the mass spectrometric analysis of ALMs produced by *Trichoderma* strains has been performed by on-line reversed-phase high performance liquid chromatography (HPLC) coupled to electrospray ionization ion trap mass spectrometry (ES-IT-MS) after the solid phase clean-up of culture extracts. Initially, 30 *Trichoderma* strains were tested with our previously developed rapid bioactivity-based pre-screening method to form inhibition zones on properly prepared *Micrococcus luteus* cultures. Inhibition zones were detected in the case of seven isolates including *T. virens, T. atroviride, T. pleuroticola, T. longibrachiatum, T. citrinoviride* and two *T. harzianum* strains, which were selected for further analytical examinations. For the proper analysis, the parameters of the MS were optimized by the continuous infusion of an ALM standard solution directly into the ESI source. Then, the component peaks of ALM F50-5, F50-6a, F50-7 and F50-8b containing the standard material were separated from each other, which were identified based on their mass spectra. Using the developed chromatographic method, the ALM components produced by the examined *Trichoderma* strains were investigated and determined. Some other possible peptaibol peaks were also found, however, the present study focused specifically on the peaks of ALM F50 components.

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TEMPERATURE-DEPENDENT TRANSFORMATION OF BIOGAS-PRODUCING MICROBIAL COMMUNITIES

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Biogas generation is based on the decomposition of organic materials carried out by complex microbial communities under anaerobic conditions. Stability of biogas production is highly dependent on the microbial communities of the bioreactors. The composition of these microbial consortia is basically determined by the nature of biomass substrate and the physico-chemical parameters of the anaerobic fermentation. Operational temperature is a major factor in the determination of the anaerobic degradation process; higher temperature has advantageous effects on the operation by shortening the hydraulic retention time, eliminating pathogens from the system and in certain cases decreasing the reactor cooling costs. Sequencing-based metagenomic approach was used to monitor the microbial community throughout an experiment where stable mesophilic fermentors (37 °C) were gradually switched to thermophilic (55 °C) using a temperature elevation rate of 0.9 °C / day^{+}. A temporary destabilization of the system was observed in response to changes in the operation temperature, indicating a lag phase in the community evolution. Characteristic changes in the fermentation ecosystem were observed, temperature adaptation resulted in a clearly thermophilic community having a generally decreased complexity compared to the mesophilic system. The changes of the microbial consortia strongly correlated with the operational temperature as well as with the level of volatile fatty acids (VFA) and biogas production. Our observations demonstrated that aceticlastic methanogens were the major methanogen participants in a mesophilic fermentation ecosystem represented by the dominance of *Methanosaeta* genus, while under thermophilic conditions the aceticlastic methanogens were replaced by the hydrogenotrophic *Methanothermobacter* and *Methanoculleus* genera.

We demonstrated that mesophilic to thermophilic transition promoted characteristic evolution of the microbial community in the bioreactor. Our findings point to the flexibility and versatility of the microbial consortia residing in biogas bioreactors and to the importance of appropriate operation conditions in order to achieve optimal performance of the anaerobic fermentation system.

CIRCOVIRUS-LIKE SEQUENCES DETECTED IN ANIMALS AND PROTOZOA

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Circoviruses and circovirus-like viruses with circular ssDNA genome seem widely distributed in animals and environmental samples. Integrated sequences similar to the replication initiator protein (Rep) encoding gene of these viruses were also described in vertebrates and invertebrates. In our studies rep-like sequences were detected by PCR in fish, reptiles, honey bees and the free-living *Platyamoeba* and *Acanthamoeba* species. Rep-like sequences of reptiles and amoebae were nearly identical to sequences detected in chimpanzee stool raising the possibility that the integrated sequences originated from intestinal unicellular organisms in these animals. Sixteen percent of 186 honey bees and six *Varroa* mite samples tested positive for circovirus sequences. Although most of the partial genomes were identical with circoviral rep-genes, our experiments proposed those to be integrated elements, conceivably deriving from *Varroa* mite contamination.

Circovirus-like sequences were detected by metagenomics in our laboratory in fecal samples of rabbits and pigs. The presence of two or more circovirus-like viruses with a genome length of 1-4 kb

could be supposed in these specimens based on the results of metagenomics and PCR followed by Sanger sequencing. The sequences showed various extent of similarities with viruses detected in turkey, fur seal and porcine fecal samples. Further investigations are needed to determine the viral genomes and the potential origin of these newly discovered viruses.

EMERGENCE OF OXA-48-LIKE CARBAPENEMASE PRODUCING *KLEBSIELLA PNEUMONIAE* IN PÉCS

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In May 2014 a carbapenemase producing *K. pneumoniae* was isolated from the blood culture of a patient treated at one of the Intensive Care Units of the Clinical Centre University of Pécs. For this isolate the minimum inhibitory concentration (MIC) value of meropenem was particularly high (32µg/ml) as compared to the endemic VIM-4 carbapenemase producing *K. pneumoniae* isolates. Besides resistance to imipenem was relatively low (MIC=4µg/ml). Concerning the unconventional results of susceptibilty testing, further investigations were performed. Those *K. pneumoniae* isolates of 19 patients that were suspected to produce some sort of carbapenemase and originated from the time periods before and after the aforementioned event were tested for the presence of *blaVIM, blaIMP, blaOXA-48-like, blaNDM* and *blaKPC* genes with PCR. In the case of 15 patients the isolates were proved to harbour the *blaVIM* gene, while in the case of 4 patients the presence of *blaOXA-48*-like gene was shown. This is the first case that a carbapenemase belonging to OXA-48 group was detected in the Clinical Centre University of Pécs where VIM type carbapenemases are considered to be endemic. Behind this event possible importation from Ukraine could be suspected, as the first patient was an Ukrainian citizen who was transferred from a hopsital in Kharkiv to the Clinical Centre University of Pécs for further treatment. Due to transfer of patients the newly emerged OXA-48-like carbapenemase producing *K. pneumoniae* spread to the Szent György Teaching Hospital, the Hospital of Szigetvár and to the Kaposi Mór Teaching Hospital as well.

SPECIAL DISTRIBUTION OF PHOTOSYNTHETIC MICROORGANISMS IN EXTREME AQUATIC HABITATS OF THE CARPATHIAN BASIN

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In the Carpathian Basin, various types of saline lakes could be found. The shallow turbid soda pans located in the Danube-Tisza Interfluve are unique in Europe, and could be distinguished from salt lakes based on their chemical composition. Transylvanian salt lakes are deeper, which allows the development of diverse and unique microbial communities, usually with special vertical

distribution. In contrast to the extreme conditions present in these aquatic habitats, they could be characterized with unusual photoautotrophic picoplankton (PPP, <2μm) richness. This study aimed to reveal the taxonomic composition of the planktonic eukaryotic algal community in a soda pan in the Danube-Tisza Interfluve (Zab-szék), and to characterize the picocyanobacterial community of saline lakes in the Transylvanian Basin (Lake Tarzan, Lake Cabdic and Lake Ursu). The mass production of picoeukaryotic algae observed in the soda pan was among the highest abundance values published, and microscopic examination of water samples taken from the three salt lakes suggested that the importance of picocyanobacteria in these was significant. PCR-based molecular biological techniques (cloning and sequencing of various regions of the ribosomal operon) were applied to identify PPP taxa.

It was revealed that potentially new green algal genera (Trebouxiophyceae, Chlorophyta) have caused the extraordinary bloom in the soda lake, while in the Transylvanian saline lakes, PPP community was dominated by picocyanobacterial phylotypes (*Synechococcus*, Cyanobacteria) characteristic to oceans and seas, which was also a unique observation.

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INVESTIGATION OF THE TRANSLATION OF AN ALTERNATIVE ORF IN GOOSE PARVOVIRUS

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Our computer analyses have revealed a long genus-specific ORF (LGORF) in all members of *Dependovirus* genus, which overlap with the ORF of VP proteins. The LGORF is located in position 3016-3618 of the goose parvovirus (GPV) genome. The probability that the LGORF has formed randomly, without function during the evolutionary process in all members of *Dependovirus* genus is unlikely. The simplest explanation of their existence is that they encode a protein. To prove that protein is expressed from the alternative ORF first he LGORF of GPV was cloned into 6His containing pET28b(+) expression vector, then the LGORF protein (LGORFP) was expressed in *E.coli*. Chickens were immunized with the purified protein and their sera were used in IF experiments to detect LGORFP in infected cells. The results showed that LGORFP is indeed expressed and accumulates in the nuclei of GPV infected cells. These results were further supported by the analysis of LGORFP sequence of the several dependoviruses by sub-cellular localisation (ESLpred) and nuclear export signal (NetNES 1.1) prediction programs. The *in vivo* expression of the LGORF was confirmed by western blot experiments. Anti-LGORFP antibodies were detected from the sera of GPV infected geese by using the purified protein. In order to identify the mRNA from which LGORFP is translated, the mRNA of the VP1 and VP2/3 proteins of GPV were cloned into pcDNA3 eukaryotic expression vectors. The constructs were transformed into AGE1.CRpIX cells and total proteins of the transfected cells were analyzed by western blot in order to probe the sera of LGORFP immunized chicken. The experiment proved that LGORFP is mainly expressed from the VP2/3 mRNA. The mechanism of translation is presently under investigation.

CANINE FECAL VIROME REVEALS A CANDIDATE NOVEL ROTAVIRUS SPECIES

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Rotaviruses (RVs) are classified within the genus *Rotavirus*, family Reoviridae. The 9th report of ICTV has listed five virus species (designated *Rotavirus* A to E), but with the availability of new sequence information, an additional three species, *Rotavirus* G to H, have been proposed. Species demarcation criteria include serological cross-reactivity, sequence similarity values and host range. In this study we report a diverse rotavirus strain identified by deep sequencing of the fecal virome of a suckling dog. Sequence and phylogenetic analysis of the genes encoding structural proteins revealed a low genetic relatedness between the strain 135/2012 and those being representatives of their respective virus species. The greatest aa similarities were seen when compared with the type strains of candidate species *Rotavirus* H. The VP1, VP2, VP3, VP4, VP6 and VP7 proteins shared 57%, 44%, 41%, 40%, 46%, and 28% respectively, although the length of the VP4 protein aligning between the two candidate species was relatively short (50%). The 46% aa identity within the VP6 gene was comparable to similarity values obtained for other species (range, RVA, 27%; RVB, 38%) and was below the 53% aa cutoff value proposed recently for differentiation of RV species. Analysis of the structural genes strongly suggests that the identified RV strain may be the representative of a novel RV species. The availability of full length structural genes will permit further investigations to uncover the host range and any potential public health implications of this unusual rotavirus.

DETECTION AND MOLECULAR CHARACTERIZATION OF CANINE ASTROVIRUSES IN HUNGARY

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High-throughput sequencing has become the basic tool for virus discovery. Recently, a number of new viruses have been identified by the approach of viral metagenomics in the fecal specimens of domesticated animals, including dogs. Dogs are among the most popular companion animals in Hungary and in other countries all around the world. These animals are in close contact with humans, other pets and wild-living animals. Viral infections of dogs manifest frequently as gastrointestinal symptoms that could also affect other susceptible organisms. In this study feces were collected from either healthy or diarrheic sheltered dogs and subjected to viral metagenomics using the Ion Torrent PGM platform. The coding sequences of five canine astrovirus strains were determined. The five, near-full length Hungarian astrovirus sequences shared 96-74% nt identity with each other and 80-97% nt identity with the overlapping regions of the reference canine astrovirus sequences deposited in the GenBank.
The Hungarian canine astrovirus strains were divided into four lineages. Differences between our and the reference sequences suggest a great diversity among canine astroviruses.

EXPRESSION ANALYSIS OF GENES ENCODING AN ALTERNATIVE TERMINAL OXIDASE IN TWO ASCOMYCETE FILAMENTOUS FUNGI EXPOSED TO STRESS

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Filamentous fungi have long been used as industrial producers of secondary metabolites and enzymes. In fungi, the final steps of the catabolism of glycolytic carbon sources take place in mitochondria and lead to the synthesis of ATP. Fungal mitochondria contain two respiratory electron transport chains. One is the cytochrome-dependent pathway that includes cytochrome oxidase as the terminal oxidase. The other is the alternative pathway that uses alternative oxidase (AOX, a quinol oxidase localized in the inner mitochondrial membrane) as its terminal oxidase. Alternative electron transport bypasses Complex III and IV, involved in proton gradient formation, resulting in less ATP production. AOX plays an important role in the fungal stress defense- and adaption mechanisms. The regulation of AOX-encoding genes is key to the fungal response to specific cultivation/environmental conditions. Here, the expression profiles of alternative oxidase-orthologs in the model *Aspergillus nidulans*, a soil-borne saprophyte, and the plant pathogen *Botrytis cinerea* were studied by Nothern blot analysis in presence of different stress factors such as high D-glucose, D-sorbitol or KCl (osmotic stress) or H_2O_2 (oxidative stress). Fungi counteract extracellular osmotic pressure by producing intracellular polyols to prevent loss of water. In *A. nidulans*, AOX expression was induced in the presence of D-sorbitol but did not respond to high KCl concentrations in the medium. H_2O_2 did not induce AOX gene expression in *A. nidulans*. Increasing concentrations of D-glucose in the growth medium correlated with gradually increasing AOX expression in *B. cinerea*. In *A. nidulans*, however, the expression level remained low and constitutive. Transcription of the alternative oxidase-encoding gene in *A. nidulans* thus appears less thoroughly regulated by stress factors than that of its homolog in *B. cinerea*, which may be due to differences in in situ exposure to stress factors of the two organisms, caused by a consequence of the different lifestyles.

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PROTECTION PROMOTED BY PGP3 OR PGP4 AGAINST *CHLAMYDIA MURIDARUM* IS MEDIATED BY CD4+ CELLS IN C57BL/6N MICE

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Urogenital tract infection with *Chlamydia trachomatis* is a leading cause of sexually transmitted infections. There is currently no commercially available vaccine against *C. trachomatis*. The highly conserved plasmid of chlamydiae has been considered to be a virulence factor and the plasmid proteins have important roles in the *Chlamydia*-specific immune response. This study was designed to evaluate the efficacy of vaccination with plasmid proteins in the prevention of *Chlamydia*

muridarum lung infection in a mouse model. C57BL/6N mice were immunised 3 times subcutaneously with recombinant pGP3 or pGP4 and infected with *C. muridarum*. The titre of recoverable *C. muridarum* was detected with indirect immunofluorescence test. To evaluate the inflammation, cytokines were measured in the lungs of mice by ELISA. Spleen cells depleted of CD4+ or CD8+ cells were administered to naive mice. After *C. muridarum* infection, the bacterial burden was determined. Naive mice were infected with *C. muridarum* treated *in vitro* with sera obtained from immunised mice or were treated *in vivo* with the sera of immunised mice before and after the challenge. Immunisation of the mice with recombinant pGP3 or pGP4 protein caused a significantly lower chlamydial burden in the lungs of the infected mice; the lower IFN-γ level indicated a reduced extent of inflammation. *In vitro* or *in vivo* neutralisation of *C. muridarum* with sera obtained from immunised mice did not reduce the number of viable *C. muridarum* in the lungs of mice. Adoptive transfer of the CD4+ spleen cells isolated from the immunised mice resulted in a significantly reduced bacterial burden.

Our results indicate that it is not the pGP3- and pGP4-specific antibodies, but the CD4+ cells that are responsible for the protective effect of the immune response to plasmid proteins.

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CORRELATION OF CARBAPENEM CONSUMPTION AND PREVALENCE OF *ACINETOBACTER BAUMANNII* CLONES IN THE UNIVERSITY OF DEBRECEN

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The aim of this study was to examine the correlation between prevalence of *A. baumannii* and antibiotic usage from 2002 to 2011. Overall and ward-specific antibiotic consumption was measured by the number of defined daily doses/100 bed-days, *Acinetobacter* prevalence was characterised by the proportion of positive samples of inpatients with *A. baumannii*. Relationship between prevalence and consumption was analysed using time series analysis. Altogether 160 *A. baumannii* isolates from different wards were collected and analysed. Carbapenemase genes blaOXA-23-like, blaOXA-24-like, blaOXA-48-like, blaOXA-51-like, blaOXA-58-like and integrons were sought for using PCR. Relatedness of isolates was assessed by pulsed field gel electrophoresis. Based on the time-series analysis, increasing carbapenem consumption led to increase in prevalence of *Acinetobacter baumannii* with a delay of three quaterly lags (nine months; $r=0.53$, $p<0.001$). Carbapenem resistance of *Acinetobacter baumannii* showed a similar correlation with carbapenem usage also with a delay of nine months ($r=0.43$, $p=0.005$ at the -3 quaterly lag). PFGE distinguished six clusters (A1, A2, B, C1, C2, D), a pair of isolates with identical patterns and two isolates with unique profiles. All clusters except the integron negative cluster A2 had characteristic integrons present in all isolates of the cluster; In426 harbouring gene cassettes aac(6')-Ib gene, a hypothetical protein and a blaOXA-20 in cluster A1, In561 containing aac(3)-Ia, two hypothetical proteins and ant(3")-Ia in clusters B, C1 and C2, In439 with gene cassette array aac(6)-Ib, catB8, and ant(3")-Ia. All isolates carried the chromosomal weak carbapenemase gene blaOXA-51 and the insertion sequence ISAba-1, however, carbapenem resistance was linked to carriage of blaOXA-23, present in all isolates in clusters A2, B, C1 and D as well as in 75.9% of isolates from cluster C2.

Carbapenem susceptible isolates in cluster C2 and the majority of isolates in the carbapenem susceptible cluster A1 originated from the Neurology Department, where the carbapenem consumption was significantly lower than in other departments characterized by different

carbapenem resistant clones. Based on these results, carbapenem usage is directly linked to spread of carbapenem resistance *A. baumannii* clones.

METABOLIC AND GENETIC DIVERSITY OF SOIL BACTERIAL COMMUNITIES INHABITING A DOLINE LOCATED IN THE AGGTELEK KARST, HUNGARY

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Soils play important roles in the geological and ecological processes of karst areas, nevertheless little information is available about the microbiological relations of karst soils. The aim of the present study was to reveal the metabolic and genetic diversity of soil microbial communities of a doline located near the Vörös-tó at the Aggtelek karst, Hungary. Samples were taken in June 2013 and April 2014 from five different locations with various exposures and vegetation types. The community level physiological profile was studied by the MicroResp™ method based on the substrate induced respiration. Altogether 15 substrates containing sugars, amino acids, carboxylic acids and a phenolic acid were used. The results of the cluster analyses showed that the samples grouped mainly according to the vegetation type but the parallel samples often mixed with each other. The genetic diversity of soil bacterial communities was compared on the basis of their 16S rDNA DGGE-patterns. The clustering of the samples showed a strong correlation with the pattern of vegetation types in the doline. The samples from the southern slope, covered by forest, could be clearly distinguished from that of the northern slope, which has a forest gradually interspersed with grassland, resulting in a forest steppe on the northern top of the doline. Due to the great number of the bands in the gel only a few taxa belonging into Acidobacteria, Alphaproteobacteria, Betaproteobacteria, Cyanobacteria, Firmicutes, and Verrucomicrobia were sequenced and identified.
This research was supported by the Hungarian Scientific Research Fund (OTKA T79135 and K108572).

ANTIBACTERIAL EFFECT OF *ALLIUM URSINUM* LEAF EXTRACTS BEFORE AND AFTER FLOWERING

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Allium ursinum (ramson or wild garlic) is a perennial herbaceous plant, growing wild both in Europe and Asia. It has been used for centuries in traditional medicine to prevent cardiovascular diseases, to detoxify the body, and to stimulate digestion. Nowadays, there is a growing interest to use this plant in foods for its mild garlic-like taste and it is also used as a dietary supplement for detoxifying. In our work, the antibacterial properties of extracts made from ramson leaves against some food spoilage bacteria and pathogens were investigated. Leaves of *A. ursinum* were purchased at a local market in Szeged in April and in early June (before and after flowering). Fresh leaves were crushed and macerated in ethanol : water (30:70) solution for 24 h. Thereafter, the extract was filtered and the liquid was evaporated at 50 °C. The solid residue was dissolved in 30% ethanol and the concentration was adjusted to 50 mg/ml. The solutions were sterile filtered and the concentration was determined again. Antibacterial effect of the sterile-filtered extracts was observed

in microtiter plates containing dilutions up to 1/32 of the stock solution. The wells were inoculated with a bacterial suspension of 10^5 CFU/ml and absorbance was measured after 24 h cultivation. MIC values were determined as the concentration where the absorbance of the treated culture was ≤10% of the non-treated culture. To determine MBC values track plate method was used. MBC was defined as the concentration where no colony growth was observed. Extracts obtained from plants before and after blooming showed different activities: in most cases MIC values were lower after flowering. The most sensitive strain was *E. coli* with bactericidal MIC values of 20 and 10.8 mg/ml before and after flowering. *Bacillus subtilis, B. cereus* and *Pseudomonas putida* showed moderate sensitivity to the extracts while *Serratia marcescens* and MRSA proved to be insensitive to *A. ursinum* extracts. According to the literature, S-methyl cysteinyl sulfoxides and their derivatives are mainly responsible for the antimicrobial effect of *Allium* species, and their distribution in plants shows spatial and temporal differences leading to different antibacterial effect.
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EXAMINATION OF SEROLOGICALLY CONFIRMED HUMAN HANTAVIRUS INFECTIONS IN HUNGARY: CONCLUSIONS OF LABORATORY DIAGNOSTICS

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Hantaviruses (genus *Hantavirus*, family Bunyaviridae) were first isolated in Hungary in 1998. These rodent-borne viral zoonoses spread most likely via inhalation of aerosolized rodent excreta, such as saliva, feces, or urine. In most of the human cases different serotypes of old-world hantaviruses (which are presented in the Eurasian region) caused haemorrhagic fever with renal syndrome (HFRS). However, differences could be observed in the course of disease and clinical outcome: for example the mild form of HFRS called nephropathia epidemica is usually associated with Puumala virus, which is the most widespread hantavirus in Europe, including Hungary. Virus specific therapeutic agents are not available, the only possible treatment for the impairment of renal function is the acute haemodialysis. The aim of our study was the analysis of serologically confirmed human hantavirus infections in the interval between 2010 and 2014. The examination covered the serological differentiation of Puumala and Dobrava-like hantavirus infections and the comparison with the severity of the disease. Another interesting aspect was to investigate the implication of haemodialysis treatment for the serological diagnosis. Furthermore, we examined the geographical distribution and the seasonality of the infections. From 2010 to 2014 the average annual number of confirmed cases was about 10, which shows a decreasing tendency compared to data from previous years (annual average: 20 cases per year). So far our experiences have shown that Puumala and Dobrava-like hantaviruses, which are endemic in Hungary can be distinguished by our in house serological tests. Most of the human infections are caused by Puumala hantavirus, the most frequently occurring hantavirus in our country.
Contrary to the published data in several Puumala cases severe symptomes could be detected. In case of application of haemodialysis treatment the virus specific antibody titers could be significantly decreased, however, in most of the cases the antibodies still remained detectable from human serum samples. Examining the distribution of verified human hantavirus infections no typical geographical cluster could be observed.

VIRULENCE AND ANTIMICROBIAL RESISTANCE DETERMINANTS OF VEROTOXIGENIC *ESCHERICHIA COLI* (VTEC) AND OF ESBL-PRODUCING MULTIDRUG RESISTANT *E. COLI* FROM FOODS OF ANIMAL ORIGIN ILLEGALLY IMPORTED TO EUROPE

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Microbial risk due to illegal food import has not been investigated so far. Here we aimed to reveal frequency, phenotype and genotype of verotoxigenic *E. coli* (VTEC) and ESBL-producing multidrug resistant (MDR) *E. coli* isolated from foods of animal origin confiscated at the EU airport borders. Of the 1500 food samples confiscated at the airports of Austria, Germany and Slovenia, the most frequent were cheese and meat products primarily from Turkey and from Balkan countries. The VTEC bacteria were isolated using ISO 16654:2001 for O157 and Ridascreen® ELISA based PCR testing of *stx* genes or ISO/ TS13136 for non-O157 VTEC, resulting in 15 isolates of VTEC (1%). In addition 600 samples from the Vienna airport were also tested for ESBL-producing MDR *E. coli*, using cefotaxime-McConkey agar. We identified 14 *E.coli* strains as ESBL/MDR *E. coli*. (0.9%) for phenotyping for antimicrobial resistance and for genotyping by microarray (Identibac®, AMR05). The 15 VTEC isolates were phenotyped as Stx toxin producing non-O157 strain. Only one isolate, from Turkish cheese, proved to be EHEC (O26:H46). The remaining 14 strains represent uncommon VTEC serotypes with *stx1* and/or *stx2* genes. Microarray analysis (Identibac®, Ec03) revealed a wide range of other non-LEE encoding virulence genes. Pulsed field electrophoresis (PFGE) showed high genetic diversity of the strains. Multilocus sequence typing (MLST) established three new ST types (ST4505, 4506 and 4507) in the MLST database, and indicated the existence of 5 small clusters with no relation to origin or serotype/genotype of the strains, but representing several human-related ST types. All VTEC isolates were sensitive to 18 antimicrobials relevant to human and/or animal health, and did not contain resistance genes. ESB/MDR *E. coli* were resistant to at least 3 classes of antimicrobials. Microarray analysis detected TEM-1 in all but one strain and a variety of genes encoding resistances to other ESBLs (CTXM-1, OXA-1), trimethromprim, tetracycline, aminoglycosides and class1/class2 integrons (8/14 isolates).

E. coli virulence microarray detected 2-6 virulence genes in all but one MDR *E. coli*, and one of the strains qualified as an atypical EPEC. Even though the frequency and attributes of isolated VTEC and ESBL/MDR *E. coli* did not represent an immediate major risk through illegal food import for the countries involved, it is suggested that the unusual serovars of VTEC as well as the virulence and antimicrobial resistance determinants of ESBL/MDR *E. coli* detected here, may indicate a future emerging threat by strains in illegally imported foods.

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INVESTIGATION OF THE METABOLIC CONTEXT OF THE SOLUBLE HYDROGENASES IN *THIOCAPSA ROSEOPERSICINA*

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The photosynthetic purple sulphur bacterium, *Thiocapsa roseopersicina* harbours four functional [NiFe] hydrogenases. Two of them are attached to the periplasmic membrane (Hyn, Hup) and the other two are apparently localized in the cytoplasm (Hox1, Hox2). It prefers to utilize reduced sulphur compounds for anaerobic photochemolithoautotrophic growth, but simple organic substrates such as glucose and acetate can also be used as carbon, energy and electron sources. There is a facultative lithoautrophic proteobacterium, *Ralstonia eutropha* which has a soluble hydrogenase gene, *hoxI*. In *R. eutropha* the *hoxI* gene has an established role in the *in vivo* photosynthetic electron transport similar to the *hox1E* gene in *T. roseopersicina*. The in frame deletion of *hox1E* gene causes complete inability of *in vivo* hydrogenase activity and in addition, this loss-of-function mutation was not complemented by *hoxI* gene. Various constructs harbouring the *hoxI* gene were transferred into different *T. roseoperscina* hydrogenase mutant strains. Functional studies aimed the investigation of the role of *hoxI* in the modulation of Hox1 enzyme. Therefore, experiments were carried out under conditions when only Hox1 hydrogenase was functional. The strain expressing the *hoxI* gene was shown to evolve significantly higher amount of hydrogen *in vivo* than the negative control (empty plasmid in the same strain). The global gene expression changes were studied in three different strains by Whole transcriptome sequencing: the strain which contains the complete Hox1 hydrogenase, the *hox1E* mutant strain and the *hox1E* mutant strain with transferred *hoxI* gene. The results consist of two main parts. The first major part deals with the expression levels of particular genes, which are decreased because of the *hox1E* deletion. This low expression is not run-down in the presence of the *hoxI* gene. The first gene codes the NADH dehydrogenase, subunit 5 protein, while the second gene codes the Hypothetical transmembrane protein coupled to NADH-ubiquinone oxidoreductase chain 5 homolog. The second major part deals with the decrease in expression levels of the genes involved in the Photosynthetic reaction center and the Light harvesting complex, due to the *hox1E* deletion, while the presence of the *hoxI* gene restores the expression levels. Currently we are investigating the metabolic background of the gene expression changes by deploying different molecular methods as well as mutant analysis studies.

EVOLUTION OF HIV – 30 YEARS OF AIDS

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Human immunodeficiency virus HIV has been proposed as etiological agent of the than newly emerging disease called now acquired immunodeficiency syndrome AIDS 30 years ago in 1984. HIV has been classified as a human lentivirus, being an RNA human retrovirus. The exceptionally human pathogenic HIV however is not a *per se* human virus, it evolved from retroviruses of various monkeys and primates. The immediate predecessors of HIV-1 are SIV of chimpanzees (SIVcpz) and western gorillas (SIVgor), which latter virus originated from spotted nose monkeys. HIV-2 strains are evolved from SIV of sooty mangabeys (SIVsmm). Old World monkeys have been naturally infected with more than 40 different lentiviruses (SIVS) more than 30.000 years ago. Several of

these SIVs have crossed species barrier to great apes and humans, generating new pathogens such as SIVmac, SIVcpz, SIVgor, HIV-1 and HIV-2. The earliest evidence of HIV was proven by a stored serum sample in Zaire (Congo) in 1959, which confirms the hypothesis HIV has been evolved in Central Africa in the last 80 years or so, and spread to worldwide. According to geographical regions HIV-1 formed major variants of M and N (O?) and has been grouped to subtypes (clades) A to K. HIV-1B proved to be the most widespread subtype in Europe and USA, while A and C were the typical virus in Africa, and E in South-East Asia. The high error rate of reverse transcriptase (RT) and recombination events contributed to the expansion of the genetic heterogeneity of HIV-1 *in vivo* and resulting emergence of circulating recombinant forms (CRF) as well as drug resistant HIV variants. Genotyping of HIV by *in situ* DNA hybridization using Line Probe Assay, direct sequencing using the Stanford AIDS database, Truegene HIV-1 Genotyping kit and OpenGene Sequencing System we identified HIV-1 subtypes, evolution of multi-drug resistant variants, emergence of non-B clades and African HIV-1 circulating recombinant forms (CRF) in Hungary, which raises serious clinical and public health consequences. By constructing recombinant psudotype HIVs, primary molecular events of virus-cell interactions can be revealed contributing to the development of more effective anti-HIV entry-inhibitor drugs. On the other hand evolution of genetic polymorphisms of the three most frequent HIV-1 resistance-conferring human alleles: CCR5-Δ32, CCR2-64I and SDF1-3'A also playing an important role in HIV-1 pathogenesis.
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THE GENOMIC PREREQUISITES OF THE CONVERGENT EVOLUTION OF YEAST-LIKE FUNGI

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Yeasts make up a polyphyletic assemblage of species with a pronounced unicellular phase in their lifestyle. They evolved several times in distantly related clades, in both the Asco- and Basidiomycota, yet the genetic bases of their evolution is not known. Phenotypic simplification associated with massive genome contractions is an attractive explanation, however, it fails to explain the level of similarity in the developmental program of distantly related yeast clades. Here, we show that the potential to develop yeast forms arose early in fungal evolution and has been conserved but latent in most clades. Yeast-like growth became the dominant growth form independently in multiple clades; these clades share the genes involved in yeast-like growth, which explains the similarities between unrelated yeast species. Our data suggest that the potential for yeast-like growth became pronounced most likely via parallel diversification of Zn-cluster transcription factors, a fungal-specific family involved in regulating yeast-filamentous switches.

We conclude that convergent evolution of yeasts happened via a single origin of the genes involved in yeast-like growth, followed by repeated modification of their regulation is disparate clades, which ultimately led to clades of predominantly yeast-like fungi.

ANTIBODY DEPENDENT ENHANCEMENT OF INFECTIVITY IN HUMAN *FLAVIVIRUS* INFECTIONS

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Antibody dependent enhancement of infectivity is a well-studied yet poorly understood characteristic phenomenon of flavivirus infections that occurs when pre-existing, but non-neutralizing antibodies are present. It is also proven that low levels of neutralizing antibodies may lead to insufficient protection and induce antibody dependent enhancement as well. Antiviral antibodies can promote viral entry into macrophages by pathways involving cellular receptors for the Fc portion of immunoglobulin or for complement components leading to increased viral replication and more severe course of the disease. Since serological cross-reactivity is very likely among the members of the family Flaviviridae, immune-enhancement of heterologous *Flavivirus* infection can occur as a result of prior vaccination or infection. Low antibody titers due to incomplete immunization can also lead to the same consequences.

In Hungary both antigenetically related flaviviruses, tick-borne encephalitis virus and West Nile virus are endemic and cause serious neurological infections annually. During serological examination of samples of patients with severe *Flavivirus* infections we found that in many cases the role of antibody dependent enhancement can be suspended. Serological evidences of both inadequate immunization with tick-borne encephalitis vaccines and secondary infections with a heterologous *Flavivirus* were detected including a case of a PCR-confirmed dengue infection with severe symptoms after a previous asymptomatic tick-borne encephalitis infection.

ANALYSIS OF THE WEE1 PROTEIN'S EVOLUTION BY BIOINFORMATICAL METHODS

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Precise regulation of the cell cycle is essential for each dividing cell. The Wee1 tyrosine kinase plays an important role in cell cycle control, it facilitates the existence of a cell cycle control point at the G2/M transition through phosphorylating the Cdk1 kinase during the G2 phase. The importance of the Wee1 kinase was revealed by the examination of the fission yeast *Schizosaccharomyces pombe*. Although there are several cell cycle control mechanisms, Wee1 exists in almost every eukaryotic cell; moreover its back-up copy, the Mik1 in fission yeasts and its paralog, the Myt1 in some higher animals, are also present. In some higher animals there are two homologs of Wee1, called Wee1A and Wee1B, where one of the two homologs exists in the embryo and the other one in the animal. The aim of this study was to perform an in silico analysis of the Wee1 protein's evolution. We constructed the phylogenetic tree of the Wee1 homologs of 18 model organisms (7 animals, 6 plants and 5 fungi) to analyze the relationship of the Wee1, the Mik1 and the Myt1

proteins. A search for homologs was performed in the database of NCBI with the BLASTp algorithm by using the amino acid sequence of the Wee1 protein of the fission yeast. In the Pfam database we checked, whether all of the homologs contain the same protein kinase domain as the fission yeast Wee1 protein. To construct the multiple alignments, we used the algorithms of both the ClustalX and the PRANK programs. We constructed the phylogenetic trees with the MEGA program with three methods: neighbor joining, maximum parsimony and maximum likelihood. In the case of the maximum likelihood method we always used the best fitting evolutionary model. We checked the reproducibility of the phylogenetic trees by bootstrap analysis. As expected, we have found that the Wee1 kinase is present in all of the examined species, while the Mik1 only in the species of the *Schizosaccharomyces* genus. Moreover, the Myt1 exists only in some animals, but does not exist in plants and in fungal species. The Wee1A and the Wee1B proteins localize on separate branches of the phylogenetic trees. In the C-terminal kinase domain of the protein we observed conserved regions and used them to check the multiple alignments.
The phylogenetic trees revealed that the formation of Mik1 and Myt1 from the Wee1 homologs was likely two distinct evolutionary events.
This project is supported by the Hungarian Scientific Research Fund (OTKA K-101323).

GENERATION AND FUNCTIONAL ANALYSIS OF MAP KINASE MUTANTS IN *FUSARIUM* SPECIES

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Fusarium proliferatum and *F. verticillioides*, members of a species complex, known as *Gibberella fujikuroi* sensu lato are economically important fungi that occur globally, infect a range of plants and produce various secondary metabolites including fumonisin mycotoxins. The aggressive expansion of the two fungi, observed in the last 15 years in Central Europe suggests that they are especially successful in adapting themselves to stressful conditions and, therefore seem to be suitable organisms for stress response studies. Stress response is mainly signaled by the mitogen-activated protein kinase (MAPK) pathways in eukaryotic organisms. Two of the three MAPK pathways identified in filamentous fungi, the HOG1 (high osmolarity glycerol) MAPK and the CWI (cell wall integrity) MAPK (homologous to Slt2/Mpk1 in yeast) pathways taking part in abiotic stress responses, like response to salt, osmotic, oxidative, and temperature stressors. The third MAPK pathway, the pathogenicity MAP kinase (PMK) pathway (homologous to Fus3/Kss1 in yeast) mediates biotic stress responses and is required for virulence including penetration and invasive growth in plant tissues. Functional analysis of separate elements of the regulatory network formed by these three MAPK cascades helps to understand the highly complicated interaction that exists in this signaling system. To achieve this goal we cloned both HOG1 (Fphog1, Fvhog1) and CWI MAPK (Fvmk2) encoding genes from *F. proliferatum* and *F. verticillioides*, produced gene-disruption mutants (ΔFphog1, ΔFvhog1, ΔFvmk2) by site-specific insertion of a hygromycin phosphotrasferase expression cassette and restored the normal MAPK phenotype by introducing the competent wild-type MAPK gene into the mutants transformation construct containing the *nptII* (neomycin phosphotransferase) gene.
The wild type strains, their gene-disruption MAPK mutants and the restored strains were tested for salt, heat, and oxidative stress tolerance, fungicide sensitivity, secondary metabolite production, invasive growth in plant tissues, asexual sporulation and mating capability.

INVESTIGATION OF THE NITRIFICATION PROCESS IN FIVE SMALL DRINKING WATER SYSTEMS WITH SPECIAL EMPHASIS ON THE AMMONIA-OXIDIZING MICROORGANISMS

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In drinking water networks ammonia, e.g. originated from well water, can be biologically oxidized to nitrate via nitrite by nitrification process performed ammonia- and nitrite-oxidizing microorganisms. The prevailing conditions in drinking water are usually appropriate for nitrification process. The aim of the study was to detect, identify and quantify nitrifying microorganisms inhabiting five different small drinking water network systems. Cultivation-based methods were applied to estimate heterotrophic plate count and Most Probable Number (MPN) of nitrifying bacteria. Further investigation was carried out to detect the selectivity of MPN test media on ammonia oxidizing and utilizing microorganisms. To explore the community structure of nitrifying microorganisms inhabiting in drinking water and MPN test media, sequence aided Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was applied, based on 16S rDNA and ammonia-monooxygenase (*amoA*) gene respectively. Statistical analysis of data was performed by Principal Component Analysis. Raw water contained only ammonium-ion as inorganic nitrogen form. Nitrite and nitrate appeared only in the distribution systems generated by nitrification processes. However in the examined water networks nitrifying bacteria's MPN was low, or even zero especially in well water. Ammonia-oxidizing Bacteria, based on *amoA* gene were detected only in one, out of the five, drinking water system. Contrary to *amoA* gene, based on 16S rDNA *Nitrosomonas oligotropha* were detectable in almost all sampling points. In addition ammonia-oxidizing Bacteria, ammonia-oxidizing Archaea based on archaeal *amoA* gene were detectable in four water network systems. Ammonia-oxidizing Bacteria were detected in the ammonia-oxidizing positive MPN test mediums merely inoculated by well water and all sampling points of two drinking water systems. Nevertheless ammonia-oxidizing Archaea were detectable in MPN test media optimized for enumeration AOB. Disparities were observed in the community structure of the ammonia-oxidizing microorganisms originated from drinking water and AOB-MPN samples.
Compared to ammonia-oxidizing microorganisms, NOB were detected in each sample of the different water networks, especially *Nitrospira defluvii* and *Nitrospira moscoviensis*. Surprisingly members of *Nitrospira* genus also were present in several AOB-MPN test media.

FOLDING FEATURES OF THE LONG-SEQUENCE HYPOMUROCIN PEPTIDES AND THEIR ANALOGS

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Hypomurocins isolated from the fungus *Hypocrea muroiana* are the members of peptaibol family, and this group of peptides covers both short-sequence and long-sequence molecules. The six hypomurocin B (HM B) peptides composed of 18 amino acids belong to the group of long-sequence peptaibols, and three molecules of them contain D-isovaline (D-Iva) residue. In the course of this study, the folding processes of HM B peptides were explored applying molecular dynamics (MD) methods. Additionally, the folding features of three analogs of the HM B molecules were also investigated, for which the D-Iva amino acid was replaced by L-Iva residue, in order to study the

effects of the chirality of this amino acid on the folding processes of peptides. On the basis of MD simulations, the evolution of different helical structures, such as, alpha-, 310- and left-handed alpha-helix, was examined, taking into consideration the entire sequence of peptides, as well as each amino acid. Nevertheless, the evolution of a variety of the intramolecular H-bonds formed between the backbone NH and CO groups was investigated. Two different groups of H-bonds could be distinguished, as follows: (1) the local (i.e. i←i+3 and i←i+4) H-bonds; and (2) the non-local (i.e. i←i+n, where n > 4; and i→i+n) H-bonds. The formation of these intramolecular interactions was studied with regard to the average numbers of H-bonds, as well as to each i←i+3 and i←i+4 H-bonds, respectively. The results obtained by the MD calculations pointed out that the occurrence of local H-bonds was in agreement with the appearance of various helical structures. Furthermore, it could be observed that the non-local H-bonds did not affect significantly the evolution of helical conformations. Based on our results, however, it could be concluded that the chirality inversion of Iva amino acid produced effects on the folding processes of peptides. In summary, the characteristic structural and folding features of long-sequence HM B peptides and their analogs were determined.

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STRUCTURAL INVESTIGATION OF BIOACTIVE PEPTAIBOL MOLECULES BY THEORETICAL METHODS

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Peptaibol molecules are fungal peptide antibiotics, which show a broad spectrum of biological activities covering antibacterial, antifungal, antiviral, immunosuppressive and neuroleptic effects. The peptaibols are composed of 5-20 amino acids, and these molecules contain non-proteinogenic amino acids (e.g. alpha-aminoisobutyric acid and isovaline), as well as an amino alcohol (e.g. leucinol, phenylalaninol and valinol) is linked at their C-terminus. The three-dimensional (3D) structure of several peptaibols has been examined mainly by experimental techniques, so far. Nevertheless, applying the appropriate theoretical methods, the structural properties and folding features of peptaibol molecules could be studied in detail. First of all, the non-proteinogenic amino acids and amino alcohols should be parameterized by means of quantum chemical calculations, and the partial atomic charges regarding these non-standard residues should be calculated. Afterwards, the characteristic structural properties and folding features of peptaibols could be identified applying a variety of theoretical methods (e.g. simulated annealing calculation, molecular dynamics simulation and multiple molecular dynamics simulation). Based on the data derived from these calculations, the 3D structure of peptaibol molecules, with regard to the backbone and side-chain conformations, as well as to the different distances, could be characterized in detail. Furthermore, on the one hand, the folding features and folding processes of peptaibols could be explored comprehensively, and on the other hand, the micelle- or membrane-bound conformations could be described. On the whole, the computational molecular modeling proves to be a suitable and useful tool to study the 3D structure and folding processes of peptaibol molecules.

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CHARACTERISTIC CONFORMATIONAL PATTERNS OF THE HARZIANIN C PEPTIDES

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Harzianins, as a microheterogeneous mixture of fungal peptides, belong to the peptaibol family, and among them, the eleven harzianin C (H C) molecules, consisting of 14 amino acids, are the members of the group of short-sequence peptaibols. The H C peptides possess a characteristic sequence pattern, namely, these peptaibol molecules contain three consecutive (Xaa-Yaa-Aib-Pro) motifs. In this theoretical study, molecular dynamics (MD) simulations were performed, in order to identify the typical conformational patterns of these short-sequence peptaibols, focusing on the secondary structural elements and intramolecular H-bonds. In the case of H C molecules, taking into account the various secondary structures, the presence of types I and III beta-turns was examined along the entire sequence of peptides, and the occurrence of beta-bend ribbon spiral structure was studied for the (Xaa-Yaa-Aib-Pro) motifs. The different beta-turns are usually stabilized by characteristic intramolecular H-bonds, thus the appearance of these interactions was investigated with regard to the tetrapeptide segments containing beta-turn structures. In order to describe the dynamic behavior of H C peptides, based on the MD trajectories, the alterations of conformational features were examined as a function of simulation time.

In the course of this investigation, it was characterized how the various structural features (i.e. secondary structural elements and H-bonds) change during the MD simulations. Based on the results, the typical conformational patterns of H C molecules were determined, and the dynamic behavior of these short-sequence peptaibols was characterized in detail.

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MICELLE-BOUND CONFORMATIONS OF SHORT-SEQUENCE PEPTAIBOL MOLECULES

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Peptaibols are fungal peptide antibiotics, which belong to the family of antimicrobial peptides. These molecules possess a variety of biological activities comprising antibacterial, antifungal and antiviral effects, as well as they are well-known as membrane-active peptides. In the present study, the micelle-bound conformations were investigated for short-sequence peptaibols, such as, trichobrachin A I, trichobrachin B I, hypomurocin A I, trichorozin I, harzianin B I, trichofumin A and trichorovin XIIa. In order to characterize the micelle-bound conformations of these short-sequence peptaibols, molecular dynamics (MD) calculations were carried out on peptide-micelle systems applying DPC micelle. On the basis of MD trajectories, the interactions between the peptaibol molecules and micelle could be characterized by different structural features, i.e., insertion depth, secondary structures, as well as intra- and intermolecular interplays. To describe the insertion processes of peptides, the alteration of distance measured between the centers of mass of the peptide and micelle was examined as a function of simulation time. In the case of micelle-bound conformations of the short-sequence peptaibols, the occurring secondary structural elements were

investigated, and the preferred rotamer states of the side-chains of amino acids were studied. Additionally, the presence of intramolecular H-bonds stabilizing the micelle-bound conformations of peptides was investigated, as well as the appearance of intermolecular interactions formed between the peptides and lipid molecules was studied, which could also contribute to the structural stability of peptides. On the basis of results derived from the MD simulations, the micelle-bound conformations, as well as the interactions between the peptides and DPC micelle were characterized for the short-sequence peptaibol molecules.

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STRUCTURAL PROPERTIES OF SHORT-SEQUENCE PEPTAIBOLS CONSISTING OF 11 AMINO ACID RESIDUES

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According to their sequence length, the peptaibol molecules can be divided into two different groups, as follows: (1) the short-sequence peptaibols; and (2) the long-sequence peptaibols. The trichorozin (i.e. TZ I, TZ II, TZ III and TZ IV), the harzianin (i.e. H B I and H K VI), the trichofumin (i.e. TF A and TF B), as well as the trichorovin (i.e. TV XIIa) peptides, consisting of 11 amino acid residues, belong to the group of short-sequence peptaibol molecules. In this study, various structural properties of the above-mentioned peptaibols were investigated comprehensively by means of simulated annealing and molecular dynamics calculations. For the short-sequence peptaibol molecules, the backbone conformations were examined, and types I and III beta-turns, as well as beta-bend ribbon spiral structures were identified in certain tetrapeptide units. Nevertheless, the occurrence of intramolecular H-bonds was studied, which played a relevant role in the structural stabilization of secondary structural elements. Beside the backbone conformations, the side-chain conformations of amino acids were also investigated, and the preferred rotamers of side-chains were determined. In the case of short-sequence peptaibols, the following types of distances were measured: (1) the end-to-end distance between the N atom of the backbone NH group of Xaa1 amino acid and the C atom of the backbone CH_2 group of Xaaol11 amino alcohol; and (2) the residue-residue distances between all residue pairs of molecules. On the basis of results, with regard to the backbone and side-chain conformations, as well as to the end-to-end and residue-residue distances, the three-dimensional structure of short-sequence peptaibol molecules were characterized, and they were compared to one another.

Our structural investigation led to the observation that the trichorozin, harzianin, trichofumin and trichorovin peptides showed typical structural properties, nevertheless, both conformational similarities and dissimilarities could be detected for these short-sequence peptaibols.

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STUDY OF SYMBIOSIS RELATED ORTHOLOG GENES IN ROOT COLONIZING DARK SEPTATE ENDOPHYTES

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The Dark Septate Endophytic (DSE) fungi are found worldwide and comprise a group of root-colonizing fungi. Although DSE are common plant-associated fungi in many ecosystems our knowledge about effects of DSE colonization of host plants are poorly understood. The main aim of the work presented here was to screen orthologous of symbiosis and/or ecological-function related genes in DSE. We studied three DSE groups found to be common and general in (semi)arid ecosystems: the so called -"Pleosporales group"-, a *Cadophora* sp. and *Periconia macrospinosa*. We studied invertase and phosphate transporter genes in these DSE strains. Invertase and phosphate transporter orthologs were collected from public databases and specific PCR primers were designed to amplify them. After the test of those primers the expression of the genes was studied using real-time PCR. Understanding of these symbiosis related orthologs from DSE, might reveal important functions and help to understand our knowledge on DSE-host plant relationship.
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DOUBLE PASTEURIZATION WITH A PLATE HEAT EXCHANGER

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Nowadays, people are developing numerous new liquid egg preservation methods and testing them in industrial settings, but such methods are beyond most small producers of egg products, mainly because of the high cost of investment and implementation. On the other hand, everyone has access to simple pasteurization equipment. In this study, we have examined how conventional double pasteurization with a plate heat exchanger affects the microbiological nature of liquid egg. For our experiment, liquid egg was prepared within two hours of starting the tests and kept at 4 °C. Then it was pasteurized in a plate heat exchanger for 5 minutes at 65 °C. Following this, the material was cooled back down to 4 °C and kept for one hour in a buffer container while the heat exchanger was cleaned. Afterward the pasteurization was repeated. We then prepared a series of 1ml decimation dilutes of three types: raw liquid egg, a once-pasteurized sample, and a twice-pasteurized sample, after which we determined the microbe count of the samples with nutrient agar poured plates. Additionally, we sequenced the 16S rDNS gene of the isolate obtained from the twice-pasteurized liquid egg. Our results, which are in agreement with the literature, show the first pasteurization caused a more significant reduction in spore count. Whereas the first pasteurization reduced by a magnitude of approximately 2 the total live spore count of aerobes in the egg liquid, the second pasteurization only reduced the count by a further 1 order of magnitude. There was yet another difference. In the double-pasteurized samples, the diversity of microflora was considerably less: only

3 populations/species could be identified: *Rhodococcus erythropolis / Rhodococcus qingshengii / Rhodococcus baikonurensis / Rhodococcus boritolerans, Nocardia coeliaca, Chryseobacterium* sp., *Brevundimonas nasdae / Brevundimonas vesicularis*. Based on our results, it can be stated that double pasteurizing liquid egg enables manufacture of microbiologically stable products. This may be a feasible alternative for smaller plants operating at less than 100% capacity, and is preferable to the more modern, and more costly technologies.

THE VELVET ORTHOLOGUE VEL1 OF *TRICHODERMA REESEI* IS ESSENTIAL FOR CELLULASE GENE EXPRESSION

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The proteins VeA, VelB and LaeA of *Aspergillus nidulans* form the VELVET complex to coordinate sexual development and production of certain secondary metabolites. Deletion of veA in Aspergilli results in the complete or partial loss of secondary metabolite production, and impairment of asexual or sexual differentiation. In this presentation we report on the role that VEL1 – the orthologue of *A. nidulans* VeA in *Trichoderma reesei* – plays in this cellulase and hemicellulase enzyme producer fungus. The Δ*vel1* loss-of-function *T. reesei* mutants displayed a distinct altered phenotype. A comparative image analysis of the fungal morphology showed that while these mutants were still able to form mycelia – albeit shorter and thicker ones than the wild-type (QM 9414) – they were unable to produce conidiospores either on minimal or complex solid media. Δ*vel1* strains also exhibited a slightly reduced growth on glucose. Growth was more significantly reduced on lactose and practically absent on cellulose. These carbon sources are related to cellulase formation. To test whether VEL1 would indeed be necessary for the formation of cellulases in *T. reesei*, we cultivated the parent and the Δ*vel1* strains on lactose, which induces cellulase expression, but its utilization – in contrast to cellulose – is independent of the secreted cellulases. Under submerged (batch) conditions, both lactose consumption as well as biomass formation was notably slower in the Δ*vel1* mutant strain than the wild-type, and most importantly, cellulase activity could not be detected at any time-point of the fermentation in the Δ*vel1* mutant. We also quantified the relative expression of the two major cellulase genes *cbh1* and *cbh2* as well as of the general cellulase regulator xyr1 by Real-Time PCR. All three genes were markedly downregulated in the mutant relative to the wild-type. In order to rule out that these findings were due to the poor growth on lactose, cellulase gene expression was also tested with sophorose (2-O-beta-D-glucopyranosyl-alpha-D-glucose) in resting cells. Results essentially similar to those obtained on lactose were obtained: the transcript levels in the wild-type strain immediately increased upon sophorose supplementation to the medium, while all three genes were downregulated in the *T. reesei* Δ*vel1* mutant. We concluded that in *T. reesei*, *vel1* appears essential in the production of cellulases and has a major impact on the morphology and differentiation of the fungus.

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TO BE PERSISTENT OR NOT TO BE PERSISTENT? THAT IS THE QUESTION FOR *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM A MEAT PROCESSING ENVIRONMENT

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Despite the progress made in recent years regarding understanding bacterial population structure and dynamics in a constant environment, identification of persistent strains of *L. monocytogenes* is still challenging. The present study analysed the *L. monocytogenes* strains isolated from a meat processing facility having in view to establishing whether or not persistence is involved in the contamination of the establishment. Forty two isolates from sixty one positive samples have been characterised by serotype multiplex PCR and PFGE in order to have an overall view of their diversity. In total 11 pulsotypes have been identified by PFGE, belonging to 2 serogroups: 1/2a/3a and 1/2c/3c. The PFGE dendrogram suggests the existence of two persistent pulsotypes (T4 and T8), if persistence is considered the isolation of the same pulsotype during several months to years. Susceptibility tests to disinfectants such as peracetic acid, benzalkonium chloride and hydrogen peroxide showed that pulsotypes T1 and T2, which have not been isolated frequently, were more resistant to benzalkonium chloride than to peracetic acid and hydrogen peroxide. No significant differences were detected between pulsotypes regarding their susceptibility to peracetic acid and hydrogen peroxide. The screening for identification of resistance genes (*qacH, brcABC*) and stress survival islet (SSI-1) revealed that both T1 and T2 pulsotypes harbour the benzalkonium resistance cassette (*brcABC*) and the SSI-1, which have been detected by other researchers in persistent strains. None of the pulsotypes have got *qacH* gene. The in-vitro adhesion ability of *L. monocytogenes* to abiotic surfaces at suboptimal temperatures (4 °C, 10 °C, 20 °C) showed that T8 pulsotype had good adhesion ability, while T4 had the same ability as the other non persistent strains. Although not displaying typical characteristics for persistence, the T4 and T8 pulsotypes were identified in all sampling occasions, both in raw materials and on surfaces, suggesting a continuous contamination of the meat processing facility with these pulsotypes. Despite displaying characteristics for persistence, the T1 and T2 pulsotypes showed a non persistent phenotype. This can be attributed to the possibility of a spontaneous switch between persisters and non persisters, a phenomenon that can occur in both directions.

In these circumstances, when referring to persistence of *L. monocytogenes* strains in an environment, it is better to use the terminology "presumed to be persistent" and "presumed to be non persistent" until clear prove is identified to support their classification.

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INVESTIGATION OF THE FATTY ACID PRODUCTION AND THE EXPRESSION OF THE Δ9 AND Δ6 FATTY ACID DESATURASE GENES IN DIFFERENT *MORTIERELLA* AND *UMBELOPSIS* STRAINS

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Mortierella and *Umbelopsis* species are regarded oleaginous microorganisms as they are particularly active in polyunsaturated fatty acid (PUFA) synthesis. PUFAs are structural components of biological membranes and precursors of prostaglandins, leukotrienes and hydroxy-fatty acids. Considerable attention has been paid to them due to their beneficial effects on human health. The aim of our work was to analyse the PUFA production and the expression of the genes encoding the Δ9 and Δ6 fatty acid desaturases in some *Mortierella* and *Umbelopsis* strains under different culturing conditions. Ten strains with different PUFA yield and profile were chosen on the basis of previous HPLC analysis. Strains of *Mortierella alpina* and *M. antarctica* were good arachidonic acid producers, whilst *Lobosporangium transversale* CBS 357.67 strain was a weak PUFA producer with the same PUFA profile. Similarly, strains of *Umbelopsis ramanniana, U. isabellina* and *U. versiformis* were good γ-linolenic acid, linoleic acid and oleic acid producers, whilst *M. longicollis* CBS 209.32 strain was a weak PUFA producer with the same PUFA profile. *M. wolfii* CBS 614.70, *M. tuberosa* CBS 210.72 and *M. simplex* CBS 243.82 isolates were good linoleic acid producers and *M. tuberosa* also showed high α-linolenic acid production. The partial sequence of the Δ9 and Δ6 desaturase encoding genes as well as the actin gene, which are used as control in the expression studies, have been identified in these strains. In some isolates 2 copies of Δ9 or Δ6 desaturase genes were identified. The effect of different culturing conditions, such as cultivation time, temperature and medium composition on the fatty acid production as well as the expression of the Δ9 and Δ6 desaturase encoding genes was investigated. The highest fatty acid production was observed after 7 or 8 day incubation on 20-25°C. PUFAs containing two or three double bonds are preferentially produced on lower temperature. The effect of medium composition on the PUFA production was also investigated. However, the biomasses were the highest in MEA and GY, PUFA production was maximal in YNB and PD broth.

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TRANSCRIPTOMIC CHARACTERIZATION OF PSEUDORABIES VIRUS USING SINGLE-MOLECULE AND HIGH-THROUGHPUT SEQUENCING METHODS

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Pseudorabies virus (PrV, also known as Aujeszky's disease) is a neurotrophic herpesvirus of the Alphaherpesvirinae family. PrV infects swine populations worldwide, causing economic losses, while also being a widely used model organism and neuronal tracer. The compact genome arrangement of PrV consists of 72 known coding regions, tightly packed into overlapping gene clusters in various orientations. During viral infection, these genes are expressed in a strictly regulated and inter-dependent manner. In order to assess the possibility of transcriptional interference between and within overlapping gene clusters, and to complement our previous qPCR gene expression studies with structural genomic information, mRNA libraries from five timepoints of lytic viral infection have been analysed using Pacific Biosciences Single- Molecule Real- Time Sequencing, yielding diverse transcript isoforms and previously uncharacterized non-coding transcripts. Illumina high-throughput sequencing of totalRNA and poly(A)+ isolates was carried out

to gather accurate data on alternative splicing, and to provide a map of alternative polyadenylation across the viral genome. Alternative isoform expression has been the focus of several recent eukaryotic transcriptome analyses, utilizing different wet-lab and bioinformatic approaches. In order to minimize systematic biases and host-organism sequence content, experiments were carried out using purification methods and primers optimized for the extreme GC-content (73.6%) of PrV, while bioinformatic analyses were also carried out with regard to the special requirements posed by the experimental model. Using the combination of single-molecule and PCR-based sequencing approaches enabled robust identification of several full-length transcripts, spliced isoforms and novel RNA species of PrV, providing a framework for further analysis of transcriptional regulatory mechanisms in species with extremely condensed and optimized genetic material.

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GENOME AND METHYLATION ANALYSIS OF PSEUDORABIES BY SINGLE MOLECULE REAL-TIME SEQUENCING

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As the throughput of second-generation sequencing platforms continues to increase while reagent- and maintenance costs sharply decrease, resolving repetitive and biased sequences continues to pose a challenge in genome assembly. This problem is more easily overcome by combining second-generation, short-read methods with newly available long-read technologies, to some extent trading off accuracy for reads that are able to span large repetitive sequences. In addition, detection of modified bases in DNA has enabled researchers to gather valuable information on the spatiotemporal regulation of gene expression, and thus provide a better understanding of the epigenetic factors of inheritance and ontogeny. The detection of methylated bases in DNA has largely been restricted to 5-methylcytosine, through bisulfite treatment. Here we present the nucleotide sequence of strain Kaplan of Aujeszky's disease virus (also known as Pseudorabies, PrV), a widely used model organism and neuronal tracer of the Alphaherpesvirinae family. In order to resolve previous uncertainties in the viral genomic sequence, and to assess potential variability among laboratory strains, we have applied a long-read sequencing approach to complete the genome, paired with preliminary analyses on possible base modifications during infection. Single-Molecule Real-Time (SMRT) sequencing is based on real-time monitoring of several thousand DNA polymerase enzymes immobilized in separate wells. Nucleotides are distinguished according to the length of the fluorescent signal emitted during incorporation (pulse width) and polymerase stalling, characteristic of modifications (interpulse duration, IPD). In order to interpret IPD ratios on eukaryotic DNA samples, differences in the mechanism of methylation have to be taken into account. While optimizing for the nucleotide composition and experimental conditions of viral infection is relatively straightforward with regard to genome assembly, the adjustment of IPD ratio-based predictions on genome methylation requires the use of novel bioinformatical approaches.

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SEROLOGIC SURVEY OF COWPOX VIRUS INFECTION AMONG RODENTS IN HUNGARY

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Vaccination against smallpox was discontinued after the late 1970s, resulting different orthopoxviruses (OPVs), such as cowpox virus (CPXV) to become a re-emerging healthcare threat among zoonotic pathogens. Human infections highly depend on virus prevalence in its rodent hosts, however in Hungary, data on CPXV-prevalence among its rodent host species have been absent. The current study introduces data of rodent trappings from March to September in 2011 and 2012, in a 149 ha area in Mecsek Mountains. Animals were trapped at 13 sampling plots. Rodent sera were collected and screened for CPXV-reactive antibodies with immunfluorescence assay (IFA). Among the 1587 tested rodents, 286 (18.0%) harboured CPXV-specific antibodies. Seroprevalence was the highest in MGL (71.4%) and AAG (66.7%).

Due to a masting event in the autumn of 2011 across Central Europe and an observed predation by red foxes towards AFL at the study area, the abundance of MGL increased drastically in the 2012 season, affecting the overall CPXV-seroprevalence. We provide the first data on CPXV-occurrence and seroprevalence in rodents in Hungary. Circulation of CPXV in rodents warrants further studies to elucidate the zoonotic potential of CPXV in humans.

IMMUNOMODULATION FOLLOWING ADENOVIRUS INFECTIONS AND VECTOR APPLICATION

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Human and animal adenoviruses are important pathogens. They also function as genetic models and applied as vectors for gene therapy. Their infection in the gastrointestinal and upper respiratory tract or eye of immunocompetent individuals are usually mild and without sequele. They establish latent infection in lymphocytes and activate in immunocompromised patients. Depending on the underlying condition, the disease course affects different organs and may lead to death. Prevalence of serotypes varies depending on age, malignancy, and medical intervention. In children suffering with severe combined immunodeficiency syndrome (SCID), species A and C serotypes induce lung, liver, bladder inflammation. Haematopoietic stem cell transplantation in children is frequently accompanied by pneumonia, enteritis, encephalitis, cystitis. In adults, after transplantation species B serotypes can destroy the transplanted organ. In HIV infected patients, D and F species serotypes might cause severe enteritis with high mortality, especially before the HAART era. Unusual recombinants of B serotypes induce urinary tract infections. Progression of lymphomas, solid tumours, lupus erythematosus might be facilitated by immunosuppressive effects of adenoviruses. Vector application of adenoviruses induce serious side effects. Pre-existing antibodies or those elicited by the vector neutralize input recombinant virions rendering them ineffective. Mediators (cytokines, chemokines) induce cytotoxic reactions which extinguish transgene expression. Through the infection process and gene therapy strategies adenovirus fiber and penton bind cell surface

receptors through special amino acid moieties, and activate secondary messengers, protein kinases, pro-inflammatory mediators. To overcome these difficulties, new strategies are required in the application of recombinant adenoviruses to redirect entry to alternative binding sites. Regarding natural reactivation, pre-emptive therapy might be used preceding medical interventions. Cidofovir derivates, ribavirin, ganciclovir, vidarabine and microRNA have been used to combat infections.
Among diagnostic procedures, serology testing is unreliable, but detection of viral DNA and quantitation of virus copy number is reliable and predictive.

HUMAN PAPILLOMAVIRUS E6 AND E7 ONCOPROTEINS ALTER THE EXPRESSION OF DIFFERENTIATION-ASSOCIATED GENES IN HUMAN KERATINOCYTES

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The E6 and E7 oncoproteins of high-risk human papillomaviruses (HPVs) (such as HPV 16) are responsible for the oncogenic potential of the virus. The replication of the virus genome is strictly linked to the differentiation of their natural host cell. The HPV infection can delay the normal differentiation program of keratinocytes, however, there are few data about the underlying mechanisms responsible for this phenomenon. The purpose of the present study was to identify genes important in keratinocyte differentiation that are regulated by the HPV oncogenes on the transcriptional level. Global gene expression profiling was used to study the mRNA expression pattern of human keratinocytes transduced by LXSN-based retrovirus vectors, expressing HPV E6, E7 or E6/E7 oncoproteins. In microarray analysis, we have identified several genes involved in cell cycle, replication, proliferation and keratinocyte differentiation, whose expression was altered by HPV 16 oncoproteins. To validate our data, we performed quantitative real-time PCR analysis on a subset of genes important in keratinocyte differentiation. Then, human keratinocytes were co-transfected using reporter plasmids containing promoter regions of selected keratinocyte differentiation genes (KRT4, SPRR1A, S100A8, DSC1) along with vectors expressing the E6, E7 oncogenes of HPV 16. In these luciferase reporter assays, the HPV 16 E6 and E7 oncoproteins were shown to down-regulate the promoter activity of these genes involved in keratinocyte differentiation. The promoter of these selected genes contains putative binding sites for AP1 and C/EBP transcription factors according to sequence analysis.
To this end, keratinocytes were transfected by luciferase reporter vectors containing multiple copies of binding sites for AP1 and C/EBP along with HPV 16 E6, E7 expression vectors. Our results suggest that the HPV 16 E6 and/or E7 oncogenes may have the potential to down-regulate the expression of several keratinocyte differentiation genes by down-regulating their promoter activity, maybe through inhibiting the activities of AP1 and C/EBP transcription factors.
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IDENTIFICATION OF A BETA-GALACTOSIDASE-LACTOSE PERMEASE GENE CLUSTER IN *ASPERGILLUS NIDULANS* AND THE REGULATION OF ITS EXPRESSION IN RESPONSE TO D-GALACTOSE AND D-GLUCOSE

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Lactose is intracellularly hydrolysed by the model filamentous fungus *Aspergillus nidulans* into D-glucose and D-galactose. In this presentation, we report on the in silico identification of the clustered intracellular beta-galactosidase (*bgaD*) and lactose permease (*lacpA*) genes, whose functions were confirmed upon deletion. Transcript formation of *bgaD* and *lacpA* occurred co-ordinately in response to D-galactose, lactose or L-arabinose, while no transcription was detectable in the additional presence of D-glucose. In contrast, *creA* loss-of-function mutants were derepressed for both genes even under repressing growth conditions. Lactose- and D-galactose induction in *creA* mutants nevertheless occurred only in the absence of D-glucose, indicating a regulatory role for CreA-independent repression. The LacpA permease is irrelevant for growth on D-galactose. D-galactose can be catabolized via two independent routes in *A. nidulans*, i.e. the Leloir- and the oxido-reductive pathways. The inductive capabilities of intermediates of the two alternative assimilation routes were addressed in loss-of-function mutants defective in a defined step in one of the two pathways. In a galactokinase (*galE9*) mutant, the cluster is induced by D-galactose, suggesting that formation of Leloir pathway intermediates is not required. The expression profiles of *bgaD* and *lacpA* were similar in wild type, L-arabinitol dehydrogenase (*araA1*) and hexokinase (*hxkA1*) negative backgrounds, indicating that intermediates of the oxido-reductive pathway downstream of galactitol are not necessary either. Furthermore, *bgaD-lacpA* transcription was not induced in any of the tested strains on galactitol, the first intermediate of the oxido-reductive pathway and the substrate of the LadA dehydrogenase. Galactokinase/hexokinase double mutants cannot grow on D-galactose but the lactose catabolic cluster remains fully inducible by externally added D-galactose in biomass grown on glycerol, a non-inducing and non-repressing growth substrate. We can conclude that the physiological inducer of the *bgaD-lacpA* gene cluster upon growth on D-galactose is the non-metabolized sugar.

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BIOLOGICAL WASTEWATER TREATMENT SOLUTION BY USE OF A COMBINED TOXICITY-PROFILING METHOD

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To meet the growing populations' needs and the strengthening regulation of urban wastewater treatment plants innovative solution used by the Organica Technologies Inc. The Organica FCR is a complete wastewater treatment solution including solids removal, biological treatment/nutrient removal, phase separation, and final treatment for reuse quality, all incorporated into a compact, single structure. The biological treatment step is accomplished via a series of reactors. The quality

and quantity of the water-soluble organic and inorganic substances has a crucial role in the water-qualification. Examination of the water quality (e.g. COD, BOD, ammonia, nitrite, nitrate, etc.) with chemical analysis is important to explore weather water purification technology follows the water quality standards. However, these data are inefficient to examine the combined effects on the ecosystem. For that reason integrate application of standard tests and ecotoxicological tests in WWTPs should be applied. In the present study Organic Food Chain Reactor (FCR) was evaluated by a combined toxicity-profiling method. Pro and eukaryotic test-organisms were used:(1)The *Aliivibrio fischeri* luminescence inhibition test to determine the toxicity of water samples and (2) *Pseudomonas fluorescens* growth inhibition test to detect the harmful effects on the soil bacteria. (3) *E. coli*-based SOS-Chromo test served to detect genotoxicity or (4) the presence of hormonal substances was revealed by the BLYAS/BLYES bio-reporters, which employs the bioluminescent genetically modified strains of *Saccharomyces cerevisiae* by the help of human estrogen/androgen receptor genes. The combined toxicity profiling method revealed substandard influent wastewater samples that were characterized by increasing quality while going through the cascade system of the FCR. Interestingly, the detoxification efficiency was not linear through the FCR, since adverse effect was detected in the middle reactors, which can be attributed to harmful intermediates and by-products created during the biodegradation.

However, the cyto/geno/endocrine disrupting effect was eliminated in the FCR and the effluent wastewater was found appropriate in an ecotoxicological point of view.

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HERPES SIMPLEX VIRUS TYPES 1 AND 2 MODULATE AUTOPHAGY IN SIRC CORNEAL CELLS

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Autophagy and apoptosis function as important early cellular defense mechanisms in infections and other diseases. The outcome of an infection is determined by a complex interplay between the pathogenic microorganism and these intracellular pathways. To better understand the cytopathogenicity of *Herpes simplex virus* types 1 and 2 (HSV-1 and -2), we have studied the effect of these viruses on the autophagic and apoptotic processes in the SIRC corneal cell line. Infection with the KOS strain of HSV-1 and a wild-type strain of HSV-2 enhanced autophagosome formation, triggered cytoplasmic acidification, increased LC3B lipidation and elevated the ratio of apoptotic cells. The autophagy inhibitor bafilomycin A1 triggered a significant increase in the apoptotic responses of HSV-1- and HSV-2-infected cells.

Thus, both HSV types affect autophagy and apoptosis in a coordinated fashion, and autophagy plays cytoprotective role in HSV-infected cells via antagonizing apoptosis. Together these data implicate autophagy in the pathogenic mechanism of herpetic keratitis.

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FACING EBOLA – LESSONS LEARNT FROM THE FIELD

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Ebola virus, the causative agent of the current outbreak in West Africa, is one of the most virulent human pathogens with 40-90% case fatality rates. The virus is spread by direct contact of infected person's blood and other body fluids or via contaminated objects. According to our knowledge the putative first case of the outbreak was a 2-year-old child in the forest part of Guinea in December, 2013; the WHO was notified in March, 2014. At the end of September there were more than 5600 cases and the outbreak is also affecting high density urban populations too. The data is grossly underestimated and the outbreak is expanding exponentially with doubling period of 15.38 day. The phylogenetic analysis of the Ebola virus from the current outbreak suggests that the virus (ZEBOV-2014WA) belongs to the Zaire Ebola virus lineage. It is likely that the last three outbreaks caused by Zaire Ebola virus have a common ancestor and the ZEBOV-2014WA diverged around 2000. The genetic similarity amongst ZEBOV-2014WA sequences indicate a single transmission from the natural reservoir followed by human to human transmission. The high percentage of nonsynonymous mutations characterizes the ZEBOV-2014WA. The observed substitution rate is two times higher in the ZEBOV-2014W than in other Zaire Ebola viruses. The genomic surveillance data contribute to better understanding of the viral transmission dynamics, facilitate the monitoring of the viral changes and adaptation and help to improve diagnostic methods. Since March 2014 and as part of the international response, the European Mobile Laboratory (EMLab) provides uninterrupted field laboratory diagnostics for the surrounding areas in Gueckedou, Guinea. Colleagues of National Biosafety Laboratory at the National Center for Epidemiology spent two months in this field laboratory. The specimens (blood, swab, urine, breast milk) are handled in a negative pressure glove box designed for the EMLab. The main diagnostic testing profile is for Ebola virus and malaria but the EMLab also has the capability to detect other endemic pathogens such as Lassa virus, Dengue, *Vibrio cholerae*, etc. In this remote area of Africa, EMLab has had to face and manage several difficulties such as electrical shortages during laboratory procedures, receiving broken blood collection tubes, unpredictable specimen numbers and arrival time, limited place and resources, other logistic problems. The psychological and emotional pressure of the team members is also remarkable: working with one of the deadliest viruses under basic conditions, consequence of the false positive or negative tests and the though living conditions.
To defeat this enormous outbreak widespread collaboration is needed and the provision of accurate Ebola diagnostics is an important part of this huge struggle.

RUBELLA VIRUS PERTURBS AUTOPHAGY

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Autophagy is a cellular catabolic process implicated in numerous physiological processes and pathological conditions, including infections. Viruses have evolved different strategies to modulate the autophagic process. Since the effects of rubella virus (RV) on autophagy have not yet been reported, we evaluated the autophagic activity in the Statens Seruminstitut Rabbit Cornea cell line infected with the To336 strain of RV. Our results showed that RV lowered the levels of

microtubule-associated protein 1 light chain 3 B-II (LC3B-II) and the autophagy-related gene 12-autophagy-related gene 5 conjugate, inhibited the autophagic flux, suppressed the intracellular redistribution of LC3B, decreased both the average number and the size of autophagosomes per cell, and impeded the formation of acidic vesicular organelles. Induction of autophagy by using rapamycin decreased both the viral yields and the apoptotic rates of infected cultures.
Besides its cytoprotective effects, autophagy furnishes an important anti-viral mechanism, inhibition of which may reorchestrate intracellular environment so as to better serve the unique requirements of RV replication. Together, our observations suggest that RV utilizes a totally different strategy to cope with autophagy than that evolved by other positive-stranded RNA viruses, and there is considerable heterogeneity among the members of the Togaviridae family in terms of their effects on the cellular autophagic cascade. Moreover, the RV-mediated inhibition of autophagy may be implicated in the pathogenesis of the congenital rubella syndrome, as functional autophagic machinery is of pivotal importance for normal fetal development.
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SPECIES IDENTIFICATION OF WINE CELLAR MOULD DERIVED FROM THE TOKAJ WINE REGION, HUNGARY

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Zasmidium cellare (wine cellar mould, Syn.: *Cladosporium cellare*) was reported previously the most frequently occurred mould on the inner surfaces of the wine cellars, but other mould genus (e.g. *Alternaria, Aspergillus, Aureobasidium, Cladosporium, Geosmithia, Penicillium*, and *Verticillium*) have been identified, too. Since very few reports have been published in this topic in Hungary, we aimed to identify some isolates derived from the inner surface of some winery from the Tokaj wine region. Mycelial samples were collected from the inner surface of two cellars near to Tokaj in 2014 spring. The samples were collected with sterile forceps and were transported in sterile centrifuge tube to the Microbiological Laboratory of Institute of Food Science (University of Debrecen). Sample preparation and microbiological examination were carried out according to the protocol of Goto et al. [1]. Following the morphological tests, the molecular biological identification of the isolates was performed with the help of ITS1 and ITS2 sequences [2]. ITS4 and ITS5 primers were used for PCR amplification of the internal transcribed spacer regions [3]. The PCR products were purified with using NucleoSpin Gel and PCR Clean-up. Purified amplicons were sequenced by Mycrosinth, Austria. The sequences were aligned with Clustal X program, and manually corrected with GeneDoc. Phylogenetic analyses were performed with MEGA 5.05. Besides *Zasmidium cellare*, other fungi, like *Aspergillus cavernicola, Penicillium spinulosum, Penicillium biourgeianum, Fusarium armeniacum* and *Trichoderma atroviridea* were also identified from the inner surface samples of the wineries.

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BACTERIAL COMMUNITY STRUCTURE OF TWO EXPERIMENTAL FIXED-BED NITRIFYING REACTORS TREATING SLUDGE LIQUOR

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An experimental pilot-scale nitrification system was set up to treat anaerobically digested wastewater sludge liquor containing extremely high concentrations of ammonium-ion (1500-3800 mg/L NH_4^+-N) and organic matter (1900-4000 mg/L O_2). The system consisted of two fixed-bed reactor columns working in line with each other, filled with zeolite and ceramic beads (CB), respectively. The aim of this study was to explore the bacterial community structure of the two different biofilm carriers, particularly in view of identifying the community members being responsible for inorganic nitrogen and organic matter removal. The inflow, furthermore liquid and biofilm from different regions of the columns was sampled. The total bacterial community was investigated by terminal restriction fragment length polymorphism (T-RFLP) and clone library based on 16S rRNA gene. Ammonia-oxidising bacterial (AOB) community was studied by the T-RFLP analysis of ammonia-monooxigenase (*amoA*) gene. Nitrite-oxidising bacteria (NOB) were detected by specific 16S rDNA-based polymerase chain reaction (PCR). Denitrifying organisms were detected by nitrite-reductase (*nirS, nirK*) specific PCR. Eubacterial, AOB and NOB organisms were detected by 16S rRNA based fluorescence in situ hybridization (FISH) as well. At the time of sampling the two reactors showed similar efficiency of inorganic nitrogen and organic matter removal (both approx. 90%). According to the total bacterial community structure the inflow was considerably distinct from all the other samples, furthermore biofilm and liquid samples differed from each other. AOB were detected on the surface of zeolite in every region while in the case of CB only in the middle regions. In the reactor filled with zeolite *Nitrospira* and *Nitrobacter* spp. were detected in every region of the biofilm and liquid as well while in the case of CB only *Nitrospira* spp. were found.

Denitrification was detected in both of the reactors, on the surfaces of the biofilm carriers and in the liquid phases as well. The biofilm of zeolite was thick and well-structured unlike CB where only minimal biofilm growth was detected. Contrary, in the liquid of zeolite reactor mainly the torn pieces of the biofilm was detected while in the case of CB the liquid phase contained more complex bacterial community and large numbers of eukaryotic organisms.

SELENITE-STRESS SELECTED PROBIOTIC BACTERIA FOR DIETARY SE SOURCE PRODUCTION

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Bacterial cells usually possess low tolerance to selenite stress and also low ability to reduce high concentrations of toxic selenite. Here, high tolerance to selenite and selenium bioaccumulation capability were developed in probiotic and starter bacteria including *Enterococcus faecium, Bifidobacterium animalis* ssp. *lactis, Lactobacillus casei* and *Lactococcus lactis* ssp. *lactis* by food-

level strain development process and clone selection. All mutant clones possessed increased glutathione concentration and glutathione reductase activity. The selenite treatment increased further the values in *L. casei* mutant strain pointing at a different selenite reduction pathway and/or stress response in this organism.
Considerable conversion of selenite to cell bound and nanoselenium forms with a concomitant high biomass production was detected in *E. faecium* and *B. animalis* ssp. *lactis* cultures. Selenium deficiency is a major health problem worldwide for about 1 billion people; therefore, these strains can be recommended in small concentration for future dietary Se delivery.

BIODETOXIFICATION OF ZEARALENONE BY CELL-FREE EXTRACTS

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Zearalenone (ZEA) is a mycotoxin produced by *Fusarium* sp. which colonize mainly maize and wheat. By means of its estrogenic effect ZEA induces reproductive disorders in farm animals and humans. Elimination of mycotoxins from food and feedstuffs is an urgent issue, and biodetoxification is an appropriate way for this purpose. Previously a bioluminescent bioassay (*Saccharomyces cerevisiae* BLYES/BLYR) was successfully used to monitor toxin degradation; the results of ZEA biodegradation experiments were confirmed by parallel HPLC-FLD and ELISA tests. The most promising strains belong to *Rhodococcus* sp. In this study further researches were carried out with cell-free extracts of the analysed strains. Our aim was to reveal the enzymatic processes of microbes which have effective mycotoxin-degradation potential. The importance of this study has been confirmed by EFSA (European Food Safety Authority) which provides an assessment system named Qualified Presumption of Safety (QPS) concept that is applicable to assess the safety of microorganisms deliberately introduced into the feed and food chain. According to this assessment EFSA maintains a list on applicable biological agents. *Rhodococcus* sp. is not included on this list, so an enzyme-based formulation as a biodetoxification agent can increase the food safety. To understand the enzymatic processes of biodegradation, cell-free extracts were applied. Cultures were grown with or without ZEA before separating supernatant and pellet to reveal induced and constitutive enzymes. Moreover, to determine extra- or intracellular enzymes play role in biodegradation, samples were treated in different ways: extracellular enzymes were tested in the supernatant, while intracellular enzymes were tested by the help of sonication to destroy cells and release enzymes in the cell free matrix.
On the base of our experiments detoxification was not manifested when untreated supernatants were applied, thus intracellular enzymes may be involved in the degradation. Applied strains did not need pre-incubation with ZEA, since cell-free extracts after sonication could cease the estrogenicity of ZEA in one hour. Results indicated that the degradation is enzymatic and the enzymes responsible for the degradation are constitutively produced.
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DETECTION AND INVESTIGATION OF PLANT GROWTH PROMOTING HORMONES FROM SOIL BACTERIAL STRAINS

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Soil bacteria play a very important role in biogeochemical cycles. Plant growth promoting rhizobacteria (PGPR) are to supply nutrients to plants, to stimulate plant growth, e.g., through the production of plant hormones, to control or inhibit the activity of plant pathogens. Indole-3-acetic acid (IAA) is an important phytohormone that coordinates different developmental processes in plants. Indole-3-acetic acid is produced in young leaves, stems and seeds by the transamination and decarboxilation reaction of tryptophan. Gibberellins are ent-kaurene-derived diterpenoid phytohormones produced by plants, fungi, and bacteria. Gibberellins are involved in a number of developmental and physiological processes in plants, like seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth. Cytokinins are adenine derivative phytohormones that control cell division, cell cycle and stimulate developmental processes in plants. Cytokinins produced by rhizosphere microorganisms that live in close proximity to the root, may also influence plant growth and development. We collected soil samples from natural habitats and ploughed lands of Hungary. We isolated hundreds of strains from the samples and among these strains there were around 100 that produced indole-acetic acid (IAA). UV-Vis Spectrophotometry is an often used method to detect auxin production in liquid bacterial cultures. We can separate and identify IAA from the other indoles with TLC method by using Ehrlich reagent. TLC is a powerful technique in the purification, separation and identification of other plant growth promoting hormones, like gibberellic acid (GA3) and cytokinins too. After we screened the strains with TLC method for different hormone productions, the final identification and confirmation was by using high performance liquid chromatography (HPLC).

We chose enteropathogenic strains, which can produce IAA, gibberellic acid and cytokinin in higher concentrations. For comparison, we used two soil inoculant bacteria, *Kocuria rosea* and *Azospirillum brasilense*, which produce phytohormones. These strains are components of some commercially available microbial soil inoculants (Biofil® products).

The study was completed within the framework of the project entitled "Development of an effective soil microbial screening system and production of an instant powder soil inoculant formula" (Grant No. KMR_12-1-2012-0200).

INVESTIGATION OF THE VIRAL INFECTIONS OF THE HONEY BEE IN HUNGARY

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The honeybee (*Apis mellifera* L.) is a major pollinator of plants and is thus of global ecological significance. In addition, the products of bee origin such as honey, wax, royal jelly or propolis stress the worldwide economic importance of this insect. The intensifying international exchange of honeybee colonies and bee products has in the last decade increased interest in infectious diseases of bees. At present, at least 18 different viruses have been identified which are able to infect the honeybee. After demonstrating the presence of the acute bee paralysis virus in Hungary for the first time in 2003, a survey on the occurrence of six honeybee-pathogenic viruses was carried out in two periods, using one-step RT-PCR assays. Samples were collected between 2000 and 2001 in 52 and

later in 2007-2008 in Hungarian apiaries located in different regions of the country. The results of the assays on samples of adult honeybees and *Varroa* destructor mites were compared to similar surveys from France and Austria. The study demonstrates geographical differences in the prevalence of honeybee viruses between Hungary and the older EU member states. The results could serve as a basis for monitoring further changes in the distribution of honeybee viruses in Europe. Besides the honey bee, different wasp species were also screened, to investigate their role as honey bee virus carriers, since either as predators or as food parasites they regularly invade bee colonies. Beside the surveys, phylogenetic analysis of 22 Black queen cell virus (BQCV) genotypes collected from honeybee colonies in Poland, Austria and Hungary was performed on a partial helicase enzyme coding region (ORF1) and on a partial structural polypeptide coding region (ORF2). While the phylogeny based on the ORF2 region showed – with the exception of one strain from Poland – clustering of the genotypes corresponding to their geographic origin, the ORF1-based tree exhibited a completely different distribution of the Polish strains: three of them clustered within a branch clearly separated from all other central European BQCVs, while four other Polish strains remained well within the central European BQCV genotypes. In order to investigate this discrepancy in more detail, the nearly complete genome sequences of the three differing Polish strains were determined, together with one Hungarian sample. The sequences were aligned to each other and to the reference strain from South-Africa. Comparison of the different genome regions revealed that the 50-UTR and the intergenic regions of the BQCV genome are highly conserved with longer homologous sections. ORF1 (non-structural protein coding region) was found more variable compared to ORF2 (structural protein coding region). The 50-proximal third of ORF1 was particularly variable and contained several deletions/insertions. The sudden changes in the similarity levels of BQCV strains in different genomic regions are indicative of preceding recombination events.

CHARACTERISATION OF EGG LAYING HEN AND BROILER CECAL AND FECAL MICROBIOTA

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In this study we characterised the development of caecal microbiota in egg laying hens over their lifespan, from the day of hatching until 60 weeks of age. Using pyrosequencing of V3/V4 variable regions of 16S rRNA genes for microbiota characterisation, we were able to define 4 different stages of caecal microbiota development. The first stage lasted for the first week of life and was characterised by a high prevalence of Enterobacteriaceae (phylum Proteobacteria). The second stage lasted from week 2 to week 4 and was characterised by nearly an absolute dominance of Lachnospiraceae and Ruminococcaceae (both phylum Firmicutes). The third stage lasted from month 2 to month 6 and was characterised by the succession of Firmicutes at the expense of Bacteroidetes. The fourth stage was typical for adult hens in full egg production aged 7 months or more and was characterised by a constant ratio of Bacteroidetes and Firmicutes formed by equal numbers of the representatives of both phyla. In the second part of this study we compared the fecal microbiota composition in egg laying hens and broilers originating from 4 different Central European countries. The core of chicken fecal microbiota was formed by 26 different families. Rather unexpectedly, representatives of Desulfovibrionaceae and Campylobacteraceae, both capable of hydrogen utilisation in complex microbial communities, belonged among this core of microbiota families. Understanding such metabolic bacterial mutualisms in complex microbiota systems may

allow for interventions which might result in the targeted interventions reducing foodborne zoonoses such as replacement of Campylobacteraceae by Desulfovibrionaceae and a reduction of *Campylobacter* colonisation in broilers, carcasses, and consequently poultry meat products.
This study was supported by PROMISE project 7th Framework Programme.

SUSCEPTIBILITY OF *ACTINOBACILLUS PLEUROPNEUMONIAE* STRAINS TO DIFFERENT ANTIBIOTICS USED FOR THE TREATMENT OF PORCINE PLEUROPNEUMONIA

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Actinobacillus pleuropneumoniae is the causative agent of haemorrhagic necrotic pneumonia and fibrinous pleuritis of swine. Antibiotic susceptibility of 15 *A. pleuropneumoniae* strains isolated from pathologic cases of in Hungary between 2006 and 2012 was examined in the disk diffusion method with 20 antibiotics and antibiotic combinations used in the practice. The strains showed susceptibility to a wide range of antibiotics. When the susceptibility of 30 freshly isolated *A. pleuropneumoniae* strains to the five most frequently used antibiotics was examined in the disk diffusion test, the majority of the strains proved to be susceptible to ceftiofur, enrofloxacin, penicillin G and tiamulin, however some strains with moderate susceptibility to ceftiofur or enrofloxacin were also found together with one enrofloxacin and two penicillin resistant ones. About one third each of the strains was susceptible, moderately susceptible to and resistant against oxytetracycline. The antibiotics had wide ranges of minimum inhibitory concentrations, on the basis of them all strains were susceptible to ceftiofur, 26 (86.7%) to enrofloxacin and 19 (63.3%) to tiamulin. A total of 16 (53.3%) of the strains was only moderately susceptible to penicillin G and 6 (20%) were resistant against it. Only one oxytetracycline sensitive strain were found, while 5 (16.7%) showed moderate susceptibility and 24 (80%) were resistant to it. Examination of the antibiotic susceptibility of *A. pleuropneumoniae* with the disk diffusion method is less sensitive, so the results of the test have to be interpreted carefully.

THE EVOLUTION OF VIRULENCE IN BACTERIA

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Host-microbe interactions are fine-tuned processes governed by both host and microbial factors. The outcome of the interactions depends on the genetic background and actual susceptibility of the host, and the virulence of the microorganism. The broad palette of interactions can range from a peaceful coexistence called commensalism to infections with lethal termination. To find host niches, establish themselves, multiply, and elicit harmful process microbes need to be armoured with housekeeping and virulence machineries. Bacteria use various mechanisms to gain virulence capacity including mutations and horizontal gene transfer, and exploit sophisticated means to adapt to a new host species or develop tissue tropism. Expansion of bacterial colonisation capacity to specific mucosal surfaces and matrix components, enhancement the power of cell and tissue invasion, and development of hypertoxic clones will be exemplified through *in vitro* models and

animal assays. Special attention will be paid to the role of pathogenicity islands (PAIs) bringing forth to quantum leap development of virulence, and not respecting genus boundaries when transferred. After-transfer recombination and mutation events stabilize the presence of the virulence genes in the new host. Interestingly, these mutations may also result in loss of gene functions, and this way governs the pathogen back toward commensalism. Regulatory mechanisms play a pivotal role in host-adapted expression of virulence through global regulators managing multiple virulence genes, and hence harmonizing the functions into a concerted action. These processes will be discussed with reference to various *Yersinia* and *Escherichia* pathotypes. Emergence and disappearance of epidemic bacterial clones are driven by several biological and socio-economical forces. This issue will also be discussed in the presentation.

OCCURRENCE OF POTENTALLY AFLATOXIN-PRODUCING *ASPERGILLUS* SPECIES IN MAIZE FIELDS OF HUNGARY

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Possessing the knowledge on fungal contamination rate of stored maize in Hungary, we studied the presence of aflatoxin-producing *Aspergillus* species in the soil of maize fields and in the maize before harvest. A database was compiled from the results obtained from the mycological cultivation of soil and maize samples, and the soil and cultivation data concerning the studied maize field of 81 villages. The *Aspergillus* isolates were identified on the base of their phenotypic characteristics and their partial calmodulin sequence. Besides, aflatoxin B_1 production and sclerotia formation of the *Aspergillus* strains belonging to the *Flavi* section were simultaneously tested. *Aspergillus section Flavi* could be detected in 28.4% of the soil samples and 40.7% of the maize samples. The majority of the strains proved to be *Aspergillus flavus*, but two of them were found to be *A. parasiticus*. 66.1% of the strains represented L type; the rest did not form sclerotia at all.

We were not able to establish any correlation between the sclerotium formation and aflatoxin production measured by SOS Chromotest.

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SENSITIVITY OF MEMBERS OF THERMOPHILIC COMPOST MYCOBIOTA TO TOXIC COMPOUNDS

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Toxic compounds that occasionally contaminate the composted wastes may decrease the microbial activities during the thermophilic phase of the composting process. On the other hand, if sufficient data are available on sensitivities of thermophilic fungi, they could be used for ecotoxicological qualifications. We tested the sensitivity of strains representing thermophilic, compost inhabiting fungal species *Myceliophthora thermophila, Rasamsonia emersonii, Scytalidium thermophilum* and *Thermomyces lanuginosus* to copper and cadmium ions using the method of colony growth on poisoned agar media. Some differences among the sensitivities of the four species were found both to copper and cadmium ions as well as to the organic compound glyphosate. The EC50 values

concerning copper and cadmium ions and glyphosate broadly ranged in the testes species. As *S. thermophilum* appeared to be the most sensitive species we tested its use as an ecotoxicological test organism. The strain *S. thermophilum* Tm01 proved to be most sensitive member of the testes fungi to the inorganic and organic toxic compounds both in the agar diffusion growth test and in the spore-germination test. Thus, it can be recommended as a test organism in ecotoxicological qualification due to its sensitivity and fast-growing nature.

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COMPARATIVE MOLECULAR CHARACTERIZATION OF *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* STRAINS ISOLATED FROM HUNGARIAN SHEEP AND GOAT FLOCKS

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The genus *Corynebacterium* belongs to the order Actinomycetales that also includes the genera *Mycobacterium, Nocardia, Streptomyces*. Although this group is very heterogeneous, most of the species share particular characteristics, such as the cell wall composition and the high G+C ratio of the genomes. *C. pseudotuberculosis* is a Gram-positive, club-shaped rod, facultative intracellular bacteria that is commonly found in the environment as well as on the skin and mucous surfaces of animals. Based on biochemical features, the strains are classified into two biovars (*ovis* and *equi*). The strains within the biovar *ovis* presented negative nitrate reduction and mainly affect sheep and goats, causing caseous lymphadenitis (CL). The strains biovar *equi* reduce nitrate and cause ulcerative lymphangitis of the distal extremities, ventral abscesses of the thorax and abdomen, and furunculosis in horses. Occasionally, both types could generate subcutaneous abscesses or visceral organs in cattle. In addition, in rare instances, other domestic or wild animal species, and even human are affected. Due to the sale restrictions of pedigree sires, the reduction of milk, meat and woollen yields, expressive losses in reproductive efficiency, and condemnation of carcasses in slaughterhouse the caseous lymphadenitis causes significant economic losses to sheep producers worldwide. While the illness is common on the tropical areas, it occurs only sporadically in the Mediterranean. In default of the appropriate import regulations it has spread throughout the world by the pedigree sires. The aim of our study was to molecular comparison of phenotypically characterized *C. pseudotuberculosis* strains have been collected form sheep and goat flocks in different areas of the country over the past twenty years (1994-2014). We opted for the multilocus sequence typing (MLST) analysis. In the absence of data from literature, we were going to use the MLST had been developed for the analysis of human pathogenic *C. diphtheriae* strains recently (2010). However the applied primers were *C. diphtheriae* specific, the developing of own system was needed. For designing of appropriate primers, we have collected and compare of sequences of 16 commonly applied housekeeping using whole genomes of *C. pseudotuberculosis* at the Genebank. During the examination of the individual genes, the *C. pseudotuberculosis* and *C. diphtheriae* strains proved to be distinct in any case and two biovars were found to various in some measure. Finally, the 600-800 bp long regions covering utmost single-nucleotide polymorphism (SNP) between the biovars and present identical phylogenetic topology of the entire genes of *dnaK, odhA*, the *groL1* and *infB* were examined in detail. Although each of our strains were classified to *C. pseudotuberculosis* biovar *ovis*, the MLST examination demonstrated that the two biovars are

separated at the level of the individual housekeeping genes. In light of the results is thought-provoking that the strains belong to *ovis* and the *equi* biovars represent the same taxonomic group despite the differences of genetic and phenotypic properties (nitrate reduction), of host spectrum (sheep, goat; horse) and of the manifestation of induced disease.

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MOLECULAR ANALYSIS OF ADHESIVE SUBSTANCES IN *PASTEURELLA MULTOCIDA*

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Pasteurella multocida is a member of the normal flora of mucosal surfaces and a widely distributed pathogen of many animal species. Molecular bases of pathogenesis and protective immunity against *P. multocida* infections are far from fully understood. Putative virulence determinants like outer membrane proteins, iron uptake and acquisition proteins, sialidases, toxins and various adhesins may play a part in these mechanisms. Adhesins have a crucial role in mediating colonization and invasion of the host. Thus, their presence on the bacterial surfaces and their morphological variability usually correlate with virulence. The aim of our study was to characterize the molecular diversity of some earlier detected adhesive appendages, like auto transporters (*hsf*1,2), filamentous haemagglutinin (*pfh*B1,2) and fimbrial structures (tight adherence macromolecular transport system, type IV pili), in *P. multocida* strains using with data generated by direct and next generation sequencing. The different types of adhesives have a role in various stages and sites of infection. The filamentous haemagglutinins and autotransporters are essential for initial colonization of trachea while the Tad system and P4T are involved in biofilm initiation and development promoting bacterial persistence in the lower respiratory tract. Lack of proper annotation of most *P. multocida* genomes in the GenBank, the PM70 and multiple alignment (progressiveMauve algorithm) of other genomes (36950, NH06, 3480, X73, P1059) were used as a reference for selection of adequate genes from our assembling genomes. Exception of *pfh*B2, both of structural and functional genes of the above mentioned structures were detectable in all strains. The functional genes presented allele differentiations in various rates. The supporting of these results comparison of further gene sequences from different strains are needed. Detection and studying of these characteristic sequence variances we obtain useful information about structural changing of adhesins of strains possessing different adhesive features and the results yield targets for prediction of virulence.

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COMPARATIVE STUDY OF *AEROMONAS* SPECIES ISOLATED FROM FRESHWATER FISH AND HUMAN CLINICAL SPECIMENS

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Aeromonas species are oxidase- and catalase-positive, glucose-fermenting, facultative anaerobic, Gram-negative, rod-shaped bacteria. They are autochthonous to aquatic environments worldwide and have long been recognized as occasional pathogens. These bacteria have a broad host spectrum, with both cold- and warm-blooded animals, including man. In fish, they cause haemorrhagic septicaemia, fin rot, soft tissue rot and furunculosis. In humans, the most common clinical manifestations of Aeromonads infection are diarrhoea, gastroenteritis. In addition, they have role in induction of primary and secondary septicaemia in immunocompromised persons, wound infections in healthy individuals, and number of less well described illnesses such as endocarditis, peritonitis, meningitis, and infection of the eyes (corneal ulcers), joints, and bones. The genus *Aeromonas* consists of 31 species and is classified historically two main groups: non-motile, psychrophilic species, best represented by *A. salmonicida*, which generally responsible for fish and reptiles infection and a large group of motile mesophilic aeromonads which are associated with both piscine and human diseases. The literature data suggest that the vast majority (≥85%) of clinical isolates attributed to three mesophilic *Aeromonas* species (*A. hydrophila, A. caviae*, and *A. veronii*). Since the available data suggest that they are the most frequently motile species in the diseased fish as well, aim of our study was to carry out comparative studies on some *Aeromonas* strains isolated from human clinical specimens (stool) and freshwater fishes (catfish, pike, sturgeon, eel). The studied species were *A. hydrophila, A. caviae, A. veronii* and some closely related spp. (*A. media, A. sobria*). The traditional identification of strains was verified with sequence-analysis. For the detection of the occurrence of different virulence genes (lateral flagella, DNase, nuclease, serine protease, lipases, and toxins) was carried out with PCR, of the antimicrobial sensitivity (ampicillin, chloramphenicol, florfenicol, gentamicin, enrofloxacin, erythromycin, oxytetracycline, furazolidone, cotrimoxazole, polymyxin B) was applied the disc diffusion technique and of the pathogenicity with *in vivo* assay was studied. Although, the results have not been able to reveal significant differences between piscine and human isolates at the studied features, the occurrence of variety virulence genes proved to be typical of each species or closely related species. These differences also manifested during the pathogenicity assays. The strains were characterised by inherited ampicillin resistance and moderate variances in the antimicrobial sensitivity against chloramphenicol, enrofloxacin. All strains were characterised by inherited ampicillin resistance and moderate differences were detectable in their antimicrobial susceptibility to erythromycin, oxytetracycline, and cotrimoxazole.Since the majority of human infection related to the aquatic environments, the similarity of *Aeromonas* isolates from fishes and man is reasonable. However, further studies are needed to clearly define the role of each species in the different type of piscine and human diseases.

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DETECTION AND IDENTIFICATION OF SYMBIOTIC BACTERIA OF ROOT ENDOPHYTIC FUNGI LIVING IN SEMIARID AREAS

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It is generally known that certain fungi live in symbiosis with various bacteria in their cytoplasm and on the surface of their hyphae. These symbionts belong to diverse bacterial phylogenetic lineages. The role of symbiotic prokaryotes in the function of fungi is not completely understood. Dark septate endophytes (DSE) are a worldwide spread group of root-colonizing fungi. Our aims were to study bacteria associated with DSE isolated from semiarid areas. Several bacterial lineages were identified from DSE strains by analyzing clone libraries of 16S rRNA gene segment (27F-519R). Additionally, the visualization of bacteria associated with DSE strains using flurescence in situ hybridization (FISH) technique will be also presented.

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RETICULATE EVOLUTION OBSCURES SPECIES BOUNDARIES IN *METSCHNIKOWIA*

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In modern fungal taxonomy, species delimitation is mainly based on sequence differences of evolutionarily conserved genes. In a comparative analysis of six DNA regions performed by an international consortium of mycologists, the nuclear internal transcribed spacer (ITS) region proved to be the DNA marker with the most clearly defined barcode gap between inter- and intraspecific variation for the broadest range of fungi [1]. It was proposed for adoption as the primary fungal barcode marker. The ITS regions are present in the rDNA arrays of the fungal genomes in multiple copies. The use of ITS sequences as species-specific barcodes is based on the assumption that all copies in a genome have identical sequences due to genetic homogenization (concerted evolution). In a previous study [2] we found that the rDNA arrays of the type strains of two pulcherrimin-producing *Metschnikowia* species (*M. andauensis* and *M. fructicola*) are not homogenized. Their arrays consist of diverse repeats that differ from each other in the D1/D2 domains of the large subunit (LSU) rRNA genes. The variable sites were concentrated in regions corresponding to back-folding stretches of hairpin loops of the mature LSU rRNA molecules [2].

Here we show that the type strains of all known pulcherrimin-producing species, but not the pigment-free *M. chrysoperlae*, have non-homogenized polymorphic rDNA repeats. The phylogenetic and network analyses of the sequences of cloned repeats indicate that the pigmented species share a continuous pool of diverse D1/D2 domains and ITS1-5.8S-ITS2 regions that appear to evolve by reticulation including horizontal interspecies exchange of sequences.

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ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* IN THE CENTRAL EUROPEAN COUNTRIES

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Recently, several European countries have invested heavily in reducing pathogenic *Campylobacter* transmission, especially via specific food (mainly poultry meat) chains, but with very limited success – campylobacteriosis with the notification rate of 55.49/100,000 in year 2012 remains the leading food-borne illness and the most frequently reported zoonosis in humans in EU. *Campylobacter jejuni* is the most prevalent cause of bacterial gastroenteritis with still increasing trend in EU [1]. Moreover, the officially reported prevalence numbers are thought to be significantly underestimated, due to underdiagnosis and underreporting in different countries. Beside high prevalence in animals and retail (broiler) meat, the increasing antimicrobial resistance is a prompt problem, including multidrug resistant *Campylobacter* transmitted via food chain [2]. The resistance to ciprofloxacin, one of the first drugs of choice for treatment of campylobacteriosis has a more pronounced increasing trend in southern EU (e.g. Spain, with 84.1% of resistance rate among human isolates in 2012) and in central EU, including A, Sl and HU with 61.2, 70.7 and 79.4% of resistance rate, respectively [3]. As an evident public health risk, *C. jejuni* was included also in the microbiological isolation scheme of the "Promise" project – analyzing products of animal origin (POAO), illegally imported by travelers to the EU. However, most illegally imported POAO were durable food products, e.g. fermented sausages and aged cheeses, and consequently, due to the low water activity and low pH, harbored no *C. jejuni*. Alternatively, a *C. jejuni* strain database was established covering also the isolates from other sources in the "Promise" partner countries (N=415). We determined antibiotic resistance profiles for seven antibiotics by broth microdilution method, with PCR detection of resistance genes and with mismatch amplification mutation assay and sequencing of specific regions like Quinolone Resistance-Determining Region (QRDR) in *gyrA* gene. Multilocus sequence typing in seven housekeeping genes (*aspA, glnA, gltA, glyA, uncA, tkt, pgm)* and PFGE was done for the isolates from human, animal, and meat sources as well as from environmental (surface water and other) samples. The isolates (n=103) were divided in groups based on the sequence of *gyrA* QRDR and compared with the MLST typing results.

Correlation was confirmed between CC21 and *gyrA* cluster 2 (87.8%), as well as between *gyrA* cluster 7 and CC353 (32.3%) and CC354 (23.5%). We conclude that the high ciprofloxacin resistance prevalence observed indicates the clonal spread of quinolone resistance with CC21, not only locally as recently published [4], but also in geographically wider EU region.

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MOLECULAR ANALYSIS OF MEMBERS OF AN INTERNATIONAL CLONE OF MULTI-RESISTANT *BACTEROIDES FRAGILIS* ISOLATES

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During our investigations a particular set of genetic constitution was frequent among carbapenem and metronidazole-resistant *B. fragilis* strains from the UK, Sweden, France, Hungary and the USA. We aimed to determine the molecular characteristics and the expected clonality of such strains (n=7). The 7 *B. fragilis* strains were examined for antibiotic susceptibilities by gradient technique (Etests, bioMerieux) (piperacillin, piperacillin/tazobactam, cefoxitin, meropenem, clindamycin, moxifloxacin, metronidazole, teracycline and tigecycline), resistance gene (*cepA*, *cfxA*, *cfiA*, *nimB*, *ermF*, *tetQ* and *tetX*) and insertion sequence (IS*1186* and IS*4351*) element contents by PCR, nucleotide sequence environment of the *cfiA* and *nimB* genes. The transferability of the *cfiA*, *nimB*, and *tetQ* genes was tested by conjugation. The clonality of the isolates was tested by ERIC PCR (ca. 20 and 12 *cfiA*-negative and positive strains were additionally included respectively) and PFGE. Most of the isolates were resistant to piperacillin, piperacillin/tazobactam, cefoxitin, meropenem, metronidazole, clindamycin and tetracycline and susceptible to moxifloxacin and tigecycline and uniformly were positive for the following genetic elements: *cfiA*, *nimB*, IS*1186* and IS*4351*. While the *tetQ* genes were transferred to a susceptible host the *cfiA* and *nimB* genes were not. The analysis of the excised circular intermediate of the tet^R elements indicated that they may be different. All the *nimB* genes and some of *cfiA* genes were preceded by IS*1186* and this latter case was true for the *ermF* IS*4351* pair. A ca. 4 kb portion nucleotide sequence surrounding the *cfiA* gene in *B. fragilis* 1672 was determined and the structure of this region has been found to be similar in the other strains by long PCR amplification. ERIC PCR typing implicated a clonal relationship of these isolates while the PFGE typing gave a diverse pattern and implicating a long evolutionary history. Because of the constant occurrence of the *cfiA*, *nimB*, IS*1186* and IS*4351* elements and because of the clonality in ERIC PCR we expect the common origin of these internationally gathered *B. fragilis* strains. However, because of the differences in antibiotic susceptibilities and other molecular characteristics (e.g. different tet^R elements and diverse PFGE patterns) we infer that this clone evolved early at least before the widespread of tet^R elements among *B. fragilis* strains.

CHARACTERIZATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE FROM *ASPERGILLUS NIDULANS*

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Gamma-Glutamyl transpeptidase (gammaGT) has been known for a long time as the only enzyme capable of degrading glutathione (gamma-L-Glu-L-Cys-Gly; GSH). Recent developments in this field demonstrated that not gammaGT but the DUG system is responsible for intracellular degradation of GSH in *Saccharomyces cerevisiae* [1] and alternative pathways for GSH degradation were described in several other eukaryotes [2, 3]. Therefore, physiological function of gammaGT

became questionable. *Aspergillus nidulans* produced high gammaGT activity in both carbon-starved and carbon-limited cultures. Accumulation of gammaGT activities were observed both in the fermentation broth and in the hyphae. Our previous experiments demonstrated that gammaGT was encoded by the AN10044 gene and this enzyme was not necessary for the bulk degradation of GSH observed in carbon stressed cultures. Amino acids and peptides could enhance the induction of AN10444 gene and functional *meaB* was necessary for this process. Extracellular gammaGT was partially purified by fractionated $(NH_4)_2SO_4$ precipitation and ion exchange chromatography. The enzyme was 55 kDa (SDS-PAGE). It worked in a relatively narrow pH (pH 7-9) and temperature (<60 °C) range. Beside of Gly-Gly it could use Cys-Gly, Cys and Glu as gamma-glutamyl acceptor and it also had hydrolase activity. Indirect evidences suggest that this enzyme can use Gln as gamma-glutamyl donor. Intracellular gammaGT was also purified partially and showed similar properties to its extracellular form. Possible physiological function of gammaGT will be discussed.

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POTENTIAL CANDIDATES FOR IMPROVEMENT OF ANAEROBIC DEGRADATION OF LIGNOCELLULOSIC BIOMASS

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Depletion of fossil fuels and increase of greenhouse effect demand the use of renewable energy carriers more extensively. Biogas is formed anaerobically during the decomposition of various organic substances. Biogas generation is the result of the concerted action of the diverse microbial community present in the closed system. Polymer degradation, volatile fatty acid production and methanogenesis comprise the major stages of the complex process. The two main components of the biogas are methane and carbon-dioxide. Cellulosic biomass is the single largest amount of biomass on Earth. Plants can harvest solar energy during photosynthesis and convert it to plant tissues. Plant tissues consist of lignocellulose as the major component. Lignocellulose is composed of cellulose, hemicellulose and lignin. Cellulose is a complex polymeric carbohydrate, cellulases are needed for its efficient decomposition. Cellulases are divided into three major groups: endoglucanases, exoglucanases and β-glucosidases. For the utilization of substrates having high cellulose content, the biogas producing microbial community should include cellulose degrading microbes, which can break down cellulose to easily utilizable sugar monomers. A strategy to adapt the community to cellulosic substrate has been developed. The experiments were carried out under thermophilic conditions at 55 °C. A-cellulose was used as substrate and the control fermentors received glucose as carbon and energy source. From the adapted microbial community cellulose degraders were isolated and were also used as inoculum in the next set of biogas experiments. The cellulose degrading microbes had positive effect, elevated the biogas and methane yield. DNA was purified from the cellulose degrading consortia and was subjected to metagenome analysis. Next generation sequencing of the total DNA samples identified the main orders being Thermoanaerobacterales and Clostridiales with 70% and 10% abundances, respectively. *Thermoanaerobacterium thermosaccharolyticum, Caldanaerobacter subterraneus, Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum* were the predominant strains. In the subsequent experiments the effect of these strains on the biogas production from lignocellulosic substrates is investigated with

the aim of constructing a defined cellulolytic consortium effective in the intensification of the thermophilic anaerobic biodegradation of lignocellulose-rich substrates.

MANNOBIOSE AND MANNOTRIOSE SYNTHESIS BY A COMMERCIAL ENZYME PREPARATION FROM *ASPERGILLUS ACULEATUS*

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Manno-oligosaccharides (MOS) that contain mannose units in α-linkages are widely used in nutrition as a natural additive and thus own a particular attention in both the science and the industry. Some clinical evidences proved that MOS might promote the health of gastrointestinal tract as well as of the whole body with enhance of efficiency of the immune system. Recently, most MOS products are derived from the cell wall of the yeast, *Saccharomyces cerevisiae*, but in nature, there are many interested saccharides formed by α-mannopyranosyl linkages such as saccharides in glycoproteins. Up to day, many studies can be found in literature dealing with glycoproteins, but synthetize and understanding functions of these types of MOS are still challenge for many research groups. In this study, the production of the mannobiose and the mannotriose by reverse hydrolytic activity of a commercial enzyme preparation (Pectinex ultra SP-L) as well as optimisation of different conditions were aimed. The effect of substrate concentration was analysed in range of 10-80 g/100ml in the pH range from pH 3.0 to 7.0. The enzyme reactions were kept at temperature range from 50 °C to 80 °C. The effect of enzyme : substrate ratios (2.7-3.9 mg protein to gram substrate) were also investigated. The bioconversion ran for 96 hours and samples were taken in 24 hour time periods. Carbohydrates were monitored by HPLC and TLC methods. The commercial enzyme preparation was able to catalyze reverse hydrolysis reaction on mannose substrate to produce mannobiose and mannotriose. The highest reverse hydrolytic activity was observed at 60 g/100 ml of substrate concentration. The optimal pH and temperature for enzyme reaction were determined to be pH 5.0 and 70 °C, respectively. Maximum productivity of mannobiose was obtained when ratio of enzyme : substrate 3.1 mg protein/g substrate. In the case of mannotriose synthesis, the highest yield was detected at 3.9 mg protein/g substrate ratio. Use of the optimal parameters resulted 10.6 g/100ml disaccharide as well as 1 g/100ml trisaccharide concentration. Our results give new ways in the synthesis of mannose based oligosaccharides. More studies are needed to better understand the mechanism of the reaction.

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CHIMAERIC GENOME STRUCTURE OF A NOVEL SHIGA TOXIN (STX1) ENCODING CONVERTING BACTERIOPHAGE FROM THE ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157:H7 SAKAI STRAIN

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Shiga toxin-producing *Escherichia coli* (STEC), and especially enterohemorrhagic *E. coli* (EHEC) are important, highly virulent zoonotic and food-borne pathogens. One of their main virulence factors, the Shiga toxin represents a family consisting of two main types (Stx1 and Stx2) encoded by lambdoid prophages. The prototypic, fully-sequenced EHEC O157:H7 Sakai strain carries both types of Stx toxin, and is known to harbour several prophages including Sp5 and Sp15, which carry the genes encoding Stx2 and Stx1, respectively. Phage induction experiments revealed a lytic phage of novel genotype carrying the stx1 gene cluster. The phage showed Podoviridae morphology, and was able to lysogenise multiple lines of the *E. coli* K-12-derived C600 and MG1655 strains. Shiga toxin production of lysogenised MG1655 lines was verified on Vero-cell cultures. We determined the whole genome sequence of the phage, which is 61,137 bp long and contains 93 open reading frames (ORF). Genome analysis revealed significant recombination between Sp5 and Sp15. The majority of the novel stx1-encoding recombinant phage genome originated from the stx2-encoding prophage Sp5, with major rearrangements in its gene order. The phage genomic region containing the *stx2* genes in Sp5 was replaced by a region containing six ORFs from prophage Sp15 including the *stx1* genes, forming a chimaera of these two prophages.

In summary, the reported chimaeric genome structure represents a new type of recombinant Stx1 converting phage from the Sakai strain, furthermore, the existence of a novel Stx phage suggests its potential dissemination in nature by phage transduction.

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CELL LENGTH GROWTH PATTERNS IN FISSION YEAST REVEAL A NOVEL SIZE CONTROL MECHANISM OPERATING IN LATE G2 PHASE

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Individual cell length growth patterns were analysed of several fission yeast strains, wild type and some cell cycle mutants by fitting different functions to the data. During the growing period, most cells' growth patterns could be best described by a bilinear function (two linear segments separated by a breakpoint, RCP2); however, linear patterns also occurred in several cases, but exponential ones only very rarely. The bilinear patterns were separated into two growing parts by RCP2, and the position of size control was examined by regression analyses of appropriate growth parameters in both segments. The existence of known size controls in late G1, in mid G2, and in late G2 during the fission yeast cell cycle was verified.

However, in spite of the former general view, we now discovered that late G2 size control is a general characteristic third event in the cycle, when cell size is monitored. The level of the critical late G2 size to be reached in an individual fission yeast cell is influenced by its growth rate, similarly to budding yeast, which suggests an evolutionary conserved mechanism.

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TAXONOMIC AND FUNCTIONAL COMPOSITION OF AN ASTATIC SODA POND PRIOR TO COMPLETE DESICCATION REVEALED BY METAGENOMIC ANALYSES

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Alkaline shallow ponds of the Carpathian Basin are unique environments, comparing to other aquatic habitats. Their physical and chemical characteristics (high salinity, high turbidity, high pH) provide an extreme environment for the aquatic life. Our study aimed to obtain a more detailed view about the structure and function of the microbial community with the tools of next generation sequencing (NGS). Water sample was taken from Büdös-szék pond (Hungary) in November 2012. After DNA extraction, an amplicon and a shotgun library was prepared and sequenced by two different NGS platforms. For the identification of bacterial taxa, primers specific for the V3-V4 region of the 16S rRNA gene were used, and amplicons were analysed on a Roche GS Junior sequencer. For the shotgun study, total environmental DNA was subjected to enzymatic fragmentation and the ~180 bp length fragments were sequenced on a benchtop Ion Torrent Machine, Life Technologies. After quality filtering, phylogenetic identification of the resulting 16S rDNA sequence reads were processed using the mothur v1.33 software using the ARB-SILVA reference database. Resulted reads of the shotgun study were assembled to contigs using the SPAdes software and metagenomics assignment was performed on the MG-RAST server.
Results showed a taxonomically and functionally complex microbial community structure dominated by members of the domain Bacteria. The most abundant groups were Proteobacteria, Actinobacteria, and Bacteroidetes, with the remarkable contribution of uncultured Actinomicrobiaceae and the genera *Gracilimonas, Hydrogenophaga, Roseovarius*, and *Belliella*. Metabolomic analysis indicated that genes involved in one carbon metabolism were abundant.
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PLANKTONIC MICROBIAL COMMUNITIES OF HELIOTHERMAL LAKES WITH DIFFERENT HUMAN IMPACT

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Some of the saline lakes possess a special thermal stratification, called heliothermy, when solar heating results in an underwater temperature maximum. In July 2013, three deep (>15 m), heliothermal, saline lakes were studied in Romania (region Transylvania), which had different human impact. Lake Fără Fund in Ocna Sibiului is a natural conservation area, Lake Ursu in Sovata, the largest heliothermal salt lake in Europe, is used as a health spa during summer, while the highly polluted, unnamed lake in Ocna Mureș was formed in 2010 due to the collapse of a salt mine-shaft that entombed a complete supermarket. Physicochemical analysis revealed that steep stratification existed in all lakes having a transition zone at around 2-4 meters depth, with the highest temperature (32.8 - 41.7 °C) and the characteristic increase of salinity (up to 260 mS cm^{-1}). In Lake

Fără Fund, the phytoplankton was dominated by *Dunaliella* sp. with maximum chlorophyll a at 1 m depth (15 µg L^{-1}). In Lake Ursu, picoeukaryotic algae (75 x 10^4 cells mL^{-1}) caused a deep chlorophyll a maximum (100 µg L^{-1}) at 2.5 m depth; below that, a bacteriochlorophyll c maximum (characteristic pigment of green bacteria) was found at 3 m (chlorophyll a + bacteriochlorophyll c was ~ 400 µg L^{-1}). The highly polluted lake in Ocna Mureş had a chlorophyll a maximum at 3.8 m depth (58 µg L^{-1}). Pyrosequencing revealed the dominance of phyla Bacteriodetes, Cyanobacteria, Proteobacteria, Firmicutes (and additionally Chlorobi in Lake Ursu) in these heliothermal saline lakes. The euphotic zone was dominated with sequences from small cyanobacteria (e.g. *Synechococcus*) and/or chloroplasts, while in the case of Lake Fără Fund genus *Salinibacter* (red-colored bacterium with a light-driven proton-pump) and in Lake Ursu genus *Prosthecochloris* (anaerobic green sulfur bacterium) contributed significantly to the bacterial community around 3 m. Strictly anaerobic genera, such as the sulfate-reducer *Desulfonauticus*, were characteristic in the deeper zones of the lakes. Our results have shown that heliothermal saline lakes with remarkably different phototrophic microbial communities may exist even within a geographically restricted area.
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DEVELOPMENT OF THREE *IN VITRO* AGE-SPECIFIC HUMAN DIGESTION MODELS

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In vitro digestion models are widely used to study the structural changes, digestibility and release of food components under simulated gastrointestinal conditions. The most accurate results usually provide the *in vivo* feeding trials, using humans or animals. However, they are time consuming and costly. The main advantage of the *in vitro* digestion models are that they rapid and inexpensive tools for investigation of food components with an acceptable precision before deeper evaluations (e.g. human clinical trials) or bringing products to the market. In this study, we report the development of three different age-specific *in vitro* digestion models. The basic digestion model simulates the gastrointestinal conditions of a healthy adult and our aim was to extend and modify this model to simulate the digestion of infants and elderly people. The basic model consists of four phases, imitating the digestion process in mouth, stomach, small intestine and large intestine. The digestion, based on physiological conditions, is typical for each compartment. A continuous colon model was set up to represent the human colonic microbiota during 96 hours of fermentation. The large intestinal phase was represented by using two potentially pathogenic (*Clostridium, Bacteroides*), two beneficial (*Bifidobacterium, Lactobacillus*) and two "ambivalent" strains (*E. coli, Enterococcus*). Selective agar plates and real-time PCR analysis were used to evaluate the composition of bacterial species. The age-specific models were slight variations of the basic adult model using different digestion enzymes and different gut microbiota population. The infant digestion model used reduced quantities of certain digestive juice components (such as bile salts and proteases), and only two bacteria (*Lactobacillus* and *Bifidobacterium*) represented the intestinal flora. The other modification simulated the digestion of elderly people. In this model less α-amylase was used in the mouth section and reduced mechanical stress in the stomach. The amount of gastric juice was reduced but the gut transit time was increased. The microbiota population was also different: the number of facultative anaerobes was increased and the amount of useful organisms was reduced. The development of age-specific *in vitro* digestion models offers a suitable alternative of clinical trials to test the potential effects of age-related foods.

CONSEQUENCES OF PERIPLASMIC INVERTASE PRODUCTION ON GROWTH, SURVIVAL AND COLONY FORMATION IN *SACCHAROMYCES CEREVISIAE*

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Saccharomyces cerevisiae produces invertase to utilize sucrose as carbon and energy source. Due to periplasmic location of the enzyme a significant amount of the formed glucose and fructose is released into the fermentation broth. These hexoses function as "public goods": they can be utilized by cells other than producers, even those which have no invertase activity. Invertase (Suc2) production of *S. cerevisiae* is a frequently used model system to study cooperation among microbial cells [1]. The cost of invertase production and the ratio of the released/produced hexoses are key parameters of mathematical models describing the behavior of "cooperators" (suc2+ cells) and "cheaters" (suc2- cells) in mixed cultures. These parameters basically govern the costs and benefits of cooperation and cheating. We experimentally investigated effects of the cost of invertase production by comparing the specific growth rates of invertase producing, overproducing and not producing cultures and determined the survival of suc2+ and suc2- cultures in carbon source free medium. Colony forming ability of single cells (precultured on either glucose or sucrose) on plates containing sucrose and/or glucose was also tested to see the effects of released/produced hexose ratio. Our results suggest that the fitness decreasing effect of invertase production is much more significant in the lag phase than during the exponential phase of growth. We also found that the weak colony forming activity of single cells on sucrose plates can be explained by the cost of invertase production rather than by the high released/produced hexose ratio.

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DIFFERENCES IN THE REGULATORY FUNCTIONS OF THE VIRAL GENOME MAY PLAY A MORE IMPORTANT ROLE THAN AMINO ACID POLYMORPHISMS IN DETERMINATION OF THE CLINICAL SEVERITY OF HPV11-ASSOCIATED RESPIRATORY PAPILLOMATOSIS

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Severity of respiratory papillomatosis is highly variable from solitary papillomas to severe recurrent papillomatosis. As human papillomavirus type 11 (HPV11) causes more severe disease than other HPVs, it is plausible that viral factors play a role in determination of disease severity. Complete genomes of seven HPV11s from seven Hungarian patients with respiratory papillomatoses of different severity were determined and compared to the 42 complete genomes available in the GenBank. None of the patients had known immune function impairment. Transactivating activity of the long control region (LCR) of the viruses as well as of LCR constructs created by site-directed mutagenesis containing the single nucleotide polymorphisms found in the different LCRs were compared in transiently transfected Hep2 cells. Phylogeny of complete genomes suggests the existence of two lineages (A1 and A2). As clinical severity data are unavailable in case of most

GenBank genomes, conclusions on virulence properties of the lineages cannot be drawn. All seven Hungarian genomes belong to putative lineage A2. Most differences are not translated to amino acid polymorphisms; altogether five amino acid polymorphisms were found between viruses from disease with different severity in the E1, E2, E4 and L1 proteins. Two (C7479T and the T deletion at position 7509) of six single nucleotide polymorphisms were universal for the Hungarian sequences. One polymorphism (T7546C) caused significant decrease of LCR activity. Five patterns were identified among Hungarian sequences; one was associated with solitary papillomas, the other four was associated with moderately (6-9 recurrences confined to the larynx) or highly (>30 recurrences with extralaryngeal appearance) aggressive papillomatosis.However, these patterns were not unequivocally associated with severity, as viruses from a moderately and a highly aggressive papillomatosis had identical LCRs. These two viruses differed only in a single amino acid in protein E4. In summary, amino acid polymorphisms in coding regions of HPV11 are rare and not consistently associated with disease severity; this was better explained by the transactivating potential of the LCR. Viruses with identical LCRs were found to be associated with different disease severity, suggesting that protein polymorphisms and differences in LCR activity may synergize to enhance virulence or pointing to the role of other viral regulatory or even host factors.

EVOLUTION OF *AGROBACTERIUM*-HOST PLANT INTERACTION

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Crown gall disease caused by tumorigenic agrobacteria causes serious economic losses on several, mainly woody crop plants. The disease can be characterized by intensive unorganized cell proliferation (*Agrobacterium tumefaciens*, *A. rubi*, *A. vitis*), or hairy root formation (*A. rhizogenes*). During the infection process a part of the pTi (pRi) plasmids, the T-DNA becomes integrated into the host chromosome resulting in stably transformed cells. The T-DNA genes direct the synthesis of plant hormones resulting in tumorous growth. Additionally, crown galls and hairy roots produce specific amino acid derivatives, called opines. Although agrobacteria are known as plant pathogens, their disarmed (non-tumorigenic) pTi and pRi derivatives are widely used in plant biotechnology as gene vectors. Colonization of plants by agrobacteria is determined by the nutrient sources provided by the plants, and the ability of the pathogen to utilize them. Thus colonization can be considered as an indirect pathogenicity factor since by itself it does not necessarily result in tumor formation. The grapevine - *A. vitis* interaction is a typical example of such metabolic adaptation. Grapevines produce tartrate and *A. vitis* is able to utilize it as a nutrient. Genes responsible for tartrate utilization are located on large plasmids called pTr. These plasmids can be conjugally transferred *in planta* to other agrobacteria, e. g., to *A. tumefaciens* strains promoting their growth in grapevine tissues. pTrs can be allocated to at least four incompatibility (*inc*) groups, suggesting their diverse evolutionary origin. This genetic diversity confers a wide ecological flexibility, as it allows them to coexist in different combinations with pTis in the same bacterial cell. Tartrate produced by the host plant and the ability of *A. vitis* to metabolize this compound may be important factors in the maintenance and propagation of pTrs as indirect virulence factors in the microbial population of grapevine. In agrobacteria, oncogenes (factors directly responsible for tumor formation) and opine synthesis genes are located on other conjugative plasmids called tumor inducing (pTi) plasmids. Like pTrs, pTis form also several *inc*-groups, although the occurrence of different pTis in the same bacterial cell is a rather rare event. These large (approx. 200 kbp) plasmids contain two type IV secretion systems. The first is responsible for conjugal plasmid transfer of pTis to other bacterial

cells (procaryote→procaryote DNA transfer), and the second encodes genes for the T-DNA transfer to plant cells (procaryote→eucaryote DNA transfer). Opines produced by the tumorous plant cells serve as selective nutrient sources for the inducing bacterium, and they may also induce conjugal pTi plasmid transfer. Thus they contribute to the maintenance and spread of pathogenicity factors by two different ways. The evolutional origin of oncogenes and opine synthesis genes is still unknown. In the early 80s' White and coworkers found genes that showed high similarity to pRiA4 T-DNA sequences in *Nicotiana glauca*. Later studies revealed the presence of such sequences, called "cellular T-DNAs", in several *Nicotiana* as well as in *Linaria* species. More recently five cT-DNAs were found in *N. tomentosiformis* and *N. otophora* with *rol*, *6b* and opine synthesis gene homologs. These sequences are probably derived from ancient transformation events that might provide ecological advantage (e. g., acclimatization to drought) to the transformed plants. The cT-DNAs are important evolutionary footprints to follow the evolution of *Nicotiana* species.

OCCURRENCE OF METALLO-BETA-LACTAMASE PRODUCING *PSEUDOMONAS AERUGINOSA* STRAINS IN TAP WATER BEFORE AND AFTER HYDROGEN PEROXIDE VAPOUR DISINFECTION IN INTENSIVE CARE UNIT

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Pseudomonas aeruginosa most frequently colonize and causes nosocomial infection in intensive care unit (ICU) patients [1, 2]. This single centre study examined tap water as a source of this bacterium in a 10-bed intensive care unit at a university hospital before and after hydrogen-peroxide disinfection [3]. 500-500 ml cold water was collected from each tap into sterile flasks which do not contain sodium thiosulphate to inhibit the effects of water chlorination. Two taps were found in social rooms for health care workers and the other five were in patients' rooms. Disinfection was performed in the whole unit. Colonies were identified after membrane filtration and culture. Antibiotic sensitivity of the strains was performed. Resistance genes were determined by PCR. No pathogenic strain was found in the tap water of social rooms. Before disinfection one tap water was sterile and *Pseudomonas aeruginosa* strains were isolated from the samples of other patients' rooms. One strain carried metallo-beta-lactamase gene (*blaVIM-1*). After hydrogen peroxide vapour disinfection the samples from social room did not contain pathogens, but *Pseudomonas aeruginosa* were isolated from each sample from the patients' rooms. Metallo-beta-lactamase producing *Pseudomonas aeruginosa* strains were isolated from three samples of patients' room. These strains were also positive *blaVIM-1* gene. Hydrogen peroxide vapour disinfection was not effective for *Pseudomonas aeruginosa* colonisation in tap and metallo-beta-lactamase producing strains appeared in other rooms' tap. Metallo-beta-lactamase gene *blaVIM-1* as a part of class 1 integrons can locate chromosomally or on plasmid [4].

This and antibiotic selective pressure in the treatment may give opportunity to transfer this gene to other bacteria in patients when use the tap water for cleaning the patients.

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HIGH RESOLUTION MELTING AND OTHER PCR-BASED METHODS FOR DISCRIMINATION OF *LACTOBACILLUS* ISOLATES

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Fermentation of food products is generally based on the activity of various *Lactobacillus* species. Though, some traditional microbiological methods (e.g. selective media or API tests) are in use every day for identification of lactobacilli, distinguishing of closely related species is not an easy task, the results are often unambiguous. DNA based molecular methods offer the potential of exact identification; however, it seems necessary to combine more than one technique in order to get clear results. In our experiments four different techniques were used for separation of *Lb.* isolates of food origin. General 16S rDNA primers were used for amplification of an approximately 1100 bp long fragment that was sequenced, and then the sequences were blasted against the NCBI GenBank database. A second portion of the fragments was digested by three restriction enzymes in order to generate unique patterns by gel electrophoresis. Species-specific primers helped in discrimination of *Lb. casei*, *Lb. paracasei* and *Lb. rhamnosus* species, each member of the '*Lb. casei*' species complex. We have designed a new primer pair that was used in High Resolution Melting experiments. Sequencing and restriction digestion are useful methods for separation of most of the *Lb.* species. Nevertheless, *Lb. casei*, *Lb. paracasei* and *Lb. rhamnosus* species were not distinguishable with these methods. In contrast to that, species-specific primers worked well and these three species were separated from each other. High Resolution Melting proved its efficiency, as well. Though, we have found that peaks of melting curves depend on the composition of the PCR mix, each *Lb.* species were separable from each other, including subspecies of *Lb. paracasei* ssp. *paracasei* and *Lb. paracasei* ssp. *tolerans*. In the near future we plan to expand HRM analysis for more *Lb.* species in order to establish a rapid and cost-efficient identification tool for laboratories.

A 6-GENE PHYLOGENY OF THE GENUS *ASPERGILLUS* FOCUSING ON SECTION NIGRI

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Aspergillus is among the economically most important fungal genera. Species belonging to section Nigri (black Aspergilli) of this genus play an important role not only as human pathogens and mycotoxin producers but also in the food and biotechnological industries due to their ability to produce hydrolytic enzymes (lipases, amylases) as well as organic acids (citric acid, gluconic acid). The genus is one of the more difficult groups concerning classification and identification. New molecular approaches have shown that there is a high biodiversity, and the closely related species are difficult to be recognized based solely on their phenotypic characters. The genus is divided to 8 subgenera and 24 sections, however until now there is no available information which could clarify the phylogenetic relationships at the subgenus or at the section levels. One of our aims was the production of a robust genus-wide phylogeny based on sequences of 6 different genes (*Acl1, MCM7, RPB1, RPB2, Cct8, Tsr1*) to get insight into the evolutionary relationships within this economically important genus with special emphasize on section Nigri. 92 species had been involved in the analysis, which represent the genus properly. According to former studies, section Nigri was

suggested to belong to subgenus Circumdati, however our data indicate that sections Cremei and Nigri are unrelated to subgenus Circumdati, and possibly represent new subgenera.

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INTERACTIONS BETWEEN *ASPERGILLUS* SPECIES IN THE PRESENCE OF ACETATE

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Aspergilli occupy a large spectrum of habitats, including soil, decaying organic materials, and indoor environments. They have a great potential to synthesize numerous secondary metabolites and antimicrobial proteins in order to inhibit the growth of competitor microorganisms. Study of these compounds is useful both in understanding the biology of microbial communities and in developing new antifungal and/or antibacterial agents. In this work we studied the interactions of *Aspergillus nidulans* and *Aspergillus niger* in the presence of acetate as an unfavorable carbon source. Strong antifungal effect was observed against *Aspergillus niger* on acetate containing medium due to diffusible compound(s) produced by *Aspergillus nidulans*. Secondary metabolites, antifungal proteins or cell wall hydrolyzing enzymes can also be responsible for this interaction. Shedding light on these compounds we studied the antifungal effect of fermentation broths of *A. nidulans* cultures growing on acetate with microdilution method. Secondary metabolites were removed from the fermentation broths by successive dialysis with membranes having different pore sizes.

The best antifungal effect against *A. niger* was found with the undialyzed samples followed by samples dialyzed by a membrane having 3600 Da-sized pores (retaining enzymes and antifungal proteins as well). Only weak antifungal activity was observed when fermentation broth was dialyzed by a membrane having 12600 Da-sized pores (retaining enzymes only). It suggests that small molecular weight antifungal protein(s) could be responsible for the observed antimicrobial effect but the role of secondary metabolites cannot be ruled out completely.

CLONING OF *STREPTOMYCES GRISEUS* AFSA TO *ESCHERICHIA COLI* EXPRESSION VECTOR

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The genus *Streptomyces* comprises Gram+, soil-dwelling, filamentous bacteria. It shows complex morphological differentiation that relates to the production of secondary metabolites. Autoregulatory molecules play a key role in controlling both of these processes. In case of *S. griseus* A-factor (2-isocarpryloyl-3R-hydroxymethyl-γ-butyrolactone) is significant. The *afsA* has prominent role in A-factor biosynthesis. Previous studies showed that the *afsA* gene is functional in the A-factor negative (AFN) mutant and it is transcripted. Our aim is to understand the background of the AFN phenotype. In this work we produced the AfsA as a GST-fusion protein in *E. coli*. Antibody production against this protein can be used for the detection of AfsA protein in the *S. griseus* AFN strain. To produce AfsA-GST fusion protein we constructed a pGEX-4T-1-afsA construct as follows. The *afsA* gene was amplified by PCR. The product was ligated to the pJET1.2 plasmid and was transformed to the *E. coli* TOP10 strain. After purification of the plasmid the *afsA* gene was cleaved

using EcoRI and NotI restriction enzymes and was cloned to the pGEX-4T-1 expression plasmid. This construct was transformed to the *E. coli* BL21 strain. To produce AfsA-GST 100 ml Luria Broth was inoculated by 1 ml of overnight culture of *E. coli* BL21 and was incubated at 30 ºC. Protein expression was induced by IPTG. Cells harvested by centrifugation were disrupted by sonication. Induction yielded insufficient amount of fusion protein in the soluble fraction due to the formation of insoluble inclusion bodies. In order to shift the inclusion bodies to the soluble fraction we decreased the growth temperature to 25 ºC but this alteration did not increase significantly the soluble form. So we tried to dissolve the inclusion bodies. The pellet obtained from the sonicate by centrifugation was incubated in a buffer containing 5 M urea. To refold the denatured AfsA-GST, urea was removed by successive dialysis. After this treatment the supernatant contained most of the fusion protein. For purification of the solubilized AfsA-GST fusion protein Gluthatione Sepharose 4B beads were used and the GST-tag was removed by thrombin protease.
In this work we successfully constructed an expression system that produces the AfsA-GST fusion protein in sufficient concentration (1.5 mg/ml). Using the antibody constructed against this protein contributes to the understanding of A-factor biosynthesis in *S. griseus*.

COMPARISON OF METABOLIC AND GENETIC DIVERSITY OF RHIZOSPHERE BACTERIAL COMMUNITIES OF HALOPHYTON PLANTS LIVING NEARBY KISKUNSÁG SODA PONDS, HUNGARY

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Genetic diversity and community level physiological profile (CLPP) of rhizosphere samples originated from four different plant communities nearby Böddi-szék, Kelemen-szék and Zab-szék soda ponds in Kiskunság National Park were investigated following a sampling in September 2013. The soil physical and chemical properties were quite different at the three sampling sites. CLPP of bacterial communities was assessed by MicroResp™ method using 15 different substrates while denaturing gradient gel electrophoresis (DGGE) was used to analyze genetic diversity. Multivariate statistics (PCA and UPGMA) revealed that Zab-szék samples could be separated from the two others according to the genetic profile which might be attributed to the geographical location and perhaps the differences in soil physical properties. Böddi-szék samples could be separated from the two others considering the metabolic activity which could be explained by their high salt and low humus contents. The number of bands in DGGE gels was related to the metabolic activity, and positively correlated with soil humus content, but negatively with soil conductivity values.
On the basis of DGGE, members of Betaproteobacteria, Gammaproteobacteria and Cyanobacteria were identified. The main finding was that geographical location, soil physical and chemical properties and the type of vegetation were all important factors influencing the metabolic activity and genetic diversity of rhizosphere microbial communities.
This research was supported by the Hungarian Scientific Research Fund (OTKA K 108572).

GENE EXPRESSION OF CHICKEN CELLS INFLUENCED BY PLASMIDLESS NON-MOTILE MUTANTS OF *SALMONELLA* ENTERITIDIS

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Salmonella Enteritidis is still a significant pathogen of human gastroenteritis, originating mainly from poultry (ECDC/EFSA supporting publication 2014). Recently we have shown that chicken embryo fibroblast cells (CEFs) proved to be useful for *in vitro* assessment of the innate immune response of chicks to *S.* Enteritidis infection. Therefore we intended to use CEFs to estimate and the significance of certain virulence determinants of *S.* Enteritidis in chicken. Our objective was to characterize the gene expression profiles of CEFs as a response to non-motile mutants of *S.* Enteritidis 11 (SE 11) lacking the serovar-specific virulence plasmid and/or the *fliD* gene in comparison to the wild type parental strain. Freshly isolated CEFs incubated with *Salmonella* for 4 hrs were used to measure the invasiveness of parental strain SE 11 and its virulence-defective mutants. We used Agilent custom 8×15K microarray to profile the expression of CEFs, with emphasis to genes related to the immune response. Expression of chicken genes identified as significantly up- or downregulated (≥3-fold) was verified by real-time PCR. Invasiveness of both mutants of SE11 proved to be significantly lower than that of parental strain. Infection with SE 11 induced 26 genes and led to the suppression of 39 genes. Out of them the colony-stimulating factor gene CSF3 and cytokine genes for IL-1β and IL-8 showed the highest upregulations. In contrast, infection with non-motile mutants lacking the virulence plasmid and/or *fliD* did not cause any significant change in host gene expression. However real-time PCR results indicated that the cell cycle G0S2 switch gene (cell division), and the enolase ENO2 gene (metabolism) were highly induced by the mutant strains, indicating that the reduced invasiveness of the mutants was concomitant to stimulated cell division and/or metabolism of the host cells. Based on these results *fliD* seems to be more important for the invasiveness of *Salmonella* Enteritidis than the serovar-specific virulence plasmid. This is in harmony with our earlier *in vivo* studies about minor significance of the virulence plasmid in the pathogenicity of S. Enteritidis 11.
In contrast, *fliD* could be considered as a modulator of the chicken response to *Salmonella* infection. Interestingly stimulation of non-immune genes such as G0S2 and ENO2 was much stronger by the virulence-defective mutants demonstrating that plasmid- and/or flagellin of *Salmonella* may influence host cell metabolism and regeneration.
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GROWTH AND FE(III)-REDUCTION OF *SHEWANELLA XIAMENIENSIS* IN SOME CARBOHYDRATES

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Bioelectricity can be generated directly from wastewater using a microbial fuel cell (MFC) while reducing the quantity of organic materials. While some members of the genus *Shewanella* are

intensively studied bio-catalytic microbe in MFC, whereas very few scientific and technological data (both physiological and biochemical features) are available in literature relating to *S. xiamenensis*, thus in this study, the effects of different carbon-sources on the Fe^{3+}-reduction of *S. xiamenensis* were focused. Marine agar and LB broth were used for inoculation of bacteria at 30°C. Different mono-, (D-glucose, D-galactose,) di- (D-maltose, D-lactose) and oligosaccharides (maltodextrin, DE:20-25) were used as carbon sources in a minimal nutrient broth containing Fe^{3+}-citrate as electron acceptor. The growth of the microbe was followed by monitoring the changes of optical density at wavelength of 600 nm with spectrophotometer and by plate pouring method. Iron-reduction was followed by the absorbance change on wavelength of 460 nm using NH_3-thiocianate at acidic milieu (pH~2). *S. xiameniensis* strain DSMZ 22215 was able to utilize and grow on wide range of carbon sources in presence of Fe^{3+} as electron acceptor. This strain exhibited similar growth kinetic parameters (growth curve, cell yield) in every investigated carbon sources, however significant differences in Fe^{3+}-reduction were observed. In the cases of maltose and maltodextrin substrates, a rapid Fe-reduction was detected at the 24th hour of cultivation and most of available Fe^{3+} ions were reduced at the 48th hour. Different glucose and maltose concentrations (0.5, 1, 2, 4 g L^{-1}) were applied to determine the microbial growth and Fe^{3+}-reduction. The increasing substrate concentration enhanced the growth rate of the microbe, but no any significant differences were detected in both carbon sources. The iron(III)-reduction rate were higher in the case of maltose (μPmax= 78.27 mg/h) comparing with the glucose (μPmax= 62.53 mg/h). While in the case of glucose about 42% of Fe^{3+}-ion were reduced after 48 hours of cultivation, whereas in the case of maltose this value was 91%. According to the results, the starch derived carbon sources may have a stimulating effect on the Fe^{3+}-reduction capacity of *S. xiameniensis*. This finding gives possibility to utilize this microbe very effective in the starch-based wastewaters opening opportunity to develop MFC technology for processing of food or paper industry wastes.

MICROBIOLOGICAL INVESTIGATIONS ON THE WATER OF GELLÉRT BATH: A STUDY FROM WELL TO POOL

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Thermal baths are unique water environments combining a wide variety of natural and anthropogenic ecological factors. There is limited information on the microbiology of thermal baths in their complexity, tracking community shifts from the thermal wells to the pools. In the present study water hygienic parameters (indicator bacteria and enteroviruses) as well as cultivable "normal" microbial community of the well and the pool water in Gellért bath was studied in detail. 10% R2A and minimal synthetic media (with "bath water") with agar-agar and Gelzan were used to isolate bacteria. Strains were identified by sequencing their 16S rRNA gene after grouping them by ARDRA. The quantity of cultivable bacteria was at least 10 fold higher in the pool as compared to the well. The number of hygienic important bacteria was low though *E. coli*, *Enterococcus* sp. and *Legionella* spp. could also be detected in the pool. Adenoviruses and Noroviruses did not appear in any cases. The most diverse bacterial communities could be cultivated on the surface of minimal medium with Gelzan and 10% R2A medium with agar-agar.

On each applied media and from each sample the dominance of α-Proteobacteria was characteristic though their diversity differed among samples. The largest groups in the pool water belonged to the *Tistrella* and *Chelatococcus* genera. The most dominant member in the well water was a new taxon, its similarity to *Rhodobium orientis* as closest relative was 93.62%. From each sample novel species

could be isolated belonging to genera *Nocardioides, Rhizobium* and *Caulobacter*. *Deinococcus* and low G+C Gram-positives appeared only in the pool water.

EXTRACELLULAR HYDROLASE ACTIVITIES OF *SMITTIUM* FUNGI

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Smittium and related species (Harpellales, Kickxellomycotina) are fungi living in the digestive tract of various arthropods, mainly in aquatic larvae of insects. These fungi have many interesting structural and functional features due to their adaptation to living in the gut of arthropods. Majority of the species are considered to symbionts or commensalists, but some of them may cause the death of insect larvae. Although several *Smittium* strains have been isolated and phylogenetically identified, their relationship with the host is not entirely known. This fungal group may also be interesting from practical aspects, such as the exploration of new enzyme activities. It is supposed that their biomass degrading hydrolases play important role in nutrient obtaining from the gut. However, extracellular enzyme activities of these fungi have not yet been investigated. Hence, the objective of the present study was to evaluate the extracellular cellulase, lipase and chitinase activities of five strains representing different *Smittium* species. To analyse the enzyme activities, *Smittium simuli, S. culicis, S. culisitae, S. tipulidarum* and *S. lentaquaticum* isolates were cultivated under inductive conditions specifically set up for the tested enzyme. Wheat bran, tributyrin and colloid chitin were used as substrates to induce cellulolytic, lipolytic and chitinolytic enzyme production, respectively. Solid medium was applied to monitor the lipase activity and the level of the enzyme production was evaluated by measuring the width of the halo around the colonies that formed by the tributyrin hydrolysis. Under this condition, the analysed fungi showed high lipolytic activity since they exhibited about 5-10 mm wide halo after 7 days incubation. For cellulase and chitinase production, isolates were cultivated in submerged systems supplemented with the appropriate inductor carbon source.

Great differences were seen between the strains regarding to the production of these enzymes; moreover, isolates with high chitinolytic and cellulolytic activity have also been identified. Anyway, besides identification of novel promising enzymes for practical use, such enzyme activity studies may also contribute to the establishment of the link between the fungi and hosts.

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DETECTION AND PHYLOGENETIC ANALYSIS OF ALKANE 1-MONOOXYGENASE GENE OF MEMBERS OF THE GENUS *RHODOCOCCUS*

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Naturally occurring and anthropogenic petroleum hydrocarbons are potential carbon sources for many bacteria. The AlkB-related alkane hydroxylases, which are integral membrane non-heme iron enzymes, play a key role in the microbial degradation of many of these hydrocarbons. Several members of the genus *Rhodococcus* are well known alkane degraders and known to harbor multiple *alkB* genes encoding for different alkane 1-monooxygenases. In the present study 48 *Rhodococcus* strains, representing 35 species of the genus were investigated to find out whether there is a dominant type of *alkB* gene which is widespread among species of the genus and can be used as a phylogenetic marker gene. PCR primer sets designed in this study enabled the amplification of *alkB* gene from majority of the representatives of the genus. Phylogenetic analysis of rhodococcal *alkB* gene sequences obtained in the present study along with sequences available in public databases indicated that a certain type of *alkB* gene is present in almost every member of the genus *Rhodococcus*. These *alkB* genes are common in a unique nucleotide sequence stretch which is absent from other types of rhodococcal *alkB* genes and encodes a conserved amino acid motif: WLG(I/V/L)D(G/D)GL. According to results of a hidden Markov Model based topology prediction this motif is part of a short outside loop connecting two transmembrane helices of the enzyme. The sequence identity of the targeted *alkB* gene in *Rhodococcus* ranged from 78.5% to 99.2%, showing higher nucleotide sequence variation at the inter-species level compared to the 16S rRNA gene (93.9% - 99.8%). Applicability of the targeted *alkB* gene as phylogenetic marker gene was analyzed in case of three clusters of the genus, in which evolutionary closely related *Rhodococcus* species can be found, sharing >99% 16S rRNA gene similarity. Our results indicated, that the investigated *alkB* gene type may be applicable (i) to differentiate closely related *Rhodococcus* species, (ii) to properly assign environmental isolates to existing *Rhodococcus* species, and finally (iii) to assess whether a new *Rhodococcus* isolate represents a novel species of the genus.

INVESTIGATION OF THE CLEARANCE OF THE HUMAN PAPILLOMAVIRUS INFECTION IN PATIENTS WITH PREMALIGNANT AND MALIGNANT LESIONS

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Persistent infection with high- risk HPV genotypes is a major risk factor for the initiation of a variety of carcinomas including head and neck malignancies. The aim of our study was to examine the HPV prevalence, genotype distribution and elimination dynamics in patients with potentially malignant and malignant lesions. Seventy- five patients with oral lichen planus (OLP), 18 patients with oral leukoplakia (OL) and 80 patients with oral squamous cell cancer (OSCC) were enrolled. Exfoliated cells were collected from the lesions, and in case of OSCC patients also from the excised tumor tissue. HPV DNA was detected by MY/GP consensus nested PCR and virus genotype was determined using restriction fragment length polymorphism or sequencing. The prevalence of HPV in lesions was 8.0% (6/75) in OLP and 27.8% (5/18) in OL. Follow up was successful in five and three HPV positive cases, respectively. During the follow-up period, the infection was eliminated in

four of five OLP cases and in one of three HPV positive OL cases. Progression of the lesion was observed in case of two and one HPV negative OLP and OL patients, respectively, HPV negative OSCC developed in all of them. Out of 80 OSCC patients, 15 carried the HPV DNA in exfoliated tumour cells and/or excised tumour biopsy samples. HPV prevalence was not significantly different between males and females (18.2%, 10/55 vs. 20%, 5/25). HPVs were mainly high-risk genotypes (six HPV 16, one HPV 33 and one HPV 66; other HPVs were untypeable). Six of the 80 patients had recurrent tumours, three of them were HPV16 positive. One of them had a second HPV16 positive recurrence during the study period in spite of the postoperative radiotherapy. Further twelve and four patients, respectively, were diagnosed with recurring tumour or metastasis during the study period; three of these patients were HPV16 positive. Six patients with primary tumors received neoadjuvant therapy. Two of them were HPV positive, both continued to carry the virus in spite of the treatment. Forty-nine patients received postoperative irradiation therapy; eight were HPV positive. Two of them remained positive after the treatment.

None of the twenty randomly selected patients with HPV negative tumour became HPV-infected during postoperative irradiation. HPV persistence in potentially malignant and malignant oral lesions seems to be an infrequent event, malignization of HPV positive potentially malignant lesions was not observed. However, HPV infection may increase the risk of tumour recurrence or metastasis. These observations warrant confirmation on larger patient cohorts.

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COMPARISON OF THE INCIDENCE OF BIOFILM PRODUCTION, TYPE 3 FIMBRIA AND *MRKA* AND *MRKD* GENES ON COMMENSAL AND WASTEWATER ISOLATES OF *KLEBSIELLA PNEUMONIAE*

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Relatively few information are available about the virulence factors of commensal and environmental *Klebsiella pneumoniae* isolates. Earlier studies have verified the pivotal role of type 3 fimbria {mannose-resistant and *Klebsiella*-like haemagglutinin (MR/K)} and biofilm production in the early phases of infection. It is also known that type 3 fimbria itself has a key role in biofilm formation. The type 3 fimbrial gens (*mrkABCDF)* were revealed to code type 3 fimbria, where *mrkA* encodes the major pilin subunit (schaft) and responsible for the biofilm production while *mrkD* encodes the tip subunit (adhesin). In our study 113 wastewater (WW) isolates were collected from 47 geographically different *sewage treatment plants* and 120 commensal (CK) strains were isolated from the stool samples of healthy individuals by the reasons of occupational medical check-up. The clonality of all the strains was checked to exclude the multiple representations in the strain collection with different DNA based methods. Presence of type 3 fimbria was detected by haemagglutination and crystal violet plate assays were performed in 96-well tissue culture plates to determine biofilm formation. Presences of *mrkA* and *mrkD* genes were shown by means of PCR. Our results show that type 3 fimbria prevalence was similar in both isolates groups CK and WW (57.5% and 49.7%, respectively). However, significantly higher prevalence was found (χ^2, p=0.001) in case of CK (93.3%) isolates compare to WW (78.8%) strains in respect of biofilm production. This result was unexpected because the fimbrial shaft subunit of type 3 fimbria responsible for biofilm formation. In addition, biofilm production was significantly higher than the prevalence of

type 3 fimbria in both isolates types (χ^2, p<0,001). The frequencies of type 3 fimbria negative but biofilm producer isolates were similar in both isolates types CK (36.6%), WW (30%). The PCR analysis showed that within this group the frequency of *mrkA*(+) and *mrkB*(+) isolates were surprisingly high in both CK (70.4%) and WW (73.5%) isolates, while the minority of the strains showed *mrkA*(+) but *mrkB*(-) genetic pattern CK (22.7%) and WW (23.5%).
This genetic arrangement is responsible for the type 3 fimbria negative but biofilm positive phenotypes. Our data suggest that, among other possibilities, the haemagglutination method may not be specific or sensitive enough to show type 3 fimbria and/or tip subunit may interfere with the thickness of capsular polysaccharide.

BACTERIOPHAGES FROM CONFISCATED FOOD, LYTIC FOR FOOD-BORNE PATHOGENS

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Bacteriophages frequently carry non essential, first of all virulence genes including Shiga toxin production encoding genes and promote the evolution of both pathogenic and non pathogenic bacteria by integration to their genomes. However these prophages could be also induced and the lytic free phages could interfere with bacterial strains present in their environment. These lytic phages could modify the sensitivity of the isolation procedure. Altogether 207 confiscated food samples of animal origin were tested for the presence of Shiga/Vero toxin producing *E. coli* (STEC) and bacteriophages. By using ISO 16654:2001 for O157 and ELISA based Ridascreen Verotoxin we could not isolate *E. coli* O157 or STEC but we were able to isolate lytic phages. By using mitomycin C at 0.5 µl/ml final concentration as inducing agent and *E. coli* K-12 C600 strain as indicator strain lytic phages were isolated from 10% (21/207) of the food samples. Electron microscopic studies revealed that the lytic phages represented different families including tailed *Myoviridae*, *Syphoviridae* and filamentous *Inoviridae*. The induced phage suspensions frequently contained more than one type of phages. The host specificity of lytic phages was tested by spot assay using *E. coli* strains representing enterohaemorrhagic (EHEC), enteropathogenic (EPEC), uropathogenic (UPEC) avian pathogenic (APEC) *E. coli* pathotypes, *Shigella sonnei, Citrobacter rodentium* and several *Salmonella* serovars including Enteritidis, Typhymurium, Hadar and Infantis. Different lytic patterns were observed and all the tested *E. coli* and *S. sonnei* strains were lysed by at least one phage. Interestingly the isolated lytic phages lysed the EHEC and atypical O157 strains tested and the *S.* Typhimurium study strain was also lysed by four phages.
Results indicate that further studies are justified to elucidate the inhibitory effect of lytic phages that might influence the isolation of food-borne pathogens from food.
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ISOLATION OF NOVEL B-DEFENSIN-LIKE ANTIFUNGAL PEPTIDES ENCODING GENES FROM FILAMENTOUS FUNGAL SPECIES BELONGING TO THE GENUS *NEOSARTORYA*

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From the second part of the 1990s several proteins with similar structure to the β-defensins have been isolated from Ascomycetes. They are very different in their amino acid sequences, but conserved homologous regions can be identified in their amino acid sequences. Based on these regions, they can be divided into two groups: proteins which contain the *Penicillium chrysogenum* antifungal protein (PAF) cluster and proteins with the *P. brevicompactum* "bubble" protein (BP) cluster. BP cluster proteins inhibit the growth of yeasts, while the PAF cluster proteins are effective against filamentous fungi. *Neosartorya fischeri* is able to produce peptides appertaining to either group. In the genome of the *N. fischeri* NRRL 181 genetic determinants of both protein groups can be identified, the PAF-cluster containing *N. fischeri* antifungal protein (NFAP) and the BP-cluster containing *N. fischeri* bubble protein (NFBP). The aim of the present study was to isolate further homologous genes to *nfap* and to *nfbp* from different *Neosartorya* species. To amplify the DNA fragments of the putative antifungal protein encoding genes polymerase chain reactions were performed with primers designed to the up- and downstream regions of NFAP and NFBP encoding genes. Genes homologous to the *nfap* were isolated from the following *Neosartorya* isolates: *N. fischeri* NRRL 4075, NRRL 4161, NRRL 4585, SZMC 1389 and *N. spathula* SZMC 1380. Furthermore, *nfbp* homologous genes were identified in the genome of the following *Neosartorya* isolates: *N. botucatensis* SZMC 2035; *N. fennelliae* SZMC 1378, SZMC 1379, SZMC 2044, SZMC 2045; *N. ferenczii* NRRL 4179; *N. fischeri* A-7223, NRRL 181, NRRL 4075, NRRL 4161, NRRL 4585; *N. glabra* NRRL 3434; *N. spinosa* NRRL 3435. Amino acid sequences of peptides encoded by the *nfap* homologous genes were identical to the previously isolated and characterized NFAP, while the isolated *nfbp* homologous genes show 79.6-100% similarity to each other. Our results provide data for the widespread presence of β-defensin like antifungal protein in the genus *Neosartorya*. The isolation and characterisation of the NFBP homologous proteins are in progress.

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CPG METHYLATION PATTERN AND ITS EFFECTS ON REPLICATION OF PARVOVIRUSES

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The main form of epigenetic modifications in eukaryote genome is DNA methylation. CpG methylation is not only important in the regulation of the hosts' life processes, but it has a major influence on the lifecycle of DNA viruses as well. The effects of CpG methylation are not well studied in parvoviruses, for this reason our main goal was to determine the methylation pattern of the porcine parvovirus (PPV) and adeno-associated virus 2 (AAV2) which are members of the Parvovirinae subfamily. CpG dinucleotides are underrepresented in PPV genome, it has only 60 CpG sites and the observed/expected CpG ratio (oCpGr) is 34%. In contrast, AAV2 has 266 CpG sites and 79% oCpGr. Furthermore, the PPV can replicate autonomously, while the AAV2, which belongs to *Dependovirus* genus, requires the presence of a helper virus for successful reproduction. Since PPV and AAV have different reproductive strategy, we expected different methylation patterns and methylation-mediated regulation. To prove this theory we investigated experimentally

the role of methylation in their lifecycles. First we determined the methylation pattern of the replicative and the packaged single-stranded form of the PPV genome originated from permissive and semi-permissive tissue culture cells by bisulfite PCR and sequencing. Both forms were found to be barely methylated, proving that PPV DNA remains hypomethylated during the entire life cycle of the virus including replication and packaging. To investigate the effects of the methylation pattern change on the viral replication, *in vitro* hypermethylated PPV genomic DNA was transfected into tissue culture cells. Its virus replication initiation capability was monitored by immunofluorescence assay and quantitative PCR. The result indicated that methylation has a modest inhibitory effect on PPV replication. In the case of AAV2 genome we examined the methylation pattern of the packaged single-stranded and integrated form in Detroit 6 cell line. The integrated form was found highly hypermethylated while the packaged single-stranded form of AAV 2 remained hypomethylated, suggesting that methylation of the integrated form does not have an inhibitory effect on the release of AAV from the integration sites and most probably does not block completely the viral gene expression. In general, our investigation of an autonomously replicating and a helper dependent parvovirus suggests that CpG methylation has limited effect on parvoviral replication and lifecycle.

PURIFICATION AND STRUCTURAL ELUCIDATION OF A BIOACTIVE COMPOUND PRODUCED BY *TRICHODERMA HARZIANUM* SZMC 1874

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Filamentous fungi can produce a large number of secondary metabolites with promising biological effects. Molecular identification of these compounds and characterization of their biological activities are among the most important goals of studies performed at the interface of chemistry and biology research. An outstanding group of the secondary metabolites are the peptaibols (PBs), produced mainly by fungal species belonging to the genus *Trichoderma*. These compounds show broad spectrum of biological activities including antibacterial, antifungal and antiviral effects. Our previous screening activities resulted in the selection of strain *T. harzianum* SZMC 1874 as a potential PB producer. The selected strain was grown on malt-extract agar. The conidia and aerial hyphae were scrapped from the agar surface and the extraction of PBs was performed in homogenizator with three 20 ml portions of chloroform. Extracts were combined, centrifuged and the supernatant was evaporated to dryness using a rotary evaporator. Ten millilitres of chloroform were added and the resulting solution was passed through silica loaded into an open glass column. The solid phase was washed with chloroform, then a stepwise gradient of methanol was applied for the elution. The evaporated fractions were applied in our bioassay and the active fractions were analyzed using HPLC-MS technique for the identification of the characteristic mass spectral elements of the PBs including y and b ions as well as the single- and double charged molecular ions. The fifth and the sixth fractions contained a total of 9 possible novel molecules. These fractions were pre-fractioned with medium pressure normal phase chromatographic method using an own-filled glass column, where the neighbouring fractions containing the same molecules were pooled and concentrated. Further purifications were carried out with RP-HPLC.

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BLOBOLOGY: THE CASE OF *THERMOPLASMA* QUINONE DROPLETS

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Cytosolic, globular droplets with an average diameter of 50 nm were observed in vitrified *Thermoplasma acidophilum* cells by means of cryo-electron tomography. These droplets were isolated by column chromatography and immuno-precipitation purification methods and subjected to chemical, biochemical and electron microscopy analyzes. LC/MS and TLC experiments revealed more than 200 small hydrophobic compounds, of which menaquinones and the polar lipid gulopyranosyl-(β1-1)-caldarchaeol (GuC) were the largest and second largest portions, respectively. LC-MS/MS based proteome analysis identified seven quinone droplet associated proteins which belong to the "hypothetical and/or uncharacterized" protein category. Out of these Ta0547 is the most abundant and unlike most of the described lipid droplet associated proteins of prokaryotic or eukaryotic cells it doesn't encode membrane spanning domain(s). The architecture of *Thermoplasma* Quinone Droplets (TaQDs) seems unique as neither lipid monolayer nor a protein coat could be detected by electron microscopy. Considering TaQD structure, we propose a model describing loosely attached Ta0547 protein filaments associated with identified lipid components. Based on present knowledge we can assume that TaQDs serve as storage particles either for newly synthesised membrane lipid precursors or on the contrary, they might be a pool of degraded gulopyranosyl-(-1-1)-caldarchaetidylglycerol(GuCGp). GuCGp is the main *T. acidophilum* polar lipid that can be degraded to GuC and glycerophosphate at 60 °C and pH 1.8 (the optimal growth condition of *T. acidophilum*) in three days. Thus, if GuC were degradation product of GuCGp then Ta0547 and/or other TaQD associated proteins might have a role in binding, detaching and delivering non-desirable GuC (together with menaquinones) from the membrane into QDs.

FLAVOBACTERIUM CAUSED FISH DISEASE IN HUNGARY

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Flavobacterium columnare is a ubiquitous bacterium in the aquatic environment. As the etiological agent of columnaris disease it affects the skin and gills both of wild and cultured freshwater fishes. This aquatic pathogen causes severe mortalities and economic losses for the fishing industry throughout the world. It favours warm water and cause problems in Hungarian fish ponds during the summer months. Increasing temperature of the freshwaters, by the global warming, elevates the risks of this disease also in natural waters.. The aim of our study was to survey the prevalence of *Flavobacterium columnare* in fishes both from wild and cultured freshwater in Hungary. The samples were collected from the skin, eye, gill and visceral organs of diseased fish and from gills of

healthy specimens. For the selective isolation, the low nutrient and high water content *Cytophaga* agar supplemented with neomycin and polymixin-B was used. The culturing of the isolates was carried out on the same media without antimicrobial agents. In the preliminary examinations we studied the morphology, motility, staining characteristics and biochemical features of the bacterial colonies. Identification of the isolates was confirmed with specific PCR based on 16S rRNA gene of *F. columnare*. In the latter assay 25 isolates from 10 different fishes (common carp, gibel carp, tench, razorfish, common bream, silver bream, perch, sander, Volga sander, and Siberian sturgeon) gave positive result. Henceforth the genotypes of the strains were determined by restriction fragment length polymorphism (RFLP) of 16S rRNA gene fragment using HaeIII and RsaI restriction endonucleases. Both the original fragment size and RFLP band patterns of our strains differed from the published ones. Twenty of 25 strains presented identical RFLP pattern, while four others varied from them using either of HaeIII or RsaI restriction endonucleases, and the last one differed from all of them using with both enzymes. The sequence analysis of about 1360 bp long 16S rRNA gene fragment identified 23 strains as a *F. johnsoniae*, closely related species of *F. columnare*, meanwhile the remaining 2 strains proved to be *Chryseobacterium* spp. Antibiograms of the strains was determined by Kirby-Bauer disc diffusion method using with 10 antimicrobial agents. The lack of international guide specific for *Flavobacterium* made the evaluation of the results difficult. The strains were multiresistant. All of them exhibited resistance to ampicillin and polymyxin B, while the 23 *F. johnsoniae* isolates were resistant also to gentamycin. High level of antimicrobial resistance was revealed to chloramphenicol as well. Only 3 separated *F. johnsoniae* strains demonstrated sensitivity to this antimicrobial agent and did it with a *Chryseobacterium* to oxytetracycline. Prevalences of resistance and sensitivity to furazolidone and cotrimoxazole of the strains were similar, with 11 and 13 resistant strains, respectively. Florfenicol, enrofloxacin and erythromycin were more effective against our isolates and only 4 strains were resistant to them.
Our results indicate that the genus *Flavobacterium* were represented by *F. johnsoniae* in addition to *Chryseobacterium* spp both in wild and cultured freshwater fishes during the studied period. Considering the prevalence of high level multiresistant strains in diseased fishes, further examinations of their epidemiological role and relation to the public health are needed.
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A SINGLE NUCLEOTIDE PRIMER EXTENSION ASSAY TO MONITOR *RHODOCOCCUS* STRAINS USED IN BIOAUGMENTATION PROCESSES

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Pollution of soil, water and groundwater environments by crude oil originated products has been, and is still today, an important problem. Crude oil is a complex mixture of thousands of different compounds. Among them alkanes generate the main fraction. Although they are chemically very inert, most of them can be efficiently degraded by several microorganisms. Bioaugmentation is a potential remediation method to use in oil contaminated environments and *Rhodococcus* strains can be used effectively in these bioremediation processes. It is a well known fact that *Rhodococcus* strains usually harbour alkane monooxygenases, which are the key enzymes in bacterial alkane degradation pathways. Our aim was to develop a technique which we can use to monitor *Rhodococcus* strains used in bioaugmentation processes. The main research questions were: (i) do

the *Rhodococcus* strains appear and/or become to be resident in the contaminated environment after usage of the inoculum; (ii) do they become to be dominant members of the bacterial community; (iii) do they take part actively in the degradation processes? For this we improved a Single Nucleotide Primer Extension (SNuPE) assay based on the detection and genotyping of alkane 1-monooxygenase gene of rhodococci. The assay was designed for monitoring two *Rhodococcus* strains (*Rhodococcus erythropolis* AK-35 and *Rhodococcus gordoniae* AK-38) which are already used in bioaugmentation processes. To develop and test the performance of the assay under *in vitro* conditions groundwater microcosm experiments were conducted with samples originating from a petroleum hydrocarbon contaminated site without bioaugmentation process. As the assay proved to be highly specific and reliable in monitoring the two different *Rhodococcus* strains, we performed investigations with groundwater samples of an AK-38 bioaugmented petroleum hydrocarbon contaminated site.
Results have shown that *R. gordonia* strain AK-38 became to be a dominant member of the microbial community at the bioaugmented contaminated site and that the developed assay provides a fast and reliable solution to detect the presence and activity of rhodococcal inoculums in petroleum hydrocarbon contaminated environments.

FURTHER INSIGHT INTO THE ANTIFUNGAL MECHANISM OF *NEOSARTORYA FISCHERI* NFAP

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The increased incidence of severe fungal infections and the fast development of drug resistant filamentous fungi causing mycoses, plant infections strongly demand for the development of new antifungal strategies. Antifungal peptides have great potential in these fields. In this respect, small, basic, cysteine-rich antifungal proteins produced by filamentous Ascomycetes are promising candidates. The antifungal protein NFAP from the *Neosartorya fischeri* NRRL 181 isolate is a novel representative of this protein group. In this study, the antifungal mechanism of NFAP heterologously expressed by *Pichia pastoris* was studied *in vitro*. Previously it was proven that the NFAP-related *Penicillium chrysogenum* PAF exerts its detrimental effect on *Aspergillus nidulans* via heterotrimeric G-protein signal transduction pathway causing apopotosis, defective actin polymerization and apolar growth. Furthermore, PAF fails to activate the mitogen-activated protein kinase (Mpk) signalling and in consequence the cell wall remodelling pathway is not induced in this fungus. Based on these findings we investigated the growth effect of NFAP on different *A. nidulans* strains carrying mutations in these signal transduction pathways. An *A. nidulans* strain carrying a dominant interfering mutation in alpha subunit of a heterotrimeric G protein (*fadA*) and a protein kinase A (*pkaA*) deletion mutant strain showed reduced sensitivity to NFAP compared to their parental strains. This result suggests that NFAP can activate the cAMP/Pka signaling cascade and triggers apoptosis similarly to PAF. The conditional alcA- protein kinase A (*PkcA*) mutant strain showed hypersensitivity towards NFAP compared to its parental strain, but interestingly, the Δ*mpkA* strain proved to be resistant to NFAP in contrast to its parental strain. The hypersensitivity of the conditional alcA-protein kinase A (*PkcA*) mutant demonstrates that PkcA/MpkA signalling cascade regulates the sensitivity towards NFAP.
The resistance of the Δ*mpkA* strain suggests that NFAP has an unknown MpkA-activated target, which could play a role in its antifungal effect. Our results indicate that the antifungal mechanism of NFAP is similar to that of PAF, however it has an MpkA-activated unidentified target.

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THE PROMISE PROJECT: PREVALENCE AND CHARACTERIZATION OF FOOD-BORNE PATHOGENS ISOLATED FROM NEGLECTED ROUTES OF TRANSMISSION TO CONSUMERS

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The 7th FP EU funded project “Protection of Consumers through Mitigation of Segregation of Expertise (PROMISE)” studies food-borne pathogens transmitted into EU-28 through less researched gates. A number of EU-28 member states participated and took samples from different possible neglected routes of pathogen transmission: food confiscates from travelers arriving at airports (Austria, Germany, Spain), food confiscated from travelers passing ground border stations (Croatia, Slovenia), food sold at black markets (Romania), and food samples from a globally acting food importer (Greece). At Austria`s main airport Wien Schwechat, more than 11 tons of food are annually confiscated from travelers hand luggage. We accompanied the authority for 8 month and took 600 food samples of animal origin carried along by passengers that flew into Vienna from destinations assignable to 33 countries worldwide. The total amount of food confiscated by these 600 checks peaked to 1278 kg. By using ISO methods, we could confirm a prevalence of *Salmonella* spp., *Listeria monocytogenes* and VTEC of 1.1% (n=7), 2.5% (n=15), and 1.3% (n=8), respectively. *Campylobacter* could not be isolated from any food commodity what was surprising since several raw poultry meat samples were confiscated. *Salmonella* isolates belonged to serotypes Telaviv, Hadar, Ohio, Brandenburg, Mbandaka, and Anatum. Two *S*. Hadar isolates from food originating from Egypt and one *S*. Anatum isolate from food originating from Ethiopia tested multidrug resistant. *Listeria monocytogenes* mainly belonged to the MLST (ST) type 9 known to be prevalent in Asian countries. All seven VTEC strains confirmed were non-0157: O2:H27, O6:H10, O8:HNM, O39:H48, O178:H7 and Orough:H7. The study shows for the first time that food shuttled illegally into EU-28 by global travelers is contaminated at frequencies similar to those usually found when domestic samples are investigated. However, yet undescribed genetic variants could be transmitted. To look into this issue, we performed a detailed analysis of virulence and occurrence of mutations in essential virulence genes in 15 *L. monocytogenes* strains isolated from a Romanian black-market served by food vendors from Moldavia. Novel virulence profiles as studied in cell culture correlated with mutations in the *hly* and *inl*A genes and with the assignment to MLST types. Conclusively, our data show that food distributed through unregulated channels may be contaminated at rates known from food samples taken from domestic markets. However, a risk is given since strains with novel virulence traits might be transmitted through such gates. That introduction of yet unknown genetic variants of pathogens may play an essential role in risk management was dramatically demonstrated by the recent EHEC outbreak in Germany and France where an obscure EHEC serovar O104 was the causal agent harboring completely novel virulence traits. The pure amount of food shuttled illegally in hand luggage implies that the information of travelers should be enforced.

ALGA BIOMASS AS BIOGAS SUBSTRATE: LABORATORY FERMENTATIONS AND METAGENOMIC STUDIES

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Microalga biomass offers a promising alternative to the commonly used maize silage in biogas technologies. There are essentially two approaches for mass cultivation of microalgae. In open ponds pure cultures are difficult to maintain, a mixture of algae and other microbes is formed. More sophisticated closed photobioreactors yield biomass of high microbiological purity but production cost may be disproportionate. Two substrates were tested for their biogas potential. A mixture of *Chlamydomonas* sp. and *Scenedesmus* sp. and syntrophic bacterial partners (mostly representatives of the genus *Rhizobium* and *Burkholderia* belonging to the phylum Proteobecteria), called MIX, and an almost 100% pure *Scenedesmus obliquus* culture, cultivated in a tubular photobioreactor, were studied in laboratory CSTR biogas reactors. Reactors fed with maize silage and a 50-50% mixture of maize silage and MIX (on organic dry substance, oDS, basis) were included in the experimental set-ups. In addition to temperature control at 37 °C, the pH and redox potential in the reactors and volumetric gas production were continuously monitored, the FOS/TAC, ammonium ion concentration, total organic carbon, total organic nitrogen, gas composition were measured regularly and the carbon/nitrogen (C/N) ratio of the input material was determined.

The composition of the microbial community was established at regular intervals through metagenomic analysis of databases obtained by next generation DNA sequencing. The MIX had a very low C/N ratio (5.3). The unfavorable C/N might be the reason why the biogas yield was lower than that of the maize silage (C/N=45). Replacing half of the MIX with maize silage improved the situation and the biogas yield was about the same as with maize silage alone. In the reactors containing MIX the Bacteria domain was quickly dominated by the Proteobacteria carried in with the substrate. The C/N content of the *S. obliquus* culture was higher (C/N=9) although still below the optimum 20-30 value. As in the case of co-fermentation with maize silage gave the best CH_4 yield. Biogas continued to evolve from the algae after discontinuing the daily substrate input, which indicated that the decomposition of the algal cell wall required longer time than that of the maize silage. In the Bacteria domain the *Bacteroidetes phylum* was most abundant. The composition of the Archea domain was not altered by the addition of the new substrates.