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RESULTS OF APPLICATION OF AN RNAi VECTOR FOR SILENCING *LIP*2 GENE OF *CANDIDA PARAPSILOSIS*

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The first evidence of RNA interference in yeasts was described in 2009 where authors proved that all necessary components of an RNAi machinery is present in *Saccharomyces castellii*, the RNAi works efficiently in *S. castellii* and the naturally RNAi negative *S. cerevisiae* could be endowed with RNAi properties upon introduction and expression of missing machinery components. They also reported that genome of *Candida albicans* was equipped with the elements of RNAi machinery and the presence of small RNA populations was observed. Despite of the indirect evidence of RNAi based gene silencing in *Candida*, application of RNAi has never been successfully developed in *Candida* species, although the method would provide a robust and efficient approach to study virulence involved gene families. Here we study the possibility of the application of RNAi to silence a virulence-related gene, LIP2 in *C. parapsilosis*, an opportunistic human pathogen.
We present the construction of autonom replicative LIP2 specific RNAi vectors that target LIP2 at three different regions, their expression in *C. parapsilosis* wild type strain and the result of observed LIP2 mRNA levels and Lip2 activity of transformants.

Supported by the Hungarian Scientific Research Fund (OTKA-K101218) and research activity of Judit Ámon was realized in the frames of TÁMOP 4.2.4.A/2-11-1-2012-0001 (EA2-ELMH-12-2-0018) „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund.

PLASMID ISOLATION FROM *THERMOPLASMA ACIDOPHILUM* HO-122 FOR SHUTTLE VECTOR CONSTRUCTION

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Convenient application of *Thermoplasma acidophilum* genetic tools is still hindered by the failure of stabile maintenance of plasmid vectors in *T. acidophilum* over a long period. Obvious solution could be the development of shuttle vectors based on endogenous, intact *T. acidophilum* plasmids. Therefore we attempted to isolate plasmids from all the available *Thermoplasma* strains isolated from solfatara (DMS 1728, JCM 17946-17956) by Qiagene Plasmid Mega Kit. Among thirteen strains we could prove that two strains (JCM 17955 and JCM 17956) harbored plasmids. Plasmids were analysed by restriction digestion and pTA1 plasmid (originated from strain JCM 19956) was chosen for the shuttle vector construction since the copy number of this plasmid was much higher than JCM 17955 plasmid. Random insertions of an *E.coli* replication origin, arr2 gene (responsible for rifampicin resistance in both organisms) and *T. acidophilum* expression elements (glucose-inducible promoter promoter and HIS-tagged version of the Ta0328 gene) containing transposon in pTA1 were generated with EZ-Tn5 Insertion Kit as described by the manufacturer. 50 vectors were recovered from *E.coli* colonies and sequenced bidirectionally from the ends of the Tn5 transposon to identify to insertion positions in the pTA1 plasmid. Ten shuttle vectors were chosen for futher

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application because insertion position of each of these vectors were different. Currently we are transforming the *T. acidophilum* type strain (DSM 1728) with these vector constructs. Additionally we adapted the transformation method to the pTA1 vector carrying *T. acidophilum* strain (HO-122) as presumably this strain might have chromosomally located genetic element(s) needed for plasmid maintenance which are missing in our DSM 1728 host.

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BIODEGRADATION OF DIHYDROTESTOSTERONE AS A MICROPOLLUTANT WITH ANDROGEN-DISRUPTING POTENCY

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The natural androgen 5α-dihydrotestosterone (DHT) is urinated by men in considerable amount daily, so it notably contributes to the androgen content of wastewaters and surface waters. DHT can cause for example reproductive disorders, so the elimination of DHT from wastewaters is essential. Using microorganisms for biodegradation is a prosperous way to eliminate this pollutant. Nevertheless, harmful intermediates may also occur during biodegradation. Hence, biological tests to evaluate hormonal or toxic effects are also essential beside analytical methods. One of the aims of this work was to screen a collection of microbes (the members of the genus *Rhodococcus* and *Cupriavidus*) for their DHT-degrading potential, and select those microbes which can completely biodegrade DHT without harmful metabolites. For this purpose, bioluminescent strains of *Saccharomyces cerevisiae* (BLYAS and BLYR) were applied. The BLYAS strain serves to measure the androgen activity and the BLYR strain to measure the cytotoxicity. The yeast bioreporters were adapted to biodegradation tests to evaluate biodetoxifying potential of microbes. Moreover, parallel analytical tests were also applied. Our other aim was to confirm that the biodegradation of DHT is mediated by enzymes and to investigate that the catabolic enzymes are produced continuously or inductively by our microbes. *R. pyridinivorans* AK37 strain was chosen to our experiments, since complete genome sequence of this strain is already available. First of all, the sensitivity of the BLYAS/BLYR test organisms to DHT was tested. According to our experiments 5,52 ng/ml DHT can increase the bioluminescence of the BLYAS strain by 50%. In the case of the BLYR strain we did not experience any changes in the bioluminescence in the examined concentration range. After screening DHT-degrading microbes with the yeast bioreporters, microbes with the highest biodegradation potential could be selected that transformed DHT without remaining harmful metabolites. Our experiments pointed out that androgen activity can be observed even after excellent DHT biodegradation. The biodegradation of DHT was proved by extracellular extracts of *R. pyridinivorans* AK37 and compared with heat- and proteinase K treated cell-free matrix.

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PATTERN OF LIGNOCELLULOSE DEGRADING ENZYME ACTIVITIES DURING COLONIZATION OF OYSTER MUSHROOM SUBSTRATE IN A LARGE SCALE PRODUCTION FACILITY

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Oyster mushroom (*Pleurotus ostreatus*) is a saprotrophic white rot fungus and it is the second most popular cultivated edible mushroom all over the world. In Hungary it is grown on composted and pasteurized wheat straw (substrate), but it can be cultivated on a wide range of other lignocellulosic materials due to its versatile enzymatic profile. The cultivation of oyster mushroom has two main parts. First, the oyster mushroom colonizes the substrate blocks in the production house at 24-26°C and relative humidity (RH) of 90-95% in dark. Second, during the fruiting body development the temperature is lowered to 16-18°C and RH is increased to 95-98 % with illumination. The aim of the present study was to monitor the lignocellulose degrading enzyme activities during colonization and fruiting body development of oyster mushroom in a large scale production facility. During the whole oyster mushroom production period (10 weeks) every week three-five samples (colonized substrate, fruiting body) were collected from five different substrate blocks. Lignocellulose decomposing enzymes were extracted from the samples with phosphate buffer (pH 7, 50 mM), enzyme activities (endocellulase, cellobiohydrolase, beta-glucosidase, endoxylanase, xylosidase, laccase, manganese peroxidase and exochitinase) and reducing sugar content were determined. Lignin decomposition enzymes showed the highest activity during the first part of oyster mushroom colonization. Activity of laccase was the highest in the first week whereas the activity of manganese peroxidase reached its peak in the third week. Endocellulase, beta-glucosidase, endoxylanase, exochitinase showed highest activity in the fourth week, which coincided with the development of fruiting bodies. Changes in cellobiohydrolase and xylosidase activity did not have any trend. The lignocellulose degrading enzyme activities of four blocks were similar during the first seven weeks. Though one block had a week delay in enzyme activity and fruiting body formation, too.

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MOLECULAR TAXONOMIC IDENTIFICATION OF LACTOBACILLI ORIGINATED FROM THE PROBIOTIC STRAIN COLLECTION OF THE DEPARTMENT OF MICROBIOLOGY, CENTRAL FOOD RESEARCH INSTITUTE (CFRI)

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Lactobacilli are of the most well-known probiotics. These microbes are lactic-acid producing bacteria (LAB), they utilise carbohydrates and produce lactic acid. Lactobacilli prefer different kinds of carbohydrates depending on their species. At the Department of Microbiology, CFRI, there is a strain collection of 41 lactobacilli, identified by classical microbiological methods. This collection was supplemented with more than 100 new isolates from the cecum of mice fed by mannane containing feed additives like BioMos, Locust bean gum and Konjac glucomannane. The strain collection was subjected to growth experiments to study their carbohydrate utilising abilities on glucose, lactose, inulin and special mannooligosaccharides. Based on the results representatives of the isolates were selected for further testing. The original strains and selected isolates were studied with Malthus conductance measuring instrument. The bacteria were grown in MRS broth without glucose supplemented with 1% glucose, 1% lactose, 1% inulin, or other

mannooligosaccharide prebiotics at 37 °C for 24 h. From the collected data growth rates and generation times were calculated. Taxonomic identification of the investigated Lactobacillus strains was done by combined molecular methods. Prior to sequencing a widely used repetitive PCR method followed by agarose-gel electrophoresis were applied for creation of lactobacilli groups. Later one or two representatives of each group were analysed by 16S rDNA identification. After molecular taxonomic identification the following species were found in our strain collection: *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus brevis*, *Lactobacillus sakei*, *Lactobacillus helveticus*, *Lactobacillus reuteri* and *Lactobacillus johnsonii*.

MONITORING MICROBIAL CONTAMINANTS OF PAPRIKA POWDER IN THE FRAME OF EU-FP7 SPICED PROJECT

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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. As Hungary is the main European paprika producing country, CFRI has the task of analysing the process from growing to packaged end product of paprika, including the definition of vulnerable points. During the three year project started this year, paprika powder samples are investigated not only by classical microbiological but by molecular taxonomical methods also. According to our preliminary experiments, the original microbial loads, determined by classical methods, of fresh paprika cultivars are approximately the same: total counts 10^8 CFU/g, moulds 10^4 CFU/g, yeasts 10^5 CFU/g, coliforms 10^4-10^6 CFU/g and *Escherichia coli* 10^2 CFU/g. As during processing 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. Beside the traditional plating technique, molecular methods were also applied to detect non-culturable contaminants of paprika powder. After total microbial DNA isolation, 16S rDNA specific PCR reactions were performed by universal primer pair (27f – 1492r). Clone libraries were created from the 16S rDNA specific community PCR products by Invitrogen TA Cloning kit. Clones were analysed by ARDRA and the group representatives were sequenced. According to our results, members of the *Bacillus* genus were the most frequent bacterial contaminants in the paprika powder.

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CORRELATION BETWEEN PLANT-SOIL-MICROBE INTERACTIONS OF WASTEWATER SLUDGE-BORNE HEAVY METALS AND THE EFFECT ON SUNFLOWER SEEDLING GROWTH

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Sunflower is one of the world's major oilseeds. The use of municipal wastewater sludge (MWS) as an organic fertilizer is an alternative disposal route to sacrificial soil disposal. Toxic elements can be transferred from soil to other portions of the ecosystem, with effects at both the ecosystem level and on human health from transmission through the web chain. Contamination of soil by potentially toxic metals from amendments of wastewater sludge is subject to strict controls in relationship to total permissible metal concentrations, soil properties, and intended use within the European Community. This study is aimed at determining the effects of municipal wastewater sludge containing high and low concentrations of Cd, Ni, Cr, and Pb in agricultural soils amended with these sludges on soil properties and plant growth. In greenhouse pot experiment, high heavy metals content of MWS (Hódmezővásárhely, Hungary) and low heavy metals content of MWS (Nyíregyháza, Hungary) at 0, 15, 30, 45% and 60% (w/w) were amended to two agricultural soils (clay loam brown forest soil; Gödöllő and chernozem meadow; Szeged) for ten weeks in greenhouse. Growth differences, heavy metal accumulation in plant parts and soil-metal concentrations were determined. The low metal sludge treatment showed the highest yield for sunflower seedlings when compared to controls. The amendment of wastewater sludge to the soil did indicate higher heavy metal content, although the increase was not as predicted, owing to the difficulty of obtaining a representative sample in the soil. Results showed that application of wastewater sludge to different soils could be useful in order to increase crop growth over a ten weeks period in the glasshouse. Results indicated that microbial biomass carbon and nitrogen, CO_2-release and some enzymatic activities determined in fresh soil samples were affected by soil types and increased by organic matter application as well as the rate of heavy metals content in the MWS, also, the investigated parameters were higher in Szeged's soil than the soil originated from Gödöllő. Results showed that applications of wastewater sludge are known to influence plant growth by various direct or indirect mechanisms. To exert their beneficial effects, wastewater sludge increases the microbial contents in rhizosphere and therefore, microbial traits required for root colonization are subsequently described. Finally, several mechanisms by which these microbial contents can act beneficially on plant growth are described. Examples of direct plant growth promotion that are discussed include biofertilization, stimulation of root growth, rhizoremediation, and plant stress control. Altogether, the use of waste sludge promoted microorganisms and the exploitation of beneficial plant-microbe interactions offer promising and environmentally friendly strategies for conventional and organic agriculture.

INHIBITION OF *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* EHEC BY ANTAGONISTIC BACTERIA ISOLATED FROM FOOD RAW MATERIAL OF ANIMAL ORIGIN

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Food-borne pathogenic bacteria can cause serious illnesses via their growth or toxin production, thus leading to significant health problems of the consumers. *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli* (EHEC) can cause infection by consumption of contaminated food and beverages, and are important causative agents of food-borne diseases, therefore inhibition or elimination of these pathogenic bacteria is an essential role for food producers. Certain microorganisms are able to inhibit the pathogens by overgrowing them or producing antibiotic

metabolites. Spoilage process of fresh meat and other protein rich raw materials represents characteristic interactions of different microorganisms when competition of saprophytic and pathogenic bacteria for the available nutrients results in succession of populations for different species. Our aims were to isolate and select bacteria from food raw material of animal origin, and test their antagonistic effect on pathogenic bacteria by microbiological test methods. Altogether 94 bacterium isolates (originated from cold stored poultry meat, fish, milk and liquid egg) were tested for antagonistic interaction with strains of *Listeria monocytogenes*, non-monocytogenes *Listeria* species and one avirulent strain of *Escherichia coli* O157:H7. Inhibition was tested by co-culturing of the test strains and the pathogens, and by determining the effect of the cell-free supernatant. Out of the 94 bacterium isolates, altogether twelve had an inhibitory effect on the examined pathogens. The antagonistic bacteria belong to *Pseudomonas fluorescens*, *P. lundensis*, *P. aeruginosa* and *P. frederiksbergensis*/*P. antarctica* species as revealed by molecular identification. They had the best inhibitory effect at 20°C, and they are members of either psychrotrophic or mesophilic bacteria. Based on the results of co-culturing it was established that in the case of *L. monocytogenes* inhibition could be achieved if the cell concentrations of the antagonistic bacteria were equal to or less than one order of magnitude compared to that of the pathogen. Significant differences between the sensitivity of the investigated *Listeria* species and strains for the antagonists were also observed. *Listeria ivanovii* subsp. *ivanovii* was the most sensitive, while one of the investigated *L. monocytogenes* strains proved to be the most resistant. The optimal ratio of the antagonistic bacteria and the enterohemorrhagic *E. coli* O157:H7 for significant inhibition was so high (approximately four orders of magnitude) that it would lead to organoleptic changes and spoilage of the food. Consequently, the direct application of antagonistic bacterial cells for *E. coli* inhibition is not adequate, and therefore the analyses of cell free supernatants are essential. Determination of the inhibitory effects of extracellular compounds produced by antagonists is still in progress.

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ISOLATION AND TOXICOLOGICAL STUDIES OF OPHIOBOLIN A

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Numerous sesterterpene-type secondary metabolites are produced by filamentous fungi belonging to the genera *Bipolaris*, *Cochliobolus*, *Drechslera* and *Aspergillus*. Among them ophiobolins are one of the most interesting family of compounds with numerous biological activities. The most intensively studied member of this group is ophiobolin A which is known as a very effective calmodulin antagonist agent. In this study a new purification procedure of ophiobolin A was described based on multi-step preparative HPLC separations from a previously selected *Bipolaris* sp. strain. Furthermore, the novel biological activity of the isolated compound was demonstrated. After incubation, the ferment broth was filtered and liquid-liquid extraction was carried out using ethyl acetate. This crude extract was concentrated at reduced pressure and fractioned by a semi-preparative normal phase HPLC system. The fractions contained ophiobolin A were pooled and was separated by two consecutive RP-HPLC chromatographic runs. The purified compound has been identified as ophiobolin A using standard reference compound and its identity was confirmed by mass spectrometric and NMR experiments. The purity of the isolated ophiobolin A was determined

by an isocratic HPLC technique and proved to be above 95%. The biological activities of ophiobolin A has been characterised via in vitro mammalian cell toxicity tests. According to the results of the porcine sperm motility inhibition experiments, ophiobolin A proved to be strongly toxic against sperm cells. Toxicity tests against somatic cells, PK-15 (porcine kidney), FFL (foetal feline lung), MNA (murine neuroblastoma) indicated that ophiobolin A is a biologically highly active representative of the ophiobolin family. The results of this work provide a good basis for the search for bioactive ophiobolin analogues and an opportunity to start an extensive assay to enlarge our knowledge of their biological effects.

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PURIFICATION AND CHARACTERIZATION OF THE TOXICITY EFFECTS OF 3-ANHYDRO-OPHIOBOLIN A

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Ophiobolins are a unique family of sesterterpene-type secondary metabolites produced by filamentous fungi belonging to the genera *Bipolaris*, *Cochliobolus*, *Drechslera* and *Aspergillus*. Until now 28 ophiobolin analogues have been described and assigned into 15 subgroups based on their characteristic structure. Ophiobolins have numerous biological activities such as antimicrobial, nematocid, calmodulin antagonist and cytotoxic. In this study a rapid preparative thin layer chromatographic (PTLC) methods were developed for the purification of an ophiobolin-isomer compound from a selected *Bipolaris oryzae* isolate. The whole procedure contained two consecutive PTLC steps using Kieselgel 60 F254 as stationary phase. The mobile phase was the mixture of ethyl acetate and n-hexane of 6:5 (V/V) for the first chromatographic run, while chloroform:acetonitrile 10:2 (V/V) was used for the last separation of the purification process. The structure of the purified compound has been elucidated and determined by mass spectrometric and NMR techniques and found to be as 3-anhydro-ophiobolin A. The purity of this compound was determined by an isocratic HPLC technique suitable for the analysis of different ophiobolin analogues and proved to be above 95%. The biological activities of this sesterterpene-type secondary metabolite has been characterised via in vitro mammalian cell toxicity tests. 3-anhydro-ophiobolin A proved to be moderately toxic compared to the effect of ophiobolin A against porcine sperm and somatic cells including PK-15 (porcine kidney), FFL (foetal feline lung), MNA (murine neuroblastoma) cells.

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MOLECULAR MICROBIOLOGICAL ANALYSIS OF BIOFILM BACTERIAL COMMUNITIES RESPONSIBLE FOR CARBON REMOVAL THROUGH A WASTEWATER TREATING REACTOR CASCADE SYSTEM

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The application of biofilm based wastewater treatment technologies is of growing interest worldwide. Compared to activated sludge systems these technologies represent a series of advantages such as low reactor footprint, less sensitivity to environmental conditions and no sludge recycling. Although biofilm based technologies are widely recognized only a few number of research is being carried out to increase their optimization possibilities. Furthermore, limited number of studies deals with exploration of biofilm bacterial community dynamics under different operational conditions. Thus, little or no information is available regarding the structure and alteration of biofilm bacterial communities responsible mainly for carbon removal as well. In order to fill this research gap molecular microbiological- and multivariate statistical analyses of biofilm bacterial communities were carried out through a biofilm based, pilot scale wastewater treatment cascade system. Results indicated a vertical as well as horizontal differentiation of biofilm bacterial communities in individual reactors and through the reactor series, respectively. The bacterial diversity and richness of biofilm samples taken from dissolved oxygen rich sections of reactors were relatively lower than samples taken from less oxygenized sections.
Our results indicated that within a particular reactor the concentration of dissolved oxygen is primarily responsible for alteration of bacterial communities. Through the cascade system set up mainly for carbon removal the initially dominant heterotrophic bacteria such as members of the genera *Acinetobacter*, *Acidovorax*, *Parabacteroides*, *Thauera*, *Desulfobacterium* and *Desulfomicrobium* were gradually replaced or supplemented by autotrophic nitrifying bacteria such as members of the genera *Nitrosomonas*, '*Candidatus Nitrotoga*' and *Nitrospira*.

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USEFULNESS OF MALDI-TOF IN CLINICAL MYCOLOGY

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Nowadays, application of MALDI-TOF (Matrix-Assisted Laser Desorption Ionization Time-Of-Flight) in identification processes in routine clinical microbiological laboratories are becoming more and more widespread around the world. In parallel, new technologies, application possibilities are expanding, so now clinical mycology - where a rapid species-level identification often give life-saving information – perhaps will be another field, where MALDI-TOF will gain a more important role in identification of yeast isolates. Our goals are to develop MALDI-TOF applications by optimalization to a reliable, usable and valuable tool in identification processes of clinical fungal isolates, comparing results obtained by different methods, verification of doubtful and rare results

with sequence analysis. More than 1000 clinical yeast isolates were examined, the results of different methods were compared. Optimalization processses were performed (different culture media, incubation time, inoculum volume, extraction methods), by this we are efficiently able to produce reliable results with laying down optimalized general processing rules for the 'in routine' occuring yeast isolates. In applying the optimalized method for identification we mainly got experience on non-albicans *Candida* species and other yeast species from different genera.
We conclude that the technology is well suited for: *i.* differentiation of closely related yeast species were differentiable so far only by DNA-based methods (*C. albicans – C. dubliniensis*, *C. parapsilosis – C. metapsilosis – C. ortopsilosis*), *ii.* identification of yeast species so far identified by slow or complicated biochemical methods and growth conditions (*C. lambica*, *C. famata*, *C. colliculosa*, *C. catenulata*, *C. pararugosa*, *C. lipolytica*), *iii.* identification of uncommon yeast isolates belonging to non-*Candida* genera (*Pichia*, *Kluyveromyces*, *Hanseniospora*, *Kloeckera*, *Geotrichum*, *Exophiala* species). The obtained results were verified by standard ITS-sequence analysis, they show a very good correlation so far.

EFFECTS OF CLARY SAGE OIL AND ITS MAIN COMPONENTS LINALOOL AND LINALYL ACETATE ON *CANDIDA ALBICANS*

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The essential oil from clary sage (*Salvia sclarea*) has been found to demonstrate antibacterial, antifungal, anti-inflammatory, antioxidant, antiviral and insecticidal properties and have been used traditionally for medicinal purposes, but have not fully examined the mode of action of oils and oil components or their target points on human pathogen yeast cells. The aim of this study was to examine the effects of clary sage oil and its main components, linalool (Lol) and linalyl acetate (La) on *Candida albicans* plasma membrane. Growth inhibition at various concentrations was measured in shaken cultures. Salvia oil and La caused the same growth inhibition while Lol was significantly less effective in this aspect at 0.125 µl ml^{-1} concentration in comparison to control. The cell growth was practically fully blocked at 0.5 µl ml^{-1} concentration. To determine the survival rates, cultures were treated with 0.125 µl ml^{-1} clary sage oil, Lol or La, which resulted in a 36%, 41% and 26% decrease, respectively, in the colony-forming ability of the cells after 60 min.Effects of clary sage oil, Lol and La on dynamic and thermodynamic properties of *C. albicans* plasma membrane were studied by electron paramagnetic resonance spectroscopy, using 5-doxylstearic acid (5-SASL) and 16-doxylstearic acid (16-SASL). The *C. albicans* protoplasts were treated with 0.125 µl ml^{-1} *Salvia* oil, Lol and La for 15 min in 0.6 M KCl solution than the plasma membrane of cells was labeled with 5-SASL and 16-SASL for 3 min. Statistical analysis of the temperature dependent I_{+1}/I_0 ratio curves showed phase transition break points at 13.15, 10.35, 12.70°C and 9.55°C after treatment. Thus, *Salvia* oil and its components induced a significant increase in fluidity, reflected by decrease in the break point temperatures, by disordering the plasma membrane of the treated cell. The vegetative cells were treated with 0.125 µl ml^{-1} Salvia oil, Lol and La for 60 min induced loss of 13.0%, 12.3% and 26.4% of intracellular metabolites absorbing at 260 nm, respectively. We exhibited a synergistic effect between clary sage oil and the antifungal compounds amphotericin B

and fluconazole. We believe that our results will contribute to understand the mode of action of *S. sclarea* essential oil and its main components on opportunist human pathogen yeasts.

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DEGRADATION OF ANILINE DERIVATIVES BY *PHANEROCHAETE CHRYSOSPORIUM* IN MANGANESE PEROXIDASE INDUCTIVE MEDIUM

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Phanerochaete chrysosporium, a basidiomycetous filamentous fungus, has outstanding pollutant-degrading capabilities with its extracellular and intracellular oxidoreductases. It is a white rot fungus which has a highly efficient lignin degrading enzyme system. With these enzyme systems the fungus can also break down different xenobiotic pollutants. In these types of degradation processes, the lignin peroxidase and the manganese peroxidase have great significance. In our present study *P. chrysosporium* strains were isolated from Hungarian habitats. We worked out a new selective medium, containing rose bengal, dichloran and carbendazim, with which the isolation of *Phanaerochete* strains from soil and from other habitats could be simply accomplished. In soil microcosm experiments the investigated *Phanerochaete* strains were able to degrade distinct herbicides and parabens in distinct soil types. One of the strains was able to degrade distinct chlorinated and alkyl derivatives of aniline in standing, manganese peroxidase inductive liquid medium. The amount of aniline derivatives was followed by colorimetry in the cultures. Aniline, 3-chloro-4-methylaniline, 3,4-dichloroaniline, 3-chloroaniline and 4-chloroaniline were degraded efficiently within two weeks, but 2,6-dimetylaniline proved resistant against the degradation.

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VERIFICATION OF HXNV PLAYS ROLE IN THE NICOTINIC ACID DEGRADATION PATHWAY IN *ASPERGILLUS NIDULANS*

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Precursor of the vital NAD+ is nicotinic acid that can be synthesized endogenously or taken up from the environment by transporters. Many microorganisms possess the capacity to utilize nicotinic acid as N-source, however the degradation of nicotinic acid was studied in only a limited number of prokaryotes. In eukaryotes the nicotinic acid degradation process is completely unknown, only the nicotinic acid hydroxylase (HxnS) of *Aspergillus nidulans* had been thoroughly studied that is responsible for the first step of the degradation. In the last years we explored the genetic background of nicotinic acid degradation and identified three genes (*hxn*X, *hxn*V and *hxn*M) of the degradation pathway downstream to *hxn*S. Here, we present the cloning, expression and functional analysis of

HxnV, a FAD-dependent enzyme. Coding sequence of HxnV was 6His-tagged, cloned into integrative pANpantoB *A. nidulans* vector and transformed into pantothenic acid auxotroph *Δhxn*V deletion strain. Pantothenic acid prototroph transformants were selected and tested for the utilization of nicotinic acid as sole N-source. His-tagged HxnV was purified by Immobilized Metal Affinity Chromatography with high yield and activity of purified enzyme was analysed.

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NOVEL GENERA OF AVIAN-ORIGIN PICORNAVIRUSES ("*GALLIVIRUS*" AND "*AVISIVIRUS*") AND THEIR UNEXPECTED GENOME FEATURES: SMALL STEPS TOWARDS TO UNDERSTAND THE COMPLEXITY OF THE PICORNAVIRUS GENOME STRUCTURE AND DIVERSITY

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Members of the family Picornaviridae are important pathogens of humans and animals, although compared with the thousands of known bird species (>10 000), only a few (n=11) picornaviruses have been identified from avian sources. During the last year complete RNA genomes of two novel enteric avian picornaviruses from turkeys (Meleagris gallopavo) were characterized in our laboratory using viral metagenomics and RT-PCR method. The phylogenetic analyses and the low sequence identity suggests that these viruses could be the founding members of two novel picornaviral genera ("*Gallivirus*" and "*Avisivirus*"), with "*Turkey gallivirus*" (TuGV) "*Turkey avisivirus*" (TuASV) as candidate type species. The complete genome analysis revealed multiple uncommon genome features in both viruses like the unusually long 3' untranslated region (UTR), the presence of at least three unrelated 2A, two in-tandem sequence repeats of 'ribosome skipping' sites (DxExNPG/P), with the first site being located at the end of the VP1 capsid protein in avisiviruses [1], or the presence of type II IRES (first identified among avian-origin picornaviruses) and a novel, potential mobile genetic element called "barbell-like structure" first identified in the 3' UTR of the galliviruses, which can be found in various sites in the 3'UTR of viruses at least two other picornavirus genera [2]. These viruses were detected in high abundance in faecal samples of healthy and affected commercial turkeys showed different manifested symptoms e.g. growth depression, skeletal disorders, enteric and/or stunting syndrome collected from eight turkey farms located in different sites of western Hungary. The high detection rate strongly suggests the endemic circulation of these viruses at least in (but probably not restricted to) this country. Furthermore, the genotype analysis of additional Hungarian strains of avisi-, and galliviruses revealed minor sequence variations among the TuGV strains, however in case of TuASV strains at least two genome clusters (probably distinct geno/serotypes) could be distinguishable. In addition, co-infection with these two picornaviruses was detected in seven (63.6%) specimens and six (75.0 %) of the investigated turkey farms. In this context, any contribution of these viruses to the development of different manifested symptoms as a single infection or part of co-infections or with other pathogens, as well as the potential worldwide distribution should be investigated.

[1] Boros, A. et al. (2012) J Gen Virol 93: 2171-2182.
[2] Boros A. et al. (2013). J Gen Virol 94: 1496-1509.

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IMPORTANCE OF HEALTH CARE ASSOCIATED INFECTIONS CAUSED BY VANCOMYCIN RESISTANT *ENTEROCOCCUS FAECIUM*; POSSIBILITIES OF PREVENTION

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Since the first descripition of vancomycin-resistant enterococci (VRE) in 1988 the importance of these multidrug resistant organisms (MDROs) is continuously increasing in the nosocomial infections (urinary tract infection, bloodstream infection, endocarditis). Treatment options for VRE-infections are limited as these bacteria are frequently resistant against most of the available antimicrobial agents. VRE is a well-known MDRO in the Health Care associated infections (HAI). Health Care associated infections caused by VRE is a problem especially in those health care settings where the prevalence of infections due to MDRO and *Clostridium difficile* is higher and the patients are cured with glycopeptides. In Europe, nosocomial outbreaks and clinical infections with VRE have emerged since 2000s. In paralell with this increase in nosocomial VRE infections, the proportion of *Enterococcus faecium* infections has increased relative to those of *E. faecalis*. Based on the results of multilocus sequence typing performed on many vancomycin-resistant *E. faecium* strains the clonal complex-17 (CC17) seems to be responsible for the worldwide emergence of nosocomial VRE, as most of the infection-related and outbreak strains belong to this complex. In Hungary the first case caused by VRE was published in 2000. The first outbreak caused by a vanB-positive *E. faecium* strain was detected in 2004. The prevalence of VRE remained very low until the beginning of this decade. However, in the reference laboratories of the National Center for Epidemiology the number of confirmed vancomycin-resistant *E. faecium* isolates leapt hundredfold from 2010 to May 2013 and three outbreaks caused by vanA-positive *E. faecium* were reported in 2012. In this period 194 vancomycin-resistant *E. faecium* (50 vanB-positive and 144 vanA-positive) and 4 vanB-positive vancomycin-resistant *E. faecalis* isolates were confirmed. 59% of the vanA-positive isolates belong to the ST203-like sequence type and 79% of the vanB-positive isolates belong to the ST192 sequence type. Both sequences types are the member of the highly virulent CC17 clonal complex. In Hungary reporting of HAIs caused by VRE through MDRO module of the National Nosocomial Surveillance System (NNSR) are mandatory. From 2006 to 2012 the number of reported VRE-infections is continuously increasing. The prevention of emergence of VRE-infections is similar as in case of all other MDROs. In hospitals the infection control practicioners aim to prohibit the emergence of VRE by antibiotic-stewardship and screening of high-risk inpatients by isolation of the infected patients and surveillance of VRE-infections.

THE IMPACT OF THE *CANDIDA* SURFACE ANTIGEN FROM THE DING PROTEIN FAMILY ON VIRULENCE OF CLINICALLY IMPORTANT YEASTS FROM THIS GENUS - A GENERAL SYNOPSIS

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Candida yeasts express many proteins contributing to their virulence and pathogenicity. The CR3-RP (complement receptor 3-related protein) is believed to be an important surface antigen participating in adherence to unrelated biotic and abiotic surfaces. Fragment of the CR3-RP has already been sequenced (DINGGGATLPQ). Based on this sequence, the CR3-RP can be involved into DING family of proteins (named after DINGGG N termini) already described like ubiquitous in eukaryotes with possible function in cell-cell communication or adherence. The *Candida* CR3-RP has been determined as 45 kDa protein expressed in different *Candida* spp. Among *Candida* spp., this protein has been the most precisely studied in *Candida albicans*. For direct detection of the CR3-RP, the polyclonal anti CR3-PR serum (anti CR3-RP pAb) was prepared by immunization of rabbit with undekapeptide DINGGGATLPG. Indirect immunofluorescence, ELISA as well as immunocytometry analysis proved that the CR3-RP is expressed not only by *C. albicans*, but also by different species of the *Candida* genus. Additionally, this protein cross-binds the monoclonal antibody (mAb) OKM1 directly reacting with the human CR3 receptor expressed on neutrophils, macrophages or monocytes suggesting idea that the CR3-RP can be assumed to be a "mimicry protein". The elevation of specific anti CR3-RP antibodies IgG, IgM, but mainly IgA after immunization of rabbits by CR3-RP as well as induction of cytokins IL-6, IL-1α and TNF-α in macrophages cultivated with CR3-RP suggested possible bioimmunogical role of this protein in the first phase of *Candida* infection. Similar results were confirmed by cytokine multiplex analysis in experiments with mice after CR3-RP immunization. Using incubation of *C. albicans* with anti CR3-RP pAb and OKM1 mAb, the CR3-RP epitope was blocked and such "pre-incubated" *C. albicans* cells were tested and compared with the standard non-pre-incubated *C. albicans* in different in vitro (measurement of kinetic of adhesion and biofilm formation on polystyrene plastic) and ex vivo model experiments (interaction with buccal epithelial cells). On the other hand, this peptide did not show cytotoxic activity on the standard cell lines HeLa a HEK 293. Summarizing all results, the CR3-PR expressed by *Candida* spp. can be postulated to be an immunodominat antigen participating in adhesion and modulation of host-pathogen interactions.

PHYLOGENETIC DIVERSITY OF THE BACTERIAL COMMUNITIES CONSTITUTING THE BIOFILMS OF TÖRÖK HYDROTHERMAL SPRING CAVE OF 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 in hydrothermal cave forming processes as well. The biofilms on the cave walls have already been studied by molecular methods regarding their bacterial diversity. The aim of this study was to continue these investigations, focusing on one specific site of the southern discharge region, the Rudas-Török hydrothermal spring, along with the examination of the iron hydroxide precipitates developing on cell surfaces using microscopy and spectroscopy methods. Molecular investigations were conducted on three sample types: biofilm, calcite rafts and water. DGGE analysis of samples taken from the same sites at two different periods showed a significant difference between water and biofilm communities, where biofilm samples proved to be more

diverse, and a diverse archaeal community was also revealed. Three clone libraries were constructed from the samples. The 16S rRNA gene sequences of the representative clones belonging to 15 major taxa showed the closest relation to uncultured clones from different environmental sources. The microbial community of the biofilms proved to be somewhat more diverse than that of the calcite rafts, while water showed a very simple community structure. The following taxa were dominant: Alpha- and Betaproteobacteria, *Nitrospira* and Chloroflexi. The percentage of molecular clones among different taxa was dissimilar compared to the examinations performed in 2009. Considering the metabolic traits of known species related to our clones, it is assumed that these communities may participate in the local sulfur and iron cycles, and may contribute to biogenic cave formation.

INVESTIGATION OF IMMUNOLOGICAL MARKERS AFTER ELECTROSTIMULATION

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Electric and magnetic fields are known to interact with living systems. These fields generally act as environmental stress factors. Changes in the activities of several enzymes in response to electric impulses were previously tested [1]. In study our aim was to determine the impact of electric stimulation on human professional immune cells at relative gene expression level. THP-1 human monocytic cells were treated by direct electric impulses and by the electric field of a commerce cell phone. Three impulses of direct current of 80 and 160 V/cm were used. The duration of the impulses was 2 ms and the repetition period was 2 s. The cell phone field was turned on for 20 minutes with continuous call. The relative expression of 22 genes were determined by real-time quantitative PCR and calculated by ΔΔCt method with GAPDH control. There were no changes in expression profile at lower electric field. At the same time, the larger electric field resulted in elevated pro-inflammatory effector molecule gene expression, such as TNFAIP6, CCL1, CXCL10 and IL-17A. Furthermore, the electromagnetic field of cell phone resulted in a robust relative gene expression up-regulation in case of IL-10, TNFAIP3 and TNFAIP8, at the same time, strong relative gene expression down-regulation was detected in case of IL-27 and IL-12.
Based on these findings, we can conclude that direct electric stimulation and the electromagnetic field have controversial effect on the relative gene expression profile of human monocytes. Direct electric stimulation seems to be a direct stress factor, however, electromagnetic stimulation is involved in the down regulation of the immune response.

[1] Filipic, B. et al. (2000) Bioelectrochemistry and Bioenergetics 52: 29-36.
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SELECTION AND APPLICATION OF MICROORGANISMS FOR MYCOTOXIN DEGRADATION

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Mycotoxins are secondary fungal metabolites that may have mutagenic, carcinogenic, cytotoxic and endocrine disrupting effects. These substances frequently contaminate agricultural commodities despite efforts to prevent them, so successful detoxification tools are needed. The application of microorganisms to biodegrade mycotoxins is a novel strategy that shows potential for application in food and feed processing. In this study we investigated the mycotoxin degradation ability of microorganims belonging to thirty-four species of fourteen genera on economically important mycotoxins: aflatoxin B1 (AFB1), zearalenone (ZEA), fumonisin B1, T2 toxin and ochratoxin A (OTA), and monitored the safety of AFB1, ZEA degradation processes and degradation products using previously developed toxicity profiling methods and animal tests, such as fish and broiler chicken for AFB1, rat for ZEA and mice for OTA. Moreover, experiments were performed to analyze multi-mycotoxin-degrading ability of the best toxin degrader/detoxifier strains on AFB1, ZEA and T2 toxin mixtures. This enabled the safest and the most effective Rhodococcus strains to be selected, even for multi-mycotoxin degradation. Results of the mycotoxin tests proved the degradation ability of thirty-two strains. Among these strains, thirty are capable for degrading two or three mycotoxins as well. According to recent studies, the multi-mycotoxin degradation ability for three mycotoxins (AFB1, ZEA, T-2 toxin) of *Rhodococcus erythropolis* NI1 is a unique property. By sequencing the complete genome sequence of *Rhodococcus pyridinivorans* AK37 and *Cupriavidus baseliensis* Őr16, the genetic base of mycotoxin degradation would be revealed; since genome projects have a major role to identify key-enzymes that play an important role in metabolic pathways of mycotoxin degradation. On the base of our study strains could be selected that are applicable as detoxifying agents in food or feedstuffs if toxicological studies (antibiotic resistance, pathogenicity, etc.) would not reveal harmful effect of these microbes. When microbes may raise toxicological problems, enzyme-based formulations have a great potential as mycotoxin detoxifying agents.

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CHARACTERISATION OF THE APPLE SURFACE MYCOTA

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The occurrence of fungi on apple fruit is important because they cause diseases by primary infections during the vegetation period and cause spoilage by secondary infections during storage, causing great damage in fruit production. Moreover, some fungi produce toxic compounds (mycotoxins), which is a great risk from the point of view of food safety. Apple, as a seasonal product, is generally stored with cold and dry technologies for prolonging the shelf-life of the product. The knowledge of the indigenous fungal communities inhabiting the apple surface contributes to the control of fruit decay as the most common postharvest disease. We examined the quantity of fungal elements and the taxonomic diversity of fungal communities on the surfaces of apple fruit at the time of harvest and during storage in 2010 and 2011. The six cultivars concerned in the investigations originated from the experimental orchard of the Corvinus University, Budapest. Malt extract agar was used for the determination of total fungal elements and for obtaining isolates.

The amount of fungal propagula on the surface of the six examined cultivars differed considerably from each other. In 2010, after storage of six months, the fungal population on the fruits of the cultivar Golden Reinders, Jonagold Rubinstar, Bőrkormos renet and Florina was 2.3×10^4 CFU cm^{-2}, 1.2×10^3 CFU cm^{-2}, 1.5×10^3 CFU cm^{-2} and 7.0×10^3 CFU cm^{-2}, respectively. On the surface of the different cultivars, 34 fungal species were identified. The majority of the species belonged to the *Penicillium* genus. *Aspergillus*, *Cladosporium*, *Alternaria*, and representatives of 3 further genera were present among the isolates. Taxonomic diversity expressed by Shannon's diversity index was the highest in the case of Jonagold Rubinstar (1.51) in 2010 and Jonagold Bio (2.06) in 2011. The quantity of fungal elements just slightly differed in 2010 and 2011. The difference can be significant in the case of higher water-content, which could increase the risk of post harvest spoilage.

PREVALENCE OF NOVEL HUMAN POLYOMAVIRUS 9, WU AND KI

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Our aim is to study the prevalence of HPyV9, WUPyV, KIPyV and to determine whether immunosuppression result in higher prevalence of these viruses. Blood, urine and respiratory samples are collected at the same time from renal transplant patients, patients with lymphoma and healthy individuals. Samples from renal transplant patients are collected monthly for 1 year after transplantation. Up to now 53 renal transplant patients, 50 patients with lymphoma and 133 healthy individuals are in the study. Presence and quantity of WUPyV, KIPyV and HPyV9 DNA is detected by nested and real-time PCR. PCR products from nested PCRs were sequenced to confirm the positivity. Different methods are compared. Frequency of KI virus is 0.5-1.7 % and it was found in all sample types. WU virus was detected only in respiratory samples with low prevalence. HPyV9 DNA was found in all sample types with higher frequency (0.8-4.5%). The prevalence of the studied viruses was usually higher among renal transplant patients. Nearly 1/3 of the transplant patients had either of the studied polyomavirus in either of the collected samples, while this proportion is less for not immunocompromsed patients. This suggests that immunosuppression may lead higher susceptibility for these viral infections or reactivations.

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CHARACTERIZATION OF THE VIRULENCE OF *CANDIDA PARAPSILOSIS* SENSU LATO SPECIES USING DIFFERENT IN VITRO AND IN VIVO INFECTION MODELS

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The clinical significance of the *Candida parapsilosis* sensu lato group, which involves the closely related species *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis*, has largely increased over the last decades. However, little is known about the virulence properties of the

members of the species complex. In this study, we tested 63 *C. parapsilosis* sensu stricto, 12 *C. metapsilosis* and 18 *C. orthopsilosis* isolates for the ability to produce extracellular proteases or lipases and to form pseudohyphae. Afterwards, nine different clinical isolates each of *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* were co-cultured with murine or primary human macrophages. *C. parapsilosis* sensu stricto isolates showed a significantly higher resistance to killing by primary human macrophages compared to *C. orthopsilosis* and *C. metapsilosis* isolates. In contrast, the killing of isolates by J774.2 mouse macrophages did not differ significantly between species. However, *C. parapsilosis* sensu stricto isolates induced the most damage to murine and human macrophages, and *C. metapsilosis* strains were the least toxic. Furthermore, strains that produced lipase or pseudohyphae were most resistant to macrophage-mediated killing and produced the most cellular damage. For in vivo experiments, we used 9 isolates each of *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* to examine the impact of each species on the survival of *Galleria mellonella* larvae. The mortality rate of larvae infected with *C. metapsilosis* was significantly lower than those infected with *C. parapsilosis* sensu stricto or *C. orthopsilosis*. Furthermore, we used a quantitative imaging technique to determine the phagocytic activity of J774.2 macrophages against one representative isolate of each *C. parapsilosis* sensu lato species. After 2 hours of incubation, the ingestion of fungal cells by J774.2 macrophages was considerably lower in case of *C. metapsilosis* compared to *C. parapsilosis* sensu stricto and *C. orthopsilosis*. Additionally, we found that after 48 hours, *C. metapsilosis* induced lower MCHII expression in human PBMCs compared to *C. parapsilosis* sensu stricto and *C. orthopsilosis*.
Taken together, our findings contribute to the better understanding of the interactions between the host and the species of the *C. parapsilosis* complex.

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DETECTION OF CARDIOTROP VIRUSES IN BIOPSY SAMPLES OF THE HEART IN DILATED CARDIOMYOPATHIES

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Some decades only enteroviruses were regarded as aetiological agents of dilatative cardiomyopathies (DCMs). In the past years other viral and rare bacterial pathogens were detected too. These viruses are ubiquitous pathogens of humans and animals. Quantitative PCR methods are suggested for the determination of viral nucleic acids in dilatative cardiomyopathies (DCMs), and some other cardiac diseases. Certain authors, have detected viral nucleic acids in autopsy samples, without cardiac diseases, at similar rates as in DCM patient samples. In our study in the past 9 years (2004-2012), 93 adult endomyocardial biopsy samples were received from the 2nd Department of Medicine and Cardiology Centre. Samples were prepared on the day of collection. After nucleic acid isolation from the clinical samples by the Qiagen method, these were frozen or amplified with adenovirus (AdV), cytomegalovirus (CMV), Epstein Barr virus EBV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), parvovirus B 19 (PV B19) and enterovirus (EV) primers. Qualitative RT PCRs, were used; 1-3 PCRs were performed on the biopsy samples/patients. Copy numbers of viruses were determinated by quantitative RT-PCRs. Ten control samples were examined by the same methods. 406 PCRs were performed. The clinical samples contained 1 viral nucleic acid each in 20 patients; 2

viral nucleic acids were positive in 2 patients, parvovirus nucleic acid in 11 patients, CMV in 4 patients, and EBV, enterovirus and HSV-1 in 3 patients each. Adenovirus and HSV-2 were not detected. Many research articles have been published on this topic, but there is not yet a validated molecular microbiological method. Not only classical cardiotropic enteroviruses have been detected in DCM, but also a high percentage of members of the Herpesviridae family. The possibility of antiviral therapy should be considered in the event of DCM of HSV, EBV or CMV origin.

STUDIES ON *ESCHERICHIA COLI* AS A PARADIGM OF BACTERIAL VERSATILITY AND VARIABILITY: IMPLICATIONS FOR PATHOPHYSIOLOGY, EPIDEMIOLOGY AND DIAGNOSTICS

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Escherichia coli is one of the major human pathogens in Europe, responsible for both, diarrheal and extraintestinal diseases. While antibiotic treatment of *E. coli* infections was highly successful in the end of the last century, during the last decade *E. coli* has become increasingly resistant to a variety of antibiotics. The recent German *E. coli* O104:H4 outbreak of haemorrhagic diarrhoea was a dramatic example of the explosive cocktail of high virulence and high resistance that can emerge in this species. In contrast, *E. coli* is also a commensal belonging to the normal intestinal flora of healthy individuals. The major impact on public health of *E. coli*, its versatility and the considerable amount of fundamental knowledge on this bacterial species make *E. coli* a paradigm to study microbial adaptation and evolution in the field of fundamental microbiology, but also in infectious disease research. To understand which traits may distinguish pathogenic from commensal *E. coli*, we are interested in virulence and adaptation mechanisms. Our current research activities include global functional and comparative studies of the *E. coli* genome content, gene regulation and physiology in order to better understand and eventually control *E. coli* infection. Upon infection, pathogenic *E. coli* face not only the host and its immunological defense systems but they also have to compete for resources and thus the strategic plan of the persisting bacteria must include overcoming or evading the host, but also adaptation to the growth conditions in the new niche. Our results indicate that *E. coli* employ colonization strategies to withstand or avoid the induction of host immune responses as well as to efficiently utilize nutrients provided in this niche. Our comparative and functional genome analyses of different pathogenic and non-pathogenic *E. coli* variants indicate that besides the rapid acquisition of new traits by horizontal gene transfer, differential expression of conserved genes can also contribute to a strain's ability to efficiently colonize certain niches and cause infection. These results further demonstrate the importance of *E. coli* genome plasticity as a prerequisite for adaptation and diversification of this species.

DISTRIBUTION OF VIRULENCE FACTORS BETWEEN MRSA AND MSSA ISOLATES FROM NASAL CARRIAGE AND STAPHYLOCOCCAEMIA

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Staphylococcus aureus is a well-armed pathogen and its prevalence as a source of nosocomial infections has increased steadily. Besides antibiotic resistance the success of many strains can be

attributed to expressed virulence factors which mediate colonization of the host, tissue invasion and dissemination. *S. aureus* accounts for approximately 13% of all nosocomial blood infections. Nasal carriage is an important risk factor for acquiring bacteremia since nearly 40% of humans may be colonized on mucosal surfaces. Most of the surveillance studies of methicillin-resistant and methicillin-susceptible isolates of *S. aureus* (MRSA and MSSA) have been performed during outbreaks, and focused mainly on the epidemiology of the strains. In the present study we compared the occurrence of selected genes coding for virulence factors in different MRSA and MSSA samples. We searched for possible differences in toxin gene profiles between colonizing isolates and isolates involved in staphylococcaemia. In 2012 the prevalence of MRSA reached 13% at the university hospitals of Debrecen which is still lower than the national prevalence. However, the rate of MRSA among *S. aureus* bloodstream infections was 27%. In our experiments we characterized MRSA and MSSA isolated in 2012 from blood samples and from the anterior nares of healthy medical personnel. The identification of *S. aureus* was based on conventional methods. In addition, all isolates were confirmed to be *S. aureus* by PCR. A fast duplex PCR was optimized for the detection of the mecA and femA genes together within 1 hour. Susceptibility to different antibiotics was tested by the disk diffusion method according to the CLSI guidelines. In case of MRSA isolates the minimal inhibitory concentration was determined by E-test for oxacillin, vancomycin and teicoplanin. Screening for virulence genes was carried out by multiplex PCR. Besides the classical five major types of staphylococcal enterotoxins (sea-see) we screened for the presence of other superantigens such as the toxic shock syndrome toxin-1 (tst) as well as the exfoliative toxins A and B (eta and etb), and the Panton-Valentine leukocidin (pvl). Nasal swabs from healthy medical personnel were collected monthly during a 5-month period. 55% of the volunteers were positive at least once but none of the isolates was methicillin resistant. One person yielded tst positive MSSA three times, another person's every isolate was seb positive. Prevalence of enterotoxins A and E were 14% and 21% respectively. Regarding the MRSA isolates from blood samples 28% were positive in combination for sea and see genes, and 28% were sed positive. As for MSSA from blood we could not detect either sea, sed or see genes but 20% were seb positive. We did not find any linkage between the virulence factor carriage and the antibiotic resistance profiles, except for the fact that the sed positive MRSA blood isolates had lower oxacillin MICs than the sea/see positive ones. In the future we would like to expand our investigations on the possible differences between the MRSA/MSSA from nasal samples of the general hospitalized population.

RE-EMERGING G9 ROTAVIRUSES IN 2012, HUNGARY

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Rotavirus strain monitoring in Hungary dates back to mid-1980s. The availability of rotavirus vaccines has resulted in an intensification of post vaccine strain surveillance efforts to gain information on the impact of vaccines on prevalence of circulating rotavirus strains. The present study was conducted during 2012 in 9 geographic areas of Hungary. Rotavirus positive stool samples were collected from diarrheic patients, mainly <5 years of age. The VP7 and VP4 genes

were amplified by single step RT-PCR assay and then sequenced by traditional chain termination technique. Sequence data were analyzed by similarity search through BLAST and RotaC servers. After eliminating non-typeable and partially typeable strains from the sample set, a total of 409 strains could be fully genotyped. The dominating strain was G9P[8] (42.3%), which was followed by G1P[8] (35.0%), G2P[4] (8.3%), G4P[8] (7.6%), G2P[8] (2.9%),G1P[4] (2.2%),G3P[8] (0.7%),G9P[4] (0.5%),G3P[4] (0.2%),and G3P[9] (0.2%). G9P[8] rotaviruses prevailed (46.2% to 83.3%) in 7 of 9 surveillance areas; exceptions included Szabolcs-Szatmár-Bereg county (G1P[8], 92.3%) and Hajdú-Bihar county (G4P[8], 43.9%). The changing epidemiology of rotaviruses reinforces the need for continued strain surveillance.

QUANTIFICATION OF FAECAL MICROFLORA IN IBD PATIENTS USING REAL TIME PCR

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Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the colon and small intestine. The major types of IBD are Crohn´s disease and ulcerative colitis. It is a complex chronic disorder encompassing several inflammations in the mucosal surface of the gut leading to damage of the gastrointestinal tract. IBD has a genetic background but also environmental factors and alternations in gut microflora are involved in IBD development. It is, therefore, important to study composition of faecal microflora in patients with IBD. The aim of the study was to quantitative assess the composition of major bacterial groups in stool samples and intestinal biopsies of IBD patients and to compare the results with other patient outcome. DNA was isolated by QIAamp Stool Mini Kit from frozen stool samples and by DNeasy Tissue Kit from colonoscopic intestinal biopsies. Already published primers complementary to 16S rRNA gene were used for real time PCR detection of major groups of bacteria: universal (Uni1359F/Uni1492R), *E. coli* (Ecoli396F/ Ecoli491R), Firmicutes (Firm934F/ Firm1060R), Bacteroidetes (Bact934F/ Bact1060R) and Actinobacteria (Act920F/Act1200R). Composition of bacterial microflora was tested in stool samples of 25 patients. We observed variable abundance of tested bacteria in faecal samples from different patients which reached values 12-180% for Firmicutes, 0.02-200% for Bacteroidetes and 0-27% for E. coli. In the case of faecal biopsies we observed variable composition of tested bacteria in biopsy samples from different patients as well as in the samples obtained from different intestinal parts from the same patient. We optimized methods for DNA isolation and real time PCR quantitation of bacteria from stool and intestinal biopsy samples.
Baceroidetes and Firmicutes were major groups in all samples. *E. coli* was less abundant in stool samples, but it was present at increased concentrations in biopsies. Numbers of Actinobacteria were low or under detection limit in all samples.

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DRIED BLOOD SPOT SCREENING METHOD IN INTRAVENOUS DRUG USERS FOR HIV, HBV, HCV

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Between 2006-2011, National Centre for Epidemiology conducted five national HIV/HBV/HCV seroprevalence studies among intravenous drug users, introducing dried blood spot screening method in this population. On the basis of the national prevalence surveys, the number of HIV positive persons is probably very low in Hungary, and the proportion of HBV positive persons is probably also below 1% in the IDU population, the national HCV prevalence rate has been around 25% since 2006. Authors analyze data deriving from the studies focusing on gender, age group, date of first injection, screening frequency, drug types, and other risk behaviors over time.

ZOONOTIC PATHOGENS TRANSMITTED BY MOSQUITOES AND SAND FLIES IN SLOVENIA

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Introduction and establishment of vectors and vector-borne pathogens in the naïve habitat presents a serious danger in Europe and worldwide. Either such events are a result of climate change, changing agricultural practices and land-use patterns or revolution of transport technologies and increasing global trade, the fact remains: we are witnessing a growing number of such events with devastating consequences. Modern examples include global circulation of Dengue and West Nile viruses, the intercontinental dissemination of *Aedes albopictus* and other mosquito species, the epidemic of Chikungunya virus in Italy, the spread of Crimean-Congo haemorrhagic fever virus in Greece, to name just a few. To prevent or to at least constrain the spread of vector-borne diseases, several factors that are the key to the epidemiology of vector-borne diseases need to be studied. Among the most important are the ecology and behaviour of the vector and the host, the genetic and phenotypic characteristics of vector-borne pathogen itself and the degree of immunity in the population. Slovenia is an endemic country for two vector-borne zoonoses: tick borne encephalitis and borreliosis. The causative pathogens of both diseases are transmitted by ticks. In addition, other medically important pathogens such as anaplasmae, rickettsiae and babesiae have been detected in ticks in Slovenia. However, despite their important role as vectors of pathogens, ticks are only second to mosquitoes when it comes to transmitting microorganisms. Female mosquitoes are the most important arthropod vectors worldwide. Although limited information about species of mosquitoes in Slovenia exists, no data about the pathogens they carry is available. Recently, Kalan and co-workers demonstrated the presence of tiger mosquito *A. albopictus* in south-east part of Slovenia. They concluded that tiger mosquitoes established a firm population in the studied area. In northern Slovenia another invasive mosquito species, *A. japonicus*, was found by Seidel and co-workers. Herein we will present the first information about the search for zoonotic pathogens transmitted by mosquitoes and sand flies sampled in Slovenia.

CHARACTERISATION OF ESBL-PRODUCING ENTEROBACTERIA IN STOOL SAMPLES OF INDIVIDUALS SCREENED FOR ENTERIC PATHOGENS

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The aim of this study was to investigate the prevalence of bacteria producing extended spectrum beta-lactamases (ESBLs) in asymptomatic individuals and to characterise the isolates. We used 1662 faecal samples from individuals to be screened for enteric pathogens between March 2009 and April 2010. Samples were from 1116 individuals screened for employment eligibility purposes (303 males and 813 females, mean age 34.8 years), and from 546 individuals applying for long-term care facilities (223 males and 323 females, mean age 67.9 years). Samples were inoculated onto eosin methylene blue agar, supplemented with 2 mg/l cefotaxime. ESBL production was confirmed using double disk synergy test. ESBL-coding genes (*CTX*-M1, *CTX*-M2, *TEM*, *SHV*) were detected by PCR, typing of *CTX*-M genes was performed by sequencing. Type one and two intergons were also sought for by PCR. For *E. coli* phylogenetic groups were determined by PCR and relatedness was assessed using ERIC-PCR. ESBL producers were isolated from 3.7% (61/1662) of the samples, these rates among individuals on employment screening and applicants for long-term care were 2.1% (23/116) and 7% (38/546), respectively. Among ESBL producers there were 46 *Escherichia coli*, 20 *Klebsiella pneumoniae*, one *Proteus mirabilis* and one *Citrobacter* spp. Genetic diversity was high in *E. coli*, 20 of 46 were unique, the remaning 26 formed nine pair/clusters of isolates. In total, 77.9% (53/68) of the ESBL genes were of *bla*CTX-M type; *E. coli* harboured *CTX*-M ESBLs more frequently than *K. pneumoniae* (37/46 vs. 12/20). Rates of strains harboring *CTX*-M-15, *CTX*-M-1, *CTX*-M-2 and *CTX*-M-32 were 60.4%, 17%, 5.7% and 1.9%, respectively. One strain harbored a combination of *CTX*-M-15 and *CTX*-M-2 (1.9%). Only *CTX*-M-15 was detected in *K. pneumoniae*. Type one integrons were common in both species 23/46 *E. coli* and 16/20 *K. pneumoniae* had type one integrons; two *E. coli* but no *K. pneumoniae* isolates carried type two integrons, while in a single *E. coli* isolate both integron types were found. Commensal *E. coli* (phylogenetic groups A and B1) was more frequent and more diverse genetically than extraintestinal pathogenic isolates (groups B2 and D). Group B2 was associated with *CTX*-M-15 and the aminoglycoside resistance gene *aac*(6')-Ib. Curiously, commensal strains carried integrons more frequently than pathogenic strains (19/32 vs. 4/14). In contrast to *K. pneumoniae*, high degree of genetic diversity among *E. coli* indicates an allodemic situation, where high rate of ESBL carriage among commensal *E. coli* strains seems to be a consequence of horizontal gene transfer rather than the dissemination of specific clones. The study highlights the importance of human commensal flora as reservoir of clinically relevant antibiotic resistance genes (including ESBLs) and transferable genetic elements.

INVESTIGATION OF THE MICS OF FIDAXOMICIN AGAINST HUNGARIAN *CLOSTRIDIUM DIFFICILE* STRAINS

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Clostridium difficile infection (CDI) usually occurs after exposure to broad-spectrum antibiotics and is the most common identifiable cause of diarrhea in hospitalized patients. Current treatment strategies are inadequate with decreased response rates to metronidazole, and high recurrence rates

with the use of metronidazole and oral vancomycin. Fidaxomicin is a new macrocyclic antibiotic, which has a narrow spectrum of activity against gram-positive anaerobes and is bactericidal against *C. difficile*. Fidaxomicin stands out as the first-in-class oral macrocyclic antibiotic with targeted activity against *C. difficile* and minimal damage on the normal colonic flora. The aim of this study was to investigate the in vitro activities of fidaxomicin against 188 *C. difficile* strains isolated in our laboratory or in different centers of Hungary. The strains' toxicity was tested routinely. The determination of MICs of metronidazole, moxifloxacin, rifampicin and vancomycin has already been done previously by E-test method. MICs of fidaxomicin were determined by agar dilution (according to the CLSI recommendations). None of the isolates were resistant for metronidazole or vancomycin. However, 35 of the 188 isolates (18.6%) proved to have an MIC>32 µg/ml for moxifloxacin and 19 (10.1%) had an MIC ≥ 32 µg/ml for rifampicin. The *C. difficile* isolates displayed minimum inhibitory concentrations (MIC) for fidaxomicin in the range of <0.008-0.5 µg/mL, with a MIC90 of 0.125 µg/mL. Only four isolates (2.1 %) had 0.5 µg/ml MICs to fidaxomicin. The detected MICs displayed an identical distribution with respect to the EUCAST database for wild-type strains. The MICs of fidaxomicin for the control *C. difficile* strain (CD 630) was 0.064 ± 1 dilution.
Overally, fidaxomicin proved to be a highly effective drug against *C. difficile* in vitro outscoring the efficiency of metronidazole and vancomycin.

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GENETIC ANALYSIS OF *BACTEROIDES* STRAINS ISOLATED AT TARGU-MURES IN 2010-2013

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Anaerobic bacteria are often neglected pathogens with regard their frequency of isolation and the serious infections they may cause. The routine diagnosis for anaerobic pathogens has been introduced at the Maros County Emergency Hospital, Targu-Mures, Romania in 2010. We aimed to confirm the local species identification by MALDI-TOF MS and conduct genetic analysis (antibiotic resistance genes and plasmid content) of the *Bacteroides* strains (n=55) isolated onward from that time. The original species identifications were confirmed by MALDI-TOF MS for almost all the strains examined. 48.3 % of the strains harboured plasmids which belonged to three main types (2.7, 4.1 and 5,6 kb). We detected 15 antibiotic resistance genes or an insertion sequence (IS) element (IS4351) from which *cep*A (normal cephalosporinase) and *tet*Q (tetracycline resistance) were the most abundant, 71 % and 74 %, respectively. The prevalence of beta-lacatamase genes displayed levels roughly equal to those that were internationally experienced (*cep*A 71%, *cfx*A 6,5%, *cfi*A 3,2%). Concerning MLS-B resistance it was interesting to find low levels of *erm*F (6.4%), IS4351 (0%) and *erm*G (9.7%). We did not find *msr*S, *mef*A, *erm*B, *lin*A, *tet*M, *tet*X, *tet*X1, *tet*36 or *nim* genes, but *bex*A (9.7%) and *sat*G (12.9%) genes were found.
As general we can say that the characteristics of the *Bacteroides* strains isolated at Targu- Mures follow the trends established for strains from other countries but bear some geographic specialities too (relative lack of *erm*F and other genes).

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MELANIZATION PROTECTS CELLS FROM CELL WALL DEGRADING ENZYMES IN *ASPERGILLUS NIDULANS*

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Several fungi produce melanin. The role of this brown to black pigment is not only to increase the mechanical strength of the cell wall and protect cells from ultraviolet radiation, but it also helps fungi to survive among harsh conditions and significantly contributes to the virulence of human and plant pathogens [1]. Here, the role of melanin in the cell wall integrity of *Aspergillus nidulans* was studied. Significant melanin production was detected after glucose depletion (carbon starving cultures) when cultures also produced extracellular chitinase and ß-1,3-glucanase. The produced melanin stained both the mycelia and the fermentation broth. Induction of *ivo*A, *ivo*B (DOPA type melanin synthesis [1]) as well as *w*A (DHN type melanin synthesis [1]) were observed by qRT-PCR. Addition of lysing enzymes of *Trichoderma harzianum* increased the pigmentation of both growing and carbon starving mycelia. Deletion of the *chi*B and *eng*A genes (encoding autolytic chitinase and ß-1,3-glucanase, respectively [2]) decreased the melanin formation in carbon starving cultures. Synthetic melanin efficiently inhibited the activity of purified *Chi*B chitinase in vitro, most likely by binding to its substrate [3]. Addition of synthetic melanin to growing cultures efficiently inhibited the pellet disorganization and hyphal fragmentation caused by *Trichoderma* lysing enzymes. Pyroquilon (an inhibitor of DHN type melanin synthesis [4]) surprisingly enhanced markedly the melanization of hyphae in carbon starving cultures and efficiently inhibited pellet disorganization and hyphal fragmentation without affecting the extracellular chitinase or ß-1,3-glucanase activities. According to these results, we assume that *Aspergillus nidulans* can protect its cells against cell wall degrading enzymes by melanin production. It can be particularly important during carbon starvation when cultures secrete chitinases and ß-1,3-glucanases [2].
Since hyphae originated from growing cultures were more sensitive to cell wall degrading enzymes than those originated from carbon starving cultures - independently of the produced melanin – melanin formation can be an important but not the only mechanism to protect cells from cell wall degrading enzymes during carbon starvation.

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LOW DIVERSITY OF AEROBICALLY CULTIVABLE BACTERIA WAS OBSERVED IN A HYPOXIC, 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 by monitoring bacterial community dynamics and meta-cleavage gene expression in a hypoxic, BTEX-contaminated groundwater. It was demonstrated that the bacterial community was dominated by Betaproteobacteria over the monitoring period, while

mRNA transcripts of a wide variety of subfamily I.2.C C23O genes could be detected constantly. By combining the results of molecular fringerprintig techniques, significant correlations were found between the community compositions and the expression of some subfamily I.2.C C23O genes. Consequently, a still uncultured member of the genus Rhodoferax and an unclassified Rhodocyclaceae bacterium were identified as possible microaerobic BTEX degraders. To verify these findings groundwater microcosm experiment and culturing approaches were performed. According to other studies enrichment and isolation of Rhodoferax and Rhodocyclaceae related strains is relatively easy using a mineral salt medium supplemented with trace elements, vitamins and acetate as sole source of carbon. These bacteria are facultative anaerobes, thus capable of using alternative electron acceptors such as Fe(III), nitrite or nitrate respectively. Therefore, groundwater microcosm was prepared by using serum bottles sealed hermetically and incubated for three weeks to ensure anaerobic circumstances in order to decrease the number of strict aerobes. After incubation acetate enrichment medium was inoculated with the groundwater microcosm sample. Agar-solidified acetate enrichment medium and R2A agar were used to isolate aerobically cultivable bacterial strains both from the microcosm and the enrichment culture. Characterizations of the bacterial communities were performed by sequence-aided terminal-restriction fragment length polymorphism (T-RFLP). Bacterial community structure analyzes showed the predominance of Betaproteobacteria both in the initial and groundwater microcosm samples. However, the three weeks of anaerobic incubation caused the rise of *Rhodoferax*-related bacteria. Significant changes in the community composition were observed after the enrichment procedure. The diversity of the microbial community was considerably decreased, since merely the presence of genera *Pseudomonas*, *Aeromonas* and *Bacteroides* was detectable. Unfortunately our attempt to cultivate *Rhodoferax* and Rhodocyclaceae related strains was failed since only members of the genera *Pseudomonas* and *Aeromonas* were cultivable under the conditions used. Thus, it can be assumed, that the diversity of easily cultivable bacteria in the investigated, BTEX-contaminated and Betaproteobacteria dominated hypoxic groundwater was considerably low. Moreover, the acetate medium used is not applicable to enrich and isolate the yet uncultured *Rhodoferax* and Rhodocyclaceae related strains in question.

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WHOLE GENOME SEQUENCING OF A PICORNAVIRUS ISOLATED FROM A HERMANN'S TORTOISE

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Picornaviruses (PVs) of tortoises have been diagnosed several times by virus isolation in Terrapene heart cell culture (TH-1; ATCC CCL-50) based on their typical lytic cytopathogenic effect and have been called Virus "X". They have usually been detected from spur-thighed tortoises (*Testudo graeca*) - one of the most popular species among exotic animal keepers - but were also found in other terrestrial species including marginated tortoises (*T. marginata*), Hermann's tortoises (*T. hermanni*), leopard tortoises (*Geochelone pardalis*) and Egyptian tortoises (*T. kleinmanni*). The whole genome sequence with the exception of the 5' UTR of a picornavirus strain TGT1A/96 (Tortoise PV 1) isolated from a spur-thighed tortoise has already been determined and characterized. Unfortunately the sequence of this virus has not been deposited to GenBank. In order

to obtain more information about tortoise PVs we decided to sequence the whole genome of a strain isolated from a Hermann's tortoise. The virus has been propagated on TH-1 cells and caused a lytic infection. After freezing and thawing the infected cell culture viral RNA was purified from the supernatant using TRIzol Reagent (Sigma-Aldrich) and the sample was prepared for next generation sequencing. Sequencing was carried out on a 316 chip using Ion Torrent semiconductor sequencing equipment (Ion Personal Genome Machine, Life Technologies). The sequence of the genome was also confirmed by Sanger sequencing method. For amplification of the 5' and 3' ends the 5'/3' RACE Kit (Roche) was applied. Using the two sequencing methods we could determine a 7078 nt long part of the genome. Unfortunately amplification of the 5' end containing partial UTR sequences remained unsuccessful. Similarly to Tortoise PV 1 a 2218 aa (6654 nt) long polyprotein with a typical picornavirus L-4-3-4 layout could be predicted. Potential protease cleavage sites have been identified and the genome organization was identical to that of Tortoise PV 1: VPg+5'UTR[L/1A-1B-1C-1D-2A/2B-2C/3A-3B-3C-3D]3'UTR-poly(A). Phylogenetic analysis of the partial polyprotein aa sequences of PVs indicated that the PV isolated from the Hermann's tortoise appeared on a clearly separate branch and was most closely related to the Mouse Mosavirus. Our results confirm that tortoise PVs belong to a proposed separate genus in the family Picornaviridae. Our plan is to sequence the whole genome of additional tortoise PV strains in order to determine the genetic diversity of these viruses and develop a new diagnostic system.

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AN OVERVIEW OF ACTIVITIES DONE BY NATIONAL FOOD CHAIN SAFETY OFFICE IN ANIBIOTHREAT BIOSECURITY PROJECT

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Bioterrorism is a real danger of our world. Deliberate spread of the so-called transboundary animal diseases (TADs) may cause untold economical losses and are also suitable to to intimidate or coerce a government or civilian population to further political or social objectives. AniBioThreat project launched in 2010 is about to improve the capacity of the European Union (EU) to counter biological animal bioterrorism threats in terms of awareness, prevention and contingency. The project has special focus on threats to living animals, feed and food of animal origin. Within this project the possibilities and ways of protection were scrutinised. To complete this objective our Office determined in cooperation with Federal Institute for Risk Assessment (Germany) and The National Veterinary Institute (Sweden) the available ways of protection against veterinary pathogens listed by the project's "Threat recognition" work package and to assess the legal background of vaccine usage together with the expected protective efficacy of available of vaccines. The methodology is based on the following steps: i.) surveying and assessment of availability, strategic stocks, time-lines to produce large-scale amount of batches of a given vaccine; ii.) data collection on the safety, efficacy and potency of the available vaccines against biological agents of agro-/bioterrorism potential; iii.) comparison of efficacy of vaccines; iv.) analysing the "weak points", when vaccine does not exist against a given biological agent of agro-/bioterrorism potential and/or the presently available vaccines have shortage in safety or efficacy.

NATIONWIDE OUTBREAK OF *SALMONELLA STANLEY* IN HUNGARY, 2012 – 2013: ROLE OF ENVIRONMENTAL INVESTIGATIONS

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In April 2012 the national surveillance system detected an increase of *Salmonella Stanley* infections from all counties of Hungary. We investigated to identify the source and prevent new cases, in the context of cases reported from other European countries. We defined a cases as a person living in Hungary and not travelling abroad two weeks before onset of illness (1 August 2011 to 30 May 2013) with at least one symptom of salmonellosis (diarrhoea, fever, abdominal pain, vomiting) and laboratory confirmation. Field epidemiologists interviewed patients by phone using structured questionnaires. As comparison of *S. Stanley* isolates (Xbal-PFGE profile) from cases, food and environmental specimens from different poultry farms pointed to turkey as the potential source of the outbreak, we compared cases (N=59) with controls (patients with enteric diseases of other etiologies matched by age and sex, N=96) in terms of consumption of various food items using odds ratios (OR) and 95% confidence intervals (CIs). 267 sporadic cases were reported (median age: 7, 52% of males), with only one cluster (two cases in a camp). The average of the days between onset and interview was 12 days. Cases and controls did not differ in terms of consumption of turkey and chicken products (OR: 1,9 95% CI: 0,65 – 5,66 and 1,5, 95% CI: 0,66 – 3,24). While environmental investigations suggested that the turkey production chain was the source of the outbreak, the analytical epidemiological studies failed to confirm our hypothesis. Early investigation of cases during outbreak is essential to prevent recall bias and identify sources of infection.

MOLECULAR SCREENING OF CIRCOVIRUSES IN FISH AND REPTILES

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Circoviruses, members of the Circoviridae family are viruses with single-stranded circular DNA genome of ~1700-1900 bases and ambisense organization. Circoviruses may cause severe infection in pigs and birds (parrot, pigeon, duck, goose, etc.) with immune suppression and death of the host. Circoviruses have also been described in primates, farm animals, dogs and dragonflies. Circoviruses replicate via rolling circle amplification and their Rep protein plays an important role in the initiation and its process. Similarly to retroviruses, circoviral rep-like endogenous elements were observed in different organisms, for example in protozoa, worms, amphibians and mammals. These sequences may be present as several identical and/or similar copies in the host genome and are generally interrupted (open reading frames with premature stop codons, frameshift, insertions, deletions) suggesting loss of their function. Novel groups of circoviruses have been detected in barbel fry (*Barbus barbus*) and European catfish (*Silurus glanis*) in Hungary in 2011-2012. In our recent studies we amplified circoviral rep-like sequences using degenerate primers in the Indian fish *Catla catla* and *Labeo rohita*, and in different reptiles including chelonian species (Testudines and

Trachemys species), Schneider's skink (*Eumeces schneideri*) and ball python (*Python regius*). The closest relatives of the rep-like sequences of *C. catla* and *L. rohita* were the two barbel fish circoviruses. The sequence of *L. rohita* encoded terminating and frameshift mutations and seemed to be integrated into a microsatellite region of the host genome. Any attempt to gain more information about *C. catla* sequences have been failed therefore the integration or the presence of whole viruses could not be determined so far. Rep-like sequences of reptiles are highly similar (97-99% amino acid and nucleic acid similarity) to a partial circovirus rep-like sequence described in a chimpanzee stool sample and are only distantly related (46-48% amino acid similarity) to other *rep* genes. These sequences do not contain any mutations that would suggest any functional modifications; however, our specifically designed back-to-back primers have not amplified any viral sequence. Treatment of the isolated DNA with Lambda exonuclease followed by PCR with the degenerated primes has not given any amplicons. These data together indicate that rep-like sequences may be present in the genome as integrated elements. Further studies are needed to elucidate whether these reptile sequences are parts of intact viruses or are integrated viral elements.

MICROBIOLOGICAL INVESTIGATION OF ORAL MALIGNANCIES BY THE MALDI-TOF MS METHOD

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Tumorigenic transformations can be linked directly to microbes including certain viruses and bacteria and indirectly to the inflammatory changes that they cause. It was earlier demonstrated that the superficial microbiota of oral tumors and the healthy unaffected sides can differ regarding mainly the anaerobic flora. Our aim was to investigate the microbiological changes during malignant transformations in the oral cavity using conventional culturing techniques and MALDI-TOF MS identification. In one case we also used comparative genomics (16S RNA gene parallel sequencing) to investigate the differences between the malignant and healthy mucosa. Biofilm samples were obtained from the central surface of the normal mucosa, malignant or premalignant lesions in 8 patients aged 59,7 (± 10,2) years, and from contiguous healthy mucosa, before any antibiotic therapy or any tumor treatment. Samples were transported in anaerobic gel transport media, suspended in brain heart infusion (BHI) broth and cultured within 1 day of removal, using general and selective, aerobic and anaerobic media. After the 2-5 day long incubation total aerobic and anaerobic colony counts (CFUs) were determined, categorized by colony morphology, and the different isolates were identified by MALDI-TOF MS. Statistical evaluations were made by the R software (Wilcoxon rank tests and Fisher's exact test). The median number of anaerobic colony forming units (CFUs/ml) at the premalignant and tumor sites (2,0×105) was significantly higher than for the healthy (control) mucosa (3,6×104; p= 0,03), and the median number of aerobic CFU/ml at the healthy sites (6,2×105) was higher then for the premalignant or malignant mucosa (2,7×105), but this result was not significant (p=0.81). We set the healthy mucosa's anaerobic flora (3,6×104) against the aerobic flora (6,2×105), where the difference was significant (p=0,009) and we also compared premalignant and malignant mucosa's anaerobic (2,0×105) and aerobic (2,7×105) microflora, but the result was not significant (p=0,678) again. The species isolated in increased numbers at and associated to the premalignant and malignant sites were *Prevotella*, *Veilonella*, *Clostridium* (anaerobes), *Campylobacter* (microaerophil), and *Corynebacterium*, *Gemella* (aerobes). The result of the comparative metagenomics indicated that at the premalignant and malignant sites *Porphyromonas*,

Eubacterium, *Haemophilus*, *Prevotella*, Clostridiales, *Bergeyella* spp. and at the healthy mucosa *Streptococcus* spp. can be found at increased relative copy numbers. We can say that the anaerobic flora is significantly more abundant at the premalignant and malignant sides compared to the uninvolved mucosa as it was evidenced by the culturing and also by the metagenomic approaches.

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SEQUENCE VARIATION OF HUMAN PAPILLOMAVIRUS (HPV) TYPE 31 E6 AND E7 ONCOPROTEINS: PHYLOGENETIC AND FUNCTIONAL IMPLICATIONS

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Papillomaviruses (PVs) are small, double-stranded DNA viruses that infect epithelial tissues. High-risk human papillomaviruses are the causative agents of cervical and other anogenital cancers. The E6 and E7 oncoproteins contribute to oncogenesis by associating with the tumor suppressor protein p53 and the cell cycle regulatory protein Rb, respectively. The aims of this study were to determine the sequence and amino acid variations of E6 and E7 oncogenes of HPV type 31, and to investigate the functional differences between the E6 and E7 variants. We examined clinical samples from women who had colposcopical atypia in the cervix. First, the E6 and E7 genes were amplified with PCR from HPV 31 positive clinical samples. In order to evaluate nucleotide and amino acid sequence variation, the HPV 31 E6 and E7 isolates were sequenced and a phylogenetical tree was constructed. We are also studying the effects of E6 variants on the level of p53 protein and the effects of E7 variants on the activity of E2F transcription factors. To this end, the different E6 and E7 variants are cloned into expression vectors, and transiently co-transfected into MCF-7 cells, along with reporter vectors containing p53 binding sites or E2F binding sites. The level of expression of the different E6 and E7 variants are examined in stably transfected MCF-7 cells by Western-blotting.

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SPLICEOSOME TWIN INTRONS REVEALED BY FUNGAL NUCLEAR GENOMES

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The spliceosome is an RNA/protein complex, responsible for intron excision of eukaryotic gene transcripts. In mitochondria and plastids intron excision does not involve the spliceosome. For classes II and III chloroplast introns “introns within introns” (twintrons) have been described. The excision of the external intron, and thus RNA maturation, necessitates the removal of the internal intron. Analogous structures have not been described for spliceosomal introns. We have predicted four putative instances of “introns within introns” in nuclear genes of ascomycetes. We call these “stwintrons” for “spliceosomal twin introns”. Putative stwintrons show a variable phylogenetic

distribution. The presence of the internal intron predicts specific splicing intermediates. We have isolated and sequenced the predicted intermediate for the splicing of an RNA encoding a putative cyclic imidine hydrolase of *Fusarium verticillioides* (Sordariomycetes, Hypocreales), where the internal intron interrupts the donor sequence between the first and second nucleotide and predicted an analogous structure for a gene encoding a sugar transporter in two Magnaportacea. In the bioDA gene (encoding an enzyme catalysing two steps of biotin biosynthesis of the Sordariomycetes), an internal intron is predicted to interrupt a donor sequence between the second and third nucleotide. This structure has been confirmed in *Trichoderma reesei* by the isolation and sequencing of the splicing intermediate. In the fourth instance the putative internal intron disrupts the donor sequence between the fourth and fifth nucleotide of the donor sequence. In this instance, the presence of the internal intron was disproved, revealing an unsuspected case of alternative splicing. The phylogenetic distributions of the stwintrons we have identified suggest that they derive from "late" events, subsequent to the appearance of the host intron.
They may well not be limited to fungal nuclear transcripts, and their existence and disappearance are relevant to hypotheses of intron origin and alternative splicing.

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LINEAR RELATIONSHIP BETWEEN GLYCOPEPTIDE USE AND THE INCIDENCE OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS*

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Colonization and infection with vancomycin-resistant enterococci (VRE) are emerging worldwide and excessive usage of glycopeptides may enhance the incidence of VRE. The aim of the study was to investigate whether the increasing incidence of VRE isolation in our patients of the University Hospital of Vienna, Austria can be correlated to the usage of glycopeptides. Within the period of January 2011 to December 2012 all patients with isolation of invasive or non-invasive VRE were included. Hospital-wide and department-specific data concerning the consumption of glycopeptides in defined daily doses (DDDs) from January 2010 to June 2012 were extracted from the hospital pharmacy computer system. A correlation between glycopeptide usage and VRE incidence was assessed using Pearson correlation coefficient. A total of 276 non-consecutive VRE including 266 *Enterococcus faecium*, 9 *Enterococcus faecalis* and 1 *Enterococcus raffinosus* were isolated from 250 patients. 75.6% of VRE-patients were admitted to inpatient wards, 19.6% to ICU units and 4.8% were outpatients. Departments most frequently involved were general surgical units (46 patients), followed by general medical units (33 patients), the bone marrow transplant unit (37 patients), the heart surgery (28 patients) and the nephrology (26 patients). Most VRE-isolates were non-invasive isolates from faeces or rectal/anal swabs (76.1%), skin swabs (36.6%), oropharyngeal swabs (23.6%) and urine (23.4%). Invasive VRE isolates were found in blood in 6.5%, in pus in 1.1%, in ascites or pleural effusion in 1.1%, respectively. Hospital-wide DDDs of the used glycopeptides vancomycin and teicoplanin were total 8770.25 in the second half year of 2010, total 18022.5 in 2011 and total 8126 in the first half year in 2012. Departments-associated VRE isolation did not correlate with vancomycin usage alone (Pearson correlation coefficient +0.45, $p>0.05$), but with the total glycopeptide usage (Pearson correlation coefficient +0.732, $p=0.001$). We

demonstrated a high positive linear correlation between the departments-associated isolation of VRE and total glycopeptide usage in the University Hospital of Vienna.

BIODIVERSITY OF ENDOPHYTIC BACTERIA ISOLATED FROM DIFFERENT *CAPSICUM ANNUUM* VAR. *GROSSUM* CULTIVARS AND THEIR EFFECT ON SEED GERMINATION

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Endophytic bacteria can positively interact with plants by producing biofilms or antibiotics that might protect the host plant against pathogenic organisms. However, interaction between endophytic and human pathogenic bacteria may contribute to the pathogen's penetration into the plant tissues. If endophytic bacteria prevent the penetration of plant pathogens, they have a protective role, but in the second case they can be responsible for food safety problems. Our goal was the selection of endophytic bacteria among the primary potential endophytic bacterial isolates originated from different organs of *C. annuum* var. *grossum* cultivars grown in soil- and hydrocultures. Isolation, phenotypic characterisation and identification of the bacteria were performed by using traditional and molecular techniques. In our recent study we aimed to further analyse the diversity and the endophytic nature of the identified bacterial strains. Altogether 174 bacterial pure cultures were isolated from seeds, vegetative tissues and fruits of *C. annuum* var. *grossum* (cultivars Ho and KPA) and were subjected to phenotypic analyses, biochemical and physiological tests and molecular analyses. Based on the results of molecular identification the isolates belonged to *Acidovorax*, *Agrobacterium*, *Brevibacillus*, *Cupriavidus*, *Delftia*, *Enterobacter*, *Leclercia*, *Nanobacterium*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Rothia*, *Serratia*, *Stenotrophomonas* and *Xanthomonas* genera. Majority of the Gram negative isolates were identified as *Pseudomonas* spp. and beside these isolates bacteria belonging to the *Paenibacillus* and *Stenotrophomonas* genera could also be detected in significant numbers. It is worthwhile to mention that molecular identification based on the 16S rDNA and rpoB sequences resulted in more than one species or even genera for one isolate in many cases. This phenomenon may refer to the fact that these isolates are not yet properly identified, and investigation of further sequences has to be involved. Majority of the isolates derived from the root tissues and just a smaller proportion originated from the above-ground plant tissues (leaves, stems and fruits). From the root tissues of hydroculture-grown plants we were able to isolate mostly Gram-negative bacteria belonging to the genera of *Pseudomonas* and *Stenotrophomonas*, while in case of soil-grown plants members of the Enterobacteriaceae family could also be isolated. Endophytes of the above-ground tissues especially that of the soil-grown plants were mainly Gram-positive bacteria. It is a general rule that the real endophytes must not be inhibitory for the host plant; therefore we tested the effect of the potential endophytic bacteria on the germination of pepper seeds. as It has been revealed that certain species of the *Enterobacter*, *Delftia* and *Leclercia* genera had strong inhibitory effect on seed germination, while some *Pseudomonas* and *Paenibacillus* species had outstanding stimulation. It can also be concluded that the stimulatory or inhibitory activity of the potential endophytic bacteria was strain-dependent in some cases because the isolates belonging to the same species were variable from this respect.

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NFAP: A NOVEL CYSTEINE-RICH ANTIFUNGAL PROTEIN FROM *NEOSARTORYA FISCHERI*

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Due to the sharply increasing number of antibiotic or pesticide resistant filamentous fungal strains causing mycoses or plant infections there is a substantial demand for new safely applicable antifungal compounds. Cysteine-rich antifungal proteins secreted by filamentous Ascomycetes are 51-58 amino acid long antifungal proteins, with a particular tertiary structure that renders these proteins highly stable. These proteins possess distinct antifungal spectra and are toxic to a wide range of filamentous fungi. Therefore, they have great potential for medical and agricultural applications where antibiotic resistance of (opportunistic) fungal pathogens poses significant problems. The antifungal protein NFAP from the *Neosartorya fischeri* (anamorph: *Aspergillus fischerianus*) NRRL 181 isolate is a novel representative of this protein group which - based on our preliminary studies - differs in its antifungal mode of action and its tertiary structure from the up to now most investigated NFAP related proteins, *Aspergillus giganteus* antifungal protein and *Penicillium chrysogenum* antifungal protein. In our work we demonstrated that NFAP effectively inhibits the growth of numerous filamentous Ascomycetes including potential human and plant pathogens, and that its antifungal effect is dose-dependent and is strongly influenced by the extracellular mono- and divalent cation concentration. In the susceptible fungi NFAP causes damage to the cell wall by destructing chitin filaments and triggers apoptotic-necrotic pathways by intracellular accumulation of reactive oxygen species. In vitro antagonism experiments with *N. fischrei* Δ*nfa*p strain indicate that NFAP play a role in the emulation for nutrients and habitat against microorganisms with similar ecological niche. After further studies NFAP could be a potential base of new synthetic, self-structure forming, effective, target specific and safely applicable antimicrobial peptides in therapy, pest control and food preservation.

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COMPARISON OF DIFFERENT MECHANICAL LYSIS METHODS FOR THE ISOLATION OF SOIL COMMUNITY DNA

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The cell lysis is a key step of total soil DNA extraction procedure. Different mechanical pretreatments (sonication, bead beating ect.) can largely improve the efficiency of in situ cell lysis and purity of extracted DNA. Four different bead-beating based mechanical cell lysis methods were compared during extraction and purification of DNA from a single soil: Mini-Beadbeater (MB), Fast- Prep-24 (FP), Vortex (ZX3) (V); Pulsing Vortex Mixer (VWR) (PV). A commercial purification kit (ZR Soil Microbe DNA MiniPrep, Zymo Research) was used for DNA extraction.

Soil samples were taken from experimental filed of ISAC CAR HAS (Martonvásár) and 0.25 g of soil were used for the in situ DNA extraction. Bead-beating technics were tested with shaking times of: 1, 3, 5 and 10 minutes. The purity and yield of DNA was measured spectrophotometrically. Efficiency of NanoDrop1000 and Tray Cell (Hellma) spectrophotometric measurement technics were compared. Yield of DNA was calculated from the A260, purity by calculation A260/A230 and A260/A280 ratios for humic acid and protein contamination respectively. Temperature gradient gel electrophoresis (TGGE) was used to demonstrate the effect of different shaking technics on the microbial diversity detected within the community DNA recovered. The maximum DNA extraction yield for MB were 38 ng/µl, for FP 35 ng/µl, for V 30 ng/µl, and for PV 24 ng µl, with optimal shaking times 3, 1, 10 and 1 minutes respectively. Protein contaminations with the ratios of 1.2 for MB and FP; 1.1 for V and 0.9 for PV were lower compared to the optimal value (>1,7). Humic acid contaminations also falls short of optimal (>2) with ratios 0.6 for BB; 05 for FP; 04 for V; 0.4 for PV. BB and FP methods gave significantly higher DNA concentration and significanly lower protein contamination compard to the PV one. As for as humic acid contamination are concerned there were no significant differences between efficiences of tested bead beating methods. NanoDrop1000 and Tray Cell (Hellma) spectrophotometric measurement technics were strogly correlated. Despite the high protein and humic acid contamination of the extracted DNA samples the PCR were not inhibited. Bacterial community fingerprints differences were determined based on 16S rRNA fragments amplified from total community DNA using universal bacterial and group-specific primers. Conclusion. Bead beating mechanical pretreatments can increase yield and protein purity of extracted soil DNA but have no effect on humic acid contamination.

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DETECTION OF CYTOKININE AND GIBBERELLIN-LIKE PLANT HORMONES IN *SCENEDESMUS OBTUSIUSCULUS* AND *CHLORELLA MINUTISSIMA* CULTURES

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Foliar fertilization is a widespread procedure in agriculture for applying inorganic fertilizers or other substances on the foliar surface of plants in order to enhance crop yield. The efficiency of foliar fertilization can be 8-20% better than solid fertilizers. Suspension of microalgae cultures has been used for long as foliar fertilizer, and according to recent research, it utilizes the hormone-producing capacity of algae. 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. In our work, the cytokinine and gibberellin-like plant hormon production of axenic cultures of *Scenedesmus obtusiusculus* BEA (Banco Espanol de Algas) D01_12, and *Chlorella minutissima* K 2012(Albitech collection) green alga strains were investigated. Both of these types of plant hormones significantly affect cell division. The gibberellins - as diterpenoids - stimulate cell division and cell growth so they promote the developing of leaves and the progress of blossoming. Additionally, these hormones induce the de novo syntesis of hydrolitic enzymes during the germination of seeds. Cytokinines delay senescence of leaves and they have morphogenetical effects as well. Detection of the mentioned plant hormones was conducted via thin layer chromatography following organic solvent extraction and evaporation. Kinetin was present in evapored extracts of *C. minutissima*, and *S. obtusiusculus* cultures as well. These evapored extract samples were observed also by HPLC-PDA. The amount of kinetin was approximately 0,8 - 1,0 µg/ml (400 ng/ml in the

algae liquid culture) and other cytokinin compontents were detected next to the kinetin in case of *S. obtusiusculus*. In the extracts of *C. minutissima* were approximately 0,1 – 0,2 µg kinetin/ml (40-80 ng/ml in the algae liquid culture). The citokinin-like activity of liquid cultures was also measured by cucumber cotyledon growing test. Samples - obtained by evaporation of *S. obtusiusculus*, a *C. minutissima* culture on low temperature - showed effects on the weight of cucumber cotyledons after 3 days of incubation time. The gibberellin-like activity was proved also by measuring amylase production of barley endosperm. Presence of gibberellin-like substances was detected in *C. minutissima* cultures. These results can serve as a good basis for the development of algae-based foliar fertilizer products and for a better understanding of their mode of action.

THE ROLE OF FUNGAL PROSTAGLANDIN-LIKE MOLECULES IN VIRULENCE AND THEIR BYOSINTHETIC PATHWAY IN *CANDIDA PARAPSILOSIS*

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Despite the increasing clinical importance, little is known about the virulence factors of *C. parapsilosis*. Here we investigated the biosynthetic pathway of fungal prostaglandin-like molecules that can be considered as putative virulence factor of *C. parapsilosis*. Mammalians produce immune response regulator prostaglandins from arachidonic acid (AA) by the contribution of COX1 and COX2 cyclooxigenases. Although fungi do not possess cyclooxigenase homologues, several pathogenic species are able to produce prostaglandins from host originated AA. In case of *C. albicans* the fatty acid desaturase homolog ole2 and the multicopper oxidase homolog fet3 enzymes were identified as potential key factors of the prostaglandin biosynthesis. Due to its ability to block Th1-type, and promote Th2-type immune response, fungal prostaglandin-like compounds can propel the host's immune response towards helping the fungi to colonize and to carry out chronic inflammation. Previously, we generated homozygous OLE2 deletion mutant in *C. parapsilosis*, and analyzed the prostaglandin profiles of the deletion strains. According to our results we found no difference between wild type and CpOLE2 deletion mutant regarding the PGE2 production in the presence of AA. This indicates that the CpOLE2 gene does not participate in the *C. parapsilosis* prostagalndin biosynthesis. To identify further genes that are implicated in the biosynthetic pathway of the *C. parapsilosis* derived prostaglandin-like molecules, we carried out a microarray analysis in the presence and absence of AA. Microarray analysis revealed 6 genes, that were differentially expressed after AA induction. We identified UGA3 a putative transcription factor with zinc cluster DNA-binding motif, *OLE*1 the fatty acid desaturase gene 1, *SOU*2 a carbonyl reductase and three further genes with unknown function. These results strongly suggest a different mechanism in *C. parapsilosis* for prostaglandin production from that of C. albicans. In order to reveal this novel mechanism we developed KO mutants for all 6 newly identified genes, by using fusion PCR technique optimized for *C. parapsilosis*. Deletion strains were monitored for prostaglandin production by our newly developed HPLC method, that provided a complete lack of prostaglandin production ability of CpUGA3 mutant. This result suggests a strong involvement of Uga3 transcription factor in the biosynthetic pathway of fungal prostaglandin-like compounds.

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ANTIDERMATOPHYTIC ACTIVITY OF A *BACILLUS MOJAVENSIS* STRAIN ISOLATED FROM MUMIJO A TRADITIONAL MONGOLIAN MEDICINE

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The worldwide distributed dermatophytes are a group of morphologically and physiologically related molds which affect the keratinous tissue of humans and other vertebrates. The incidence of dermatophytoses has been increasing continuously during recent years as a consequence of the rise in the number of immunocompromised patients. Therefore there is a substantial demand for new safely applicable antifungal compounds without side-effects. Mumijo is a traditional medicine used in Central Asia thousands of years. This material is the fossil deposits of snow petrel stomach and is located in the high mountainous regions of Central Asia. The beneficial effects of mumijo are exerted on metabolic processes and on the human immune system and it also has antiseptic activity. Previously we observed that a Mongolian mumijo preparation can effectively inhibit the growth of different dermatophytes. In this work we isolated a *Bacillus mojavensis* strain from this preparation, and investigated its antidermatophytic effect against five dermatophyte isolates (*Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum* and *T. tonsurans*) on agar plate test. The antifungal activity of *B. mojavensis* ferment broth from LB medium was also investigated in agar diffusion test. The isolated *B. mojavensis* strain inhibited the growth of *M. canis*, *M. gypseum*, *T. rubrum* and *T. tonsurans*; and its ferment broth also exerted an antidermatophytic activity on these isolates. In our test *T. mentagrophytes* proved to be resistant in these susceptibility tests. After identification and further investigation of the antidermatophytic compounds of the *B. mojavensis* ferment broth, this could be a promising agent in the treatment of dermatophytosis.

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INVESTIGATION OF ATRAZINE DEGRADATION BY BACTERIAL STRAINS AND CONSORTIA

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a member of s-triazine group of herbicides. Atrazine is used mainly for pre and post emergence control of broadleaf and grassy weeds in maize, sorghum and corn as a photosystem II inhibitor. Atrazine is one of the most widely used herbicide in the world while it has been banned in the European Union since 2004. It is the most commonly detected pesticide contaminant of ground and surface water due to a long half-life, heavy usage and high persistence under reductive conditions. Several studies have shown that atrazine is a potent endocrine disruptor in fish, amphibians, reptiles and human cell lines. The herbicide seems to be most potent in amphibians, where it is active at levels as low as 0.1 ppb. Atrazine has a low affinity for androgen and estrogen receptor but it reduced androgen synthesis and

increases estrogen production via several mechanisms. The herbicide increases aromatase levels by inhibiting phosphodiesterase, resulting in elevated cAMP. This results in increased transcription of the aromatase gene CYP19a1, increased aromatase activity and ultimately increases estrogen production. Furthermore, other study has reported that atrazine is a ligand for steroidogenic factor 1 (SF-1) which binds to aromatase promoter II. The two most common metabolites, desethyl-atrazine and deisopropyl-atrazin also are able to increase aromatase activity in some human cell lines. In our study we investigated the atrazine (50 ppm) degradation ability of forty-four non-pathogenic bacterial strains and seventeen consortia were able to degrade aliphatic and aromatic hydrocarbons and several mycotoxins in our previous studies. Sixteen strains and fourteen consortia had >50% atrazine degrading capacity. Three strains that representatives of two *Rhodococcus* species and two consortia were able to degrade more than 90% of the atrazine.
We developed a testing method using a human carcinoma cell line combined with a fast and simple luminescence based yeast estrogen screen (BLYES) to investigation the aromatase induction effect of end-products and metabolites from atrazine biodegradation.

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COMPLETE DEGRADATION OF ACETANILIDE BY A CONSORTIUM OF MICROBES ISOLATED FROM RIVER MAROS

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A range of pesticides used extensively in agriculture are the derivatives of acetanilide and aniline, which thus enter surface waters in large amounts. Both compounds can impair the environment severely and furthermore may cause serious diseases in humans as well. Certain microbes that occur in soils and waters are able to decompose these substances, however, their degradation products often possess higher toxic effects than the original compounds, and therefore their complete degradation is of great importance. *Pseudomonas mendocina* and *Rhodococcus erythropolis* strains with the capability of decomposing acetanilide were isolated from water samples of the River Maros, however, they failed to degrade aniline (the degradation product of acetanilide) to any further extent, which thus accumulated in the culture medium. A fungal isolate with the same origin, identified by *Aspergillus ustus*, was found to be able to break down aniline with high efficacy. When the acetanilide- and aniline-degrading microbes were co-cultured in a medium containing 50 μg/ml acetanilide as a sole carbon and nitrogen source, HPLC analysis revealed the complete degradation acetanilide, while the detected concentration of aniline was 24 μg/ml after 7 days of incubation. Extended cultivation might result in the entire decomposition of aniline as well.

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ESTABLISHMENT OF A POLLUTANT-DEGRADING MICROORGANISM COLLECTION (PDMC) AT THE DEPARTMENT

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Large quantities of xenobiotics with human origin enter surface waters, and several of them represent high risk to human health in addition to being toxic to the environment. Therefore, the removal of these pollutants from waters is extremely important: bioaugmentation is suggested as a possibile approach to solve this problem. Numerous soil- and water-inhabiting microbes are known to be able to degrade various xenobiotics, thus the purpose of this project was the establishment of a collection of microbes with the ability to degrade certain chemical pollutants (Pollutant-Degrading Microorganism Collection, PDMC). Water samples were taken from 10 sites along the Romanian and Hungarian parts of River Maros in every 3 months over a 1-year period. The samples were plated on solid minimal medium supplemented with 30 different xenobiotics separately as sole carbon and in the case of nitrogen-containing compounds also nitrogen sources. The appearing colonies, considered as pollutant-degraders, were deposited in the PDMC. At present the collection consists of 140 bacterial and fungal strains, being able to decompose acetanilide, aniline, 2,6-dimethylaniline, 4-isopropylaniline, chlorpropham, diuron, phenoxyacetic acid, 2,4-dichloro-phenoxyacetic acid, phenol, m-cresol, p-cresol, resorcinol, Na-benzoate, 4-hydroxybenzoate, 3,4-dihydroxybenzoate and methylparaben, selectively. The isolates with the best acetanilide-degrading potential were identified through the sequence analysis of certain regions of the ribosomal DNA as *Pseudomonas mendocina* and *Rhodococcus erythropolis*, while the fungal strain showing the highest ability to decompose aniline was confirmed as *Aspergillus ustus*. The isolates in PDMC might be applied in approaches for the bioaugmentation of pollutants. The results of their identification at the species level the will be presented and discussed.

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DEGRADATION OF ANILINE-DERIVATIVES BY MICROBES ISOLATED FROM RIVER MAROS

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Chemicals used widely in agricultural pest management enter surface waters at enormous amounts in every growing season. Several of these xenobiotics and their degradation products, which might have even higher toxicity on the environment as well as humans than the original substances, are aniline compounds and their removal from surface waters has significant importance. A total of 20 aniline-degrading bacteria and fungi were isolated from the water samples of River Maros on solid medium supplemented with 0.5 mg/ml aniline as a sole carbon and nitrogen source. The isolates were transferred to media containing 0.5 mg/ml 3-chloroaniline, 4-chloroaniline, 3,4-

dichloroaniline, 3-chloro-4-methylaniline, 2,5-dimethylaniline, 2,6-dimethylaniline, 4-isopopylaniline and diuron selectively as sole carbon and nitrogen sources. The growth of fungi was inhibited entirely by Cl-containing compounds, while the bacterial strains VCs9 and VCs10 were able to grow in the presence of all the substances tested. The degradation of the pollutants is being quantified by spectrophotometry and HPLC analysis. The microbes isolated in this study might be potential candidates for the bioaugmentation of aniline and its derivatives under field conditions.

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INVESTIGATION OF INTERACTION BETWEEN PROBIOTIC BACTERIA AND POTENTIAL PATHOGEN MICROORGANISMS

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Lactic acid bacteria (*Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc*) are used in the production of fermented foods. Some of these strains show strong antagonistic interaction against many different bacteria strains (closely related or non-cognate spoilage and pathogen organisms). Beside lactic acid bacteria several strains of bifidobacteria are able to inhibit the growth of other microorganisms. The inhibition in many cases caused by organic acids (mainly lactic acid and acetic acid), hydrogen-peroxide and other by-product of the metabolism. However in some cases proteinaceous components with bacteriostatic and bactericid properties can be detected which are responsible for the microbial inhibition. Antimicrobial effect of these proteins as preservatives is well demonstrated in food fermentation. The antimicrobial components such as bacteriocins produced by probiotic bacteria are valuable ingredients in the food industry. Our goal based on these arguments was a selection of *Lactobacillus* and *Bifidobacterium* strains in respect of antimicrobial effects. Furthermore, the elaboration of a methodology to demonstrate antimicrobial activity: optimization of agar spot, agar diffusion and microtiter methods; environmental parameters (media composition, fermentation time) and effect of induction. Main results: The most sensitive indicator strains were *L. monocytogenes* 4ab, *E. coli* O157:H7, *Ec. faecalis* NCAIM B01312, and *Eb. cloacae* NCAIM B02073 for the inhibition of both bifidobacteria and lactobacilli, when they were cultivated in MRS based medium. The best inhibition was obtained by using *Lc. lactis* spp. *lactis* B02121 against *L. monocytogenes* test strain, which was 3.66 (Inhibition zone/colony zone). In the case of *Bifidobacterium* strain the *B. longum* A4.8 strain was the best and 2.77 inhibition was shown against *E. coli* O157:H7. The most intensive inhibition was observed in the cases of *Lactobacillus* strains at the 18th – 20th hour of cultivation and in the cases of *Bifidobacterium* strains it was at the 24th hour. Antagonistic activity of the selected strains was caused primarily by acidic effect. Important antimicrobial activities could be demonstrated in the case of all tested strains in the presence of killed indicator strain. When killed *L. monocytogenes* 4ab was used as an inducer for production of antimicrobial agents the gained supernatant expressed the highest inhibitory effect against all applied indicator strains. Among the investigated bacteria *Lactobacillus* strains exhibited the most outstanding inhibitory effect, probably because they produced antimicrobial components in high concentration during their metabolism. These findings may report on gain proteinaceous antimicrobial compounds, which can be applied as food preservatives.

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SUGAR FACILITATED HYDROLYZATION OF UNTREATED CELLULOSIC WASTE

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Hydrogen is a promising energy carrier, since its utilization/combustion does not yield environmentally harmful products. It can be produced by numerous approaches including biological tools. Hydrogen can be evolved by phototrophic and heterotrophic cells. In the latter case, the cells utilizes various organic substrates, such as pretreated energy plants, agricultural wastes etc. The most abundant biomass sources are composed of cellulose which must be converted into simple sugars for these fermentative bacteria. Despite of the simple chemical composition of cellulose, it is a recalcitrant substrate but several organisms, principally fungi and aerobic/anaerobic bacteria can degrade it efficiently. The extreme thermophilic Gram+ bacterium *Caldicellulosiruptor saccharolyticus* can use a very broad spectrum of sugars, including mono-, oligo and polysaccharides. Under strict anaerobic conditions it ferments these sugars into hydrogen, acetic acid carbon dioxide and few other by-products. The ability of the cells to produce hydrogen from various cellulosic substrates was previously demonstrated. However, in most cases the pretreatment of biomass was required for efficient conversion. In this work, we studied the conversion of untreated paper and paper sludge into hydrogen by *C. saccharolitycus*. It was recognized that monomeric sugars can promote the cellulose hydrolysis as well as the hydrogen production. The substrate conversion, cell number, pH and H_2 were monitored during the fermentations. For better understanding the molecular background of the phenomenon we also made a whole transcriptome study to disclose the main players participating in the bioconversion processes.
The positive effect of monomeric sugars for the conversion of cellulosic substrate into hydrogen was demonstrated for paper industrial samples, as well.

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FUSARIUM NAPIFORME, A NEW EMERGING PATHOGEN FROM HUMAN KERATOMYCOSIS

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Fusarium species are common soil saprophytes and plant pathogens and also the most commonly isolated filamentous fungi from human keratomycosis in Southwest India. Keratomycosis is a serious corneal infection that can lead to blindness because of a misidentified causative agent and the subsequently applied inappropriate antifungal therapy. In 2010-2011 70 *Fusarium* isolates from

human fungal keratitis were isolated and morphologically identified at the Aravind Eye Hospital (Coimbatore, Tamilnadu, India). Three of them were confirmed as *F. napiforme* based on molecular methods. The partial sequences of the β-tubulin and translation elongation factor 1α genes were compared with similar sequences available in the National Center for Biotechnology Information and FUSARIUM-ID databases using the Basic Local Alignment Search Tool. These analysis revealed 98.5-99.3% similarity to the sequence of F. napiforme NRRL 13604 strain. To confirm the results a combined phylogenetic tree was also estimated based on the two sequences mentioned above, including 67 other clinical isolates from the same years and the *F. cerealis* NRRL 25805 strain as an outgroup. On the generated tree the *F. napiforme* strains formed a separate and well-supported group. The in vitro antifungal susceptibilities of the isolates to amphotericin B, clotrimazole, econazole, itraconazole, natamycin (NTM), terbinafine (TRB) and voriconazole were determined by the broth microdilution method. The isolates showed relatively high minimal inhibitory concentration (MIC) values to the examined agents. The lowest MICs were observed in the case of NTM and TRB. To determine the interaction between these two most effective antifungals the checkerboard microdilution method was used. Based on the calculated fractional inhibitory concentration index values the combined application had a better antifungal activity on the *F. napiforme* isolates than the compounds alone, NTM and TRB interacted with each other synergistically. In conclusion, we report the first isolation of *F. napiforme* from human keratomycosis. The MICs of the 3 isolates were determined to seven conventional antifungal agents. The combination of the two most effective drugs, NTM and TRB could be a promising base of an effective therapy for keratitis caused by *F. napiforme*.

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CELL LENGTH GROWTH IN FISSION YEAST IS BILINEAR AND TENDS TO HAVE A SMOOTH TRANSITION SEGMENT

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Fission yeast (*Schizosaccharomyces pombe*) is an extensively studied model organism since the 1950s. During their mitotic cycle, cylindrical fission yeast cells grow exclusively at their tips, therefore cell length is proportional to cell volume. Length growth starts at birth and halts at mitotic onset when the cells begin to prepare for division. The classical method to study the growth of individual cells is time-lapse microscopy; cells are growing on the surface of an agar pad in a thermostated photomicroscope, and later on the growth pattern of cell length can be simply studied by a projector. While the growth pattern was initially considered to be exponential, during the last three decades an increasing amount of evidence indicated that it is rather a bilinear function (two linear segments separated by a rate change point (RCP)). The main focus of this work was to clarify this and to elucidate the further question of whether the rate change occurs abruptly at the RCP or more smoothly during a transition period around it. We have analyzed the individual growth

patterns obtained by time-lapse microscopy of 60 wild type cells separately as well as that of the 'average' cell generated from their superposition. Linear, exponential, and bilinear functions were fitted to the data, and their suitability was compared using objective model selection criteria like Akaike Information Criterion (AIC). We developed a novel method to determine whether the transition is smooth, (i.e. it has a transition period with accelerating growth rate) or sharp, (i.e. without curved transition). This analysis found the overwhelming majority of the cells (70%) to have a bilinear growth pattern with close to half of them showing a smooth and not an abrupt transition. The growth pattern of the average cell was also found to be bilinear.

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NON-STANDARD RESIDUES OF PEPTAIBOLS: THEIR APPLICATION FOR MOLECULAR MODELING STUDIES

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Peptaibols are secondary metabolites constituting a family of fungal peptide antibiotics, which is constantly growing since the first member of this family was isolated, and more than 1000 peptaibol molecules have been described to date. These peptides are composed of 5-20 amino acid residues and they are generally produced in microheterogeneous mixtures. Peptaibols show a broad spectrum of biological activities including antibacterial, antifungal and antiviral effects, as well as immunosuppressive and neuroleptic properties. They contain non-proteinogenic amino acids (e.g. alpha-aminoisobutyric acid, Aib; isovaline, Iva), and their N-terminal residues are usually acetylated, and furthermore, an 1,2-amino alcohol (e.g. leucinol, Leuol; phenylalaninol, Pheol; valinol, Valol) is linked by an amide bond at their C-terminus.

Because of the unusual amino acid content of peptaibols, these molecules could not be easily modelled applying the currently available force fields and softwares. In order to study the three-dimensional structure of peptaibols, as well as to identify their typical conformational and folding features by means of different theoretical methods, the non-standard residues should be parameterized. Therefore, in the course of present study, several non-proteinogenic amino acids (e.g. Aib, Iva), as well as C-terminal amino alcohol residues (e.g. Leuol; Pheol; Valol) were parameterized using various quantum mechanical calculations. In the case of parameterization, a detailed procedure was developed to calculate accurately the partial charges with regard to the non-standard residues of peptaibols. Applying these charges obtained by quantum mechanical calculations, preliminary molecular dynamics (MD) simulations were carried out on peptaibol molecules, in order to assess their suitability for the molecular modelling calculations.

Based on the results, it could be concluded that the applied parameterization procedure, as well as the calculated partial charges proved to be suitable to study the three-dimensional structure of peptaibols by theoretical methods.

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GENE OVER-EXPRESSION STRATEGY FOR THE HUMAN PATHOGEN YEAST *CANDIDA PARAPSILOSIS*

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Over recent years there has been exponential growth in the number of yeast genome sequences. Despite the growth of sequence information, a large number of fungal genes remain uncharacterized and the function of genes are based on sequence homology. In fungi such as the opportunistic pathogen *Candida parapsilosis* which is constitutively diploid and lacks of sexual cycle, gene knockout methods can be time consuming. Another approach for studying gene function is to generate strains that over-express the genes of interest under the control of a strong promoter. Previously over-expression strategies have been applied to investigate *Candida albicans* gene function, interaction with the host and virulence attributes. In our previous work several fungal transcriptional factors have been identified using RNA-Seq data, that were overexpressed during host-pathogen interactions. Based on these data and using the GatewayTM technology we were able to generate *C. parapsilosis* strains that over-express our genes of interest. For this, *C. parapsilosis* CLIB 214 leu- strain was used. Using the caSAT1 flipper system we have integrated the RP10 locus of *C. albicans* SC5314 to the RP10 locus of *C. parapsilosis* CLIB 214 leu- strain. With this integration we were able to adopt the TDH3p-CLP10 over-expression system established in *C. albicans*. A TDH3p-CLP10-GFP constuct was used to test wether this system is able to express the genes of interest in *C. parapsilosis*. For entry vectors the pDONR 207 was used, while for destination vectors the TDH3p-CLP10 containing vectors were applied. All of the transformants were barcoded using a 20bp tag. This over-expression method and the gene of function phenotype is able to identify new gene functions reponsible for virulence in host-pathogen interactions.

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IMPACT OF MICROBIOLOGICAL PREPARATIONS ON SOME PARAMETERS OF HUMOUS SANDY SOIL

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In pot experiment the effect of different microbiological preparations on some soil properties and on the biomass of onion (*Allium cepa*, L.) were studied. The experiment was carried out in 2013 at the University of Debrecen, Institute of Agricultural Chemistry and Soil Science. The studied soil type was humus sandy soil, from the Debrecen-Pallag area. The soil had the following parameters: upper limit of plasticity according to Arany (KA)>32; pHKCl: 5.3; pH_{H2O}: 6.0; humus %: 1.1; AL-P_2O_5: 200 mg kg-1; AL-K_2O: 240 mg kg-1, showing that the soil was mildly acidic, of sandy texture, with medium supplies of nitrogen, phosphorus and potassium. The treatments in the experiment were control (1), NPK fertilizer (2), simple dose of mycorrhizal fungi preparation (A1) (3), NPK+simple dose of A1 (4), double dose of A1 (5), NPK+double dose of A1 (6), simple dose of mycorrhizal fungi preparation (A2) (7), NPK+simple dose of A2 (8), double dose of A2 (9), NPK+double dose of A2 (10), Foliar fertilizer (GA) sprinkled in every 10 (11), 14 (12) and 21 days (13), NPK+G1 (14), Amino acid preparation (AM) (15) and GA+AM (16) combination. The A1 and A2

preparations contained AM-27 Glomus intraradices mycorrhizal fungi. The GA was a foliar fertilizer with green algae (*Chorella vulgaris*). The 16 treatments in the experiment were arranged in a random block design with three replications. In our laboratory some physical, chemical and microbiological parameters of soil were determined. The soluble nutrient content of soil was examined: the NO_3^- N content of soil based on the sodium-salicylate method and the soluble P_2O_5 and K_2O content of soil were measured based on the ammonium-lactate method. The total number of bacteria and the number of microscopic fungi (on peptone-glucose agar) were determined from soil-water suspension by the plate method. The total number of nitrifying and cellulose decomposing bacteria were also measured, and the CO_2 production, urease and phosphatase enzyme activities of soil were examined. The biomass of onion was determined (the weight of onion leaves, the weight of onion bulbs and roots, the total weight of onion per pot). For the examination of the statistically justifiable differences between the average values of the results we applied one-factor analysis of variance on statistical data which showed the average values and LSD5%, and significance level.

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MOLECULAR CHARACTERIZATION OF DOBRAVA AND KURKINO GENOTYPES OF DOBRAVA-BELGRADE HANTAVIRUS DETECTED IN HUNGARY AND NORTHERN CROATIA

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Among the *Hantavirus* genus, Saaremaa virus (SAAV) has been the subject of numerous taxonomical debates. The International Committee on Taxonomy of Viruses declares SAAV as a distinct species, while several research groups believe that SAAV is in fact a genotype of Dobrava-Belgrade virus (DOBV). Some researchers who focus on European hantaviruses believe that DOBV is carried by three rodent species: *A. flavicollis*, *A. agrarius* and *A. ponticus*. They proposed a way to distinguish various genotypes within the DOBV species. Under this proposal, they call for the introduction of Dobrava, Saaremaa, Kurkino and Sochi genotypes within the Dobrava-Belgrade species, depending on the locality of the first identification/isolation. In the current study we present S-segment-based phylogenetic analysis of eight DOBV strains that were previously identified in rodents from Croatian and Hungarian trapping areas. Whole S-segment amplicons (1290 nucleotides) were used for sequencing and phylogenetic comparisons. Depending on their host species, DOBV strains detected in Hungary form two, clearly divided groups. Three novel strains that originate from *Apodemus agrarius*, are highly identical to a Croatian isolate which was earlier recognized as SAAV, probably due to using a too short S-segment sequence for analysis. Our data show that it is essential to compare adequately long sequences in phylogenetic studies to answer hypotheses in virus taxonomy. Based on the present study, we state that only Dobrava and Kurkino genotypes of DOBV species are circulating in the country, and there is no evidence for the presence of Saaremaa genotype in Hungary so far. Accordingly, we do not share the view that SAAV should be considered as a separate species inside the *Hantavirus* genus. We are commited to the hypothesis that not all hantavirus sequences derived form *Apodemus agrarius* in Europe belong to SAAV.

IDENTIFICATION OF HANTAVIRUS INFECTION BY WESTERN BLOT ASSAY AND TAQMAN PCR IN PATIENTS HOSPITALIZED WITH ACUTE RENAL FAILURE

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Hantaviruses (HTVs), one of the causative agents of viral hemorrhagic fevers, represent a considerable healthcare threat. In Hungary, Dobrava-Belgrade (DOBV) and Puumala (PUUV) are the main circulating HTV species, responsible for the clinical picture known as hemorrhagic fever with renal syndrome (HFRS), a disease that may be accompanied by acute renal failure, thus requiring hospitalization with occasionally greatly prolonged recovery period. Although national serosurvey of HTVs is ongoing since the year 2001, the annual number of human infection cases is probably underestimated due to the low awareness of the disease by the physicians. During a two-year period, a total of 188 persons were hospitalized with acute renal failure in the university hospital of Pécs, among whom 20 patients displayed clinical signs and laboratory findings directly suggestive for hantavirus infection. Patient sera were tested using an immunoblot assay, based on complete viral nucleocapsid proteins to detect patients' IgM and IgG antibodies against DOBV and PUUV. The total three acute positive cases were also tested by one-step real time TaqMan RT-PCR to confirm infection and to determine the causative hantavirus serotype. In a single case, follow-up sera were also applied to monitor changes in antigen response.
We present here the first Hungarian clinical study spanning across two years and dedicated specifically to assess acute renal failures, in the context of HTV prevalence.

PEACH RUSTY SPOT IS CAUSED BY THE APPLE POWDERY MILDEW FUNGUS, *PODOSPHAERA LEUCOTRICHA*

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Peach rusty spot, an economically important disease of peach (*Prunus persica* var. *persica*), appears as necrotic spots on fruit. The etiology of the disease was not well understood, although it has long been suspected that the causal agent is the apple powdery mildew pathogen, *Podosphaera leucotricha*. This hypothesis was confirmed based on cross-inoculation experiments and analysis of rDNA internal transcribed spacer sequences polymerase chain reaction amplified from rusty spot and peach powdery mildew lesions [1]. Cross-inoculations of apple and peach with *P. leucotricha* and *P. pannosa*, the causal agent of peach powdery mildew, showed that (i) young peach fruit, up to

5 cm in diameter, developed symptoms typical of rusty spot following inoculation with *P. leucotricha*; (ii) leaves of 'Jonagold' apple seedlings developed powdery mildew infections when inoculated by touching young rusty spot lesions to their surfaces; (iii) *P. leucotricha* sporulated on young peach fruit up to 5 cm in diameter; and (iv) peach leaves and young shoots were not susceptible to *P. leucotricha*, whereas *P. pannosa* infected all the green parts of peach. A field experiment revealed that there was only a 2- to 3-week period of time during early peach fruit development when the epidermis was susceptible to *P. leucotricha*. An outcome is that a clear distinction can be made between the symptoms caused by *P. pannosa* and *P. leucotricha* on peach.

[1] JANKOVICS, T. ET AL. (2011) Plant Disease 95: 719-724.
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COMPARISON OF THE HUMORAL IMMUNE RESPONSES AFTER INTRADERMAL OR INTRAMUSCULAR IMMUNIZATION OF INFLUENZA A H5N1 AND H7N9 VIRUSES

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Most vaccines are delivered by the intramuscular (IM) or subcutaneous routes using a needle and syringe; the intradermal (ID) route is widely used only for the administration of Bacille Calmette-Guérin and rabies vaccines. However there is renewed interest in ID vaccine delivery, driven by the fact that the dermis and epidermis of human skin are rich in antigen-presenting cells, suggesting that delivery of vaccines to these layers, rather than to muscle or subcutaneous tissue, should be more efficient and induce protective immune responses with smaller amounts of vaccine antigen. The ID administration route is especially important in the avian influenza vaccine development because the H5N1 and H7N9 viruses are poorly immunogenic in mammals.

Comparison of the humoral immune responses using whole, formaldehyde inactivated influenza virus concentrates for immunization of guinea pigs by different administration routes (IM, ID). The Influenza A/NIBRG-14(H5N1)- and Influenza A/Anhui/01/2013(H7N7)-containing materials were prepared for immunization under BSL3 containment. Guinea pigs were immunized by IM or ID route with 10000 HAU inactivated whole virus. Sera was collected three weeks later, analyzed by HI assay (CDC protocol), NI assay (NCE protocol) and MN assay (modified CDC protocol). The ID administrated Influenza A/NIBRG-14(H5N1) virus raised significantly higher humoral immune responses comparison with the IM immunization: Geometric mean of HI titers: 97 or 37; NI titers: 303 or 66; and MN titers: 57 or 8, respectively. Humoral responses showed similar results in Influenza A/Anhui/01/2012(H7N9) experiment: HI titers: 108 or 45; NI titers: 283 or 141; and MN titers: 40 or 7, respectively. Conclusions.

Our data showed more effective humoral immune responses after ID immunization when compared with the IM route of immunization. ID immunization could be an alternative route for antigen sparing, especially in a potential pandemic situation.

MODIFYING CD4 BINDING SITE BY THYOLATED PYRIMIDINE NUCLEOTIDES RESULTS IN SELECTIVE CYTOTOXICITY OF HIV INFECTED CELLS

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The initial molecular events of HIV infection, determined by gp120 and gp41 viral surface glycoproteins, as well as cellular receptors (CD4,CCR5) are with primary significance for HIV replication. Earlier we demonstrated significant in vitro anti-HIV effect of our new compounds: thyolated pyrimidine nucleotides termed UD, and derivatives. The aim of present study was to determine acute and chronical cytotoxicity of these compounds on monolayer and suspension cells, non-infected and infected acutly by HIV-1. Six chemically modified derivatives of thyolated pyrimidine nucleotides (UD29, UD29-new, UD30 ,UD31, Tox-1494 and Tox-2) 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. As we earlier demonstrated, 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 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 Tox-2 may function as an effective, new generation entry inhibitor for HIV.

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MECHANISM OF THE INDUCTION OF CELLULASE GENES IN *TRICHODERMA REESEI* GROWN ON LACTOSE: A METABOLOMICS APPROACH

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Lactose (1,4-0-β-D-galactopyranosyl-D-glucose) is used as a soluble carbon source for the production of cellulases and hemicellulases for – among other purposes – use in biofuel and biorefinery industries. The mechanism how lactose induces cellulase formation in *T. reesei* is enigmatic, however [1]. Previous results from our laboratories raised the hypothesis that intermediates from the two galactose catabolic pathway may give rise to the accumulation of intracellular oligogalactosides that could act as inducer. Here we have therefore used HPAEC-MS to study the intracellular galactoglycome of *T. reesei* during growth on lactose, in *T. reesei* mutants impaired in galactose catabolism, and in strains with different cellulase productivity. Lactose, allo-lactose and lactulose were detected in the highest amounts in all strains, and two trisaccharides (Gal-ß-1,6-Gal-ß-1,4-Glc/Fru and Gal-ß-1,4-Gal-ß-1,4-Glc/Fru) also accumulated to significant levels. Glucose and galactose, as well as four further oligosaccharides (Gal-ß-1,3/1,4/1,6-Gal; Gal-ß-1,2-Glc) were only detected in minor amounts, In addition, one unknown disaccharide (Hex-ß-1,1-Hex) and four trisaccharides were also detected. The accumulation of the unknown hexose disaccharide was shown to correlate with cellulase formation in the improved mutant strains as well as the galactose pathway mutants, and Gal-ß-1,4-Gal-ß-1,4-Glc/Fru and two other unknown hexose trisaccharides correlated with cellulase production only in the pathway mutants, suggesting that these compounds could be involved in cellulase induction by lactose. The nature of these oligosaccharides suggests their formation by transglycosylation rather than by glycosyltransferases. Based on our results (detailed in [2]) the obligate nature of both galactose catabolic pathways for this induction must have another biochemical basis than providing substrates for inducer formation.

[1] Fekete, E. et al. (2008) PNAS 105: 7141-7146.
[2] Karaffa, L. et al. (2013) Appl Microbiol Biotechnol 97: 5447-5456.
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THREE NOVEL BACTERIAL TAXA ISOLATED FROM THE ULTRAPURE WATER OF A HUNGARIAN POWER PLANT

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A Gram-positive actinobacterium and two groups of Alphaproteobacteria were isolated from the ultrapure water of the water purification system of a Hungarian power plant. Strains belonging to the Alphaproteobacteria were cultured by using newly developed, highly oligotrophic media, while the new actinobacterium was isolated from R2A medium. Phylogenetic analysis based on the 16S rRNA gene sequences revealed that strain IV-75T belonged to the suborder Micrococcineae and clustered with members of the family Intrasporangiaceae. Its closest phylogenetic relative is *Arsenicicoccus bolidensis* CCUG 47306T (94.3%). Strains PII_14, PII_23 and PIII_1aT exhibit the highest sequence similarity value to *Ferrovibrio denitrificans* Sp-1T (92.5%), similarity to all other taxa is less than 91.0%. The closest relatives of strains PI_31, PI_25 and PI_21T are *Bosea minatitlanensis* AMX51T (93.43%) and *Bosea thiooxidans* DSM 9653T (93.36%), similarity to all other taxa is less than 93.23%. Description of these novel bacteria was based on polyphasic

taxonomical studies. The strain IV-75T exhibited a rod-coccus cell cycle, it was strictly aerobic, non-motile, catalase-positive and oxidase-negative. The peptidoglycan of this strain contained meso-diaminopimelic acid and MK-10(H(4)) was the major menaquinone. The polar lipid pattern contained phosphatidylglycerol, two unidentified phospholipids, one glycolipid and several other lipid components. The major fatty acids were anteiso- C15:0, C18:1 w9c and C16:0. The members of the two Alphaproteobacteria groups were regular Gram negative rods, strictly aerobic and rather inactive in degradation of various carbon sources. The major isoprenoid quinone of these strains was Q-10, the major cellular fatty acids of strains PI_31, PI_25, PI_21T were C18:1w7c and 11Me18:1w7c and were C16:0, C18:1 w7c and C19:0cy w8c of strains PII_14, PII_23, PIII_1aT. The characteristic diamino acid in their cell wall was meso-diaminopimelic acid. Based on the moderate levels of 16S rRNA gene sequence similarity and the unique combination of chemotaxonomic characteristics, strain IV-75T was considered to represent a novel species of a new genus, for which the name *Aquipuribacter hungaricus* gen. nov., sp. nov. was proposed.
The proposed name of the two Alphaproteobacteria groups is *Retineobacter lacuortus* - the type strain is PIII_1aT(=DSM 25522T=NCAIM B 02509T) and *Freatibacter oligotrophus* - the type strain is PI_21T(=DSM 25521T =NCAIM B 02510T), respectively.

EFFECT OF ESSENTIAL OILS AND THEIR MAIN COMPONENTS ON THE FORMATION OF *LISTERIA MONOCYTOGENES* BIOFILMS

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The most common form of microorganisms in the environment is in a community of cells attached to a substrate. This community is referred to as biofilm and this shows greater resistance to antibiotics and disinfectants than the free-floating cells because of the thick matrix that holds the cells together. Biofilms of both spoilage and pathogenic microflora are related to problems of food contamination, leading to lowered shelf-life of products and transmission of diseases.
Therefore, it is a priority for food-processing industry to apply sanitation strategies which minimize the risk of biofilm contamination. *Listeria monocytogenes* is a Gram positive bacterium, which is pathogenic to both humans and animals. It causes listeriosis, a serious condition caused by eating contaminated food. The disease primarily affects pregnant women, newborns and adults with weakened immune systems. *Listeria monocytogenes* has become a difficult pathogen to control because of its unusual mechanisms of survival under adverse environmental conditions and its ability to form biofilms. Because of consumer demands of decreasing the use of chemical preservatives, more attention has been paid to the search for natural substances able to act as antimicrobials or decontaminants. Essential oils have gained widespread interest in the search to identify the alternatives for microbial control because of their antiseptic qualities.

The present study focuses on the effect of six essential oils (cinnamon, clary sage, juniper, lemon, marjoram and tyme) and their main components (cinnamon-aldehyde, α-pinene, limonene, linalool, terpinene-4-ol and tymol) on *L. monocytogenes* biofilms. Determination of the MIC values and biofilm formation was carried out in 96 well plates. For biofilm formation, cell suspension (108) was added into the wells, and after 4 h of incubation, unattached cells were washed and EOs or components were added in MIC/2 concentration. Wells were washed again after 24 h, and the cells were fixed with methanol and stained with crystal violet. Acetic acid was used for dissolving the dye and absorbance was measured at 590 nm. Our results showed that most of the oils used in this study have good anti-biofilm forming activity on *L. monocytogenes*. The best inhibitor resulted to be cinnamon oil. The EOs and components used seem to be good candidates for prevention of biofilm formation and could be used as preservatives or/and surface disinfectants in the food industry.

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OUTER MEMBRANE PROTEIN LOSS MEDIATES COLISTIN RESISTANCE IN *KLEBSIELLA PNEUMONIAE* ST258

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K. pneumoniae is a frequent nosocomial pathogen and found increasingly resistant to several antibiotics including colistin. In Hungary, colistin resistant *K. pneumoniae* emerged in 2008 in an outbreak of KPC-2 producing *K. pneumonaie* ST258. In this study a colistin susceptible and a resistant isogenic KPC-2 producing *K. pneumoniae* ST258 strains were investigated. The outer membrane proteins from each strain were analyzed by two-dimensional electrophoresis and mass spectrometry. The loss of LysM/domain BON superfamily with peptidoglycan bindig function was detected in the resistant strain, and it lacked DNA starvation proteins too.

The susceptibility of the strains were tested to protamin, lactoferrin, lyzozim and the colistin resistant strain showed 10-15% higher bacterial cell count than the counterpart strain. The susceptibility of the strains were analyzed to serum and liquor derived from rats where the two strains showed similar susceptibility. The PhoP-pmrD-arn regulatory system was investigated by real-time PCR where the relative expression of the coding genes in the colistin resistant strain showed elevated mRNA synthesis.

Colistin resistant *K. pneumoniae* emerged and the ST258 clone seems prone to develop colistin resistance by the loss of LysM/domain BON superfamily. The colistin resistance is associated with tolerance to lyzozym, lactoferrin and to an antimicrobial peptide protamin. The PhoP-pmrD-arn regulatory system showed elevated expression in the resistant strain. This is the first investigation of the resistance mechanisms in a colistin resistant *Klebsiella pneumoniae*.

DIVERSE FITNESS COST ASSOCIATED WITH FLUOROQUINOLONE RESISTANCE GOVERNS CAPACITY OF CLONAL EXPANSION OF *KLEBSIELLA PNEUMONIAE* IN ADULT HOSPITAL WARDS AND SELECTS FOR CTX-M-15 TYPE EXTENDED SPECTRUM-B-LACTAMASE

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Varying fitness cost associated with resistance to fluoroquinolones was recently demonstrated to play a prominent role in the clonal dynamic of methicillin-resistant *Staphylococcus aureus* in the health care setting. We investigated whether or not a similar mechanism may account for the clonal expansion of *Klebsiella pneumoniae*. The fitness of *K. pneumoniae* isolates from major international clones (ST11, ST15, ST147) already resistant to fluoroquinolones and of strains from small clones (ST25, ST274, ST1028) in which fluoroquinolone resistance was induced in vitro was tested in a propagation assay. All strains from major clones showed significantly less fitness cost than fluoroquinolone resistant derivatives of small clone isolates. In addition all isolates from major international *K. pneumoniae* clones – displaying high MIC values to fluoroquinolones (≥64 µg/ml) - retained their CTX-M-15 type extended spectrum ß-lactamases (ESBLs) while SHV type enzymes from small clone strains were lost during induction of fluoroquinolone resistance. Among two ST274 small clone isolates carrying the respective types of ESBLs the CTX-M-15 ß-lactamase was retained while the SHV-2a ESBL lost in the course of induction. Strains from major international clones mutated differently in the *gyr*A and *par*C genes than small clone isolates which could have resulted in diverse fitness cost. The Ser80ILe substitution in *par*C was found common for each major clone strain while conspicuously absent from all small clone isolates. Our findings can account for both the widespread dissemination of the major international *K. pneumoniae* clones in adult hospital wards where fluoroquinolone type antibiotics are used extensively and explain the predominance of the CTX-M-15 type ESBL in these isolates.

WHEAT BRAN AS SUBSTRATE FOR PRODUCTION OF LIPASE ENZYMES BY MUCOROMYCOTINA FUNGI

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Lipase enzymes (E.C. 3.1.1.3) have potential in various industrial applications wherein both of their hydrolytic and synthetic activities were utilized. In our previous studies, screening of lipolytic activity of Mucoromycotina fungi on tributyrin containing media was performed. Furthermore, the effect of lipid materials on lipase production was also tested in submerged cultures. Based on these experiments, many strains with good enzyme producing ability have been identified from the genera *Rhizomucor*, *Rhizopus*, *Mucor*, *Umbelopsis* and *Mortierella*. The use of food and agro-industrial wastes and by-products as substrates in fermentation processes is an environmentally friendly and economic way to utilize these residues. Fermentation on wheat bran is a frequently used technique to produce lipase enzymes by filamentous fungi. This substrate contains adequate amount of proteins, fats, amino acids and minerals that are essential for the appropriate enzyme production and

fungal growth. The goal of the present study was to investigate the possibility of using wheat bran as substrate for high-yield lipase production by the identified good enzyme producer Mucoromycotina strains. Additionally, our aim was to analyze the effect of various supplements such as mineral salts and olive oil on the product yield. Submerged and solid-state fermentations were performed to analyze the lipase production. The level of the enzyme activity was determined with p-nitrophenyl-palmitate chromogenic substrate. For submerged cultures, mineral growth fermentation medium supported with equal amount of wheat bran and olive oil was used. Only about the half of the strains tested exhibited higher volumetric activities in wheat bran based submerged fermentation than that in minimal cultures supplemented with lipid materials as sole carbon source. This indicates that in submerged cultures wheat bran does not always increase the enzyme production by zygomycetes strains. For solid cultures, two fermentation conditions were applied to evaluate the enzyme production: a simple medium which has only distilled water to moisturize the substrates, and a supplemented one which contains mineral salts and olive oil. In general, higher product yield have been achieved with solid-state fermentation than that presented in submerged assays. Furthermore, addition of mineral salt solution and olive oil to the solid fermentation medium resulted in at least 1.5-fold increment in the enzyme activities.

This research forms part of the project TÁMOP-4.1.1.C-12/1/KONV-2012-0012, and was realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund. The work was also supported by the mobility grant of the Hungarian-French Intergovernmental S&T Cooperation Programme (TÉT_10-1-2011-0747).

EFFECTS OF YEAST STRAINS AND ACIDS ON ALCOHOLIC FERMENTATION OF PEAR

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Recently, according to regulation from EU (EEC No 1576/89) Pálinka is a Hungaricum and it is a double-distilled fruit brandy produced in Hungary and in four regions of Austria. No doubt that quality of pálinka is depending on the quality of the raw material known as fruits, but the applied making technology especially mashing and fermentation process also plays an important role on aroma profile of spirits. In this study effects of yeast strains and acids on fermentation process were investigated. Bosch Cobak pear was collected from Göcsej region and mashed using standard method. Briefly, pear fruits were washed by tap water and then ground. The mash then was treated by polygalactoronase enzyme preparation. The pH of mash was adjusted to pH 3 using different acids: 25% phosphoric acid, 50% lactic acid and 25% sulphuric acid, respectively. Three yeast strains were applied: *Sacharomyces cerevisiae* strain 228 and 342 as well as bake yeast (YS4). In the case of *S. cerevisiae* strain 342, the shortest lag-phase was detected (8 hours) based on the formation of gas. The alcoholic fermentation process has got intensive after 12-15 hours inoculation. Among investigated acids, lactic acid appeared to be the best one because all three yeast strains were able to do fermentation in very short time. In the case of phosphoric acid at least 24 hours are needed for the fermentation process to get into the intensive stage, meanwhile in the case of sulphuric acid, it needs three days. All investigated yeast strains were able to ferment sugar in pear mash to ethanol. The highest alcohol content (5.1 % v/v) was detected in the case of addition of sulphuric acid with *S. cerevisiae* strain 342. Analysis of fermented mash and distilled spirits showed that supplementation with lactic acid had given products in rich of aroma components. More studies

are needed to clear the individual effects of each acids and yeasts, but our results are very promising for development of pálinka-making technology that may guarantee standard quality of products.

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RELATIONSHIP BETWEEN THE SUBSTRATE C/N RATIO AND THE COMPOSITION OF MICROBIAL COMMUNITY IN BIOGAS REACTOR

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Facing energy crisis, the world is in need of green, efficient, carbon-neutral energy sources to replace fossil fuels. Biogas is one of the major renewable energy sources ensuring an adequate energy supply for the next generations which is formed by anaerobic digestion of organic materials, provides sustainable, renewable energy. It is essential for optimizing the biogas-forming process to know detailed information about the diversity and a deeper knowledge of composition of the participating microbial community structure. In most cases biogas technologies commonly apply natural anaerobic consortia of microbes. We demonstrated earlier that the composition of microbial community of the anaerobic reactor changed after feeding with protein-rich substrates as the sole carbon source. In this work biomethane production from a mixture of protein-rich meat-extract and cellulose-rich ground corn leaf and stalk in different ratios was studied in batch fermentors at 37°C. We demonstrated that biogas fermentation can be intensified by changing carbon/nitrogen ratio and added protein-degrading bacteria into the system. The quantitative polymerase chain reaction was employed to identify the three added bacteria and relative abundance of these microorganisms of the microbial community. Proteinase activities of the consortia were monitored regularly. The changes in ammonium-nitrogen concentrations were followed during the fermentations. Volatile fatty acid compositions (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic acids) were determined using HPLC to monitor the microbiological activity in the reactors. Total carbon and total organic carbon contents have been examined to determine the C/N ratio of the biomass. Volumetric biogas yields gave information about the efficacy of the anaerobic digestion process. The composition of the evolved gas was determined by gas chromatography. The findings extend the range of organic "waste" substrates suitable for renewable energy production and predict routes for rational design of more effective microbial communities for industrial scale biogas production.

DETECTION OF AUXIN TYPE INDOL COMPOUNDS IN BACTERIA ISOLATED FROM SOIL AND THE DETERMINATION OF INDOL-3-ACETIC ACID CONCENTRATION OF CULTURES

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Microorganisms excrete stimulating and growth inhibiting materials and these influence plant development. The importance of these PGPR (Plant Growth Promoting Rhizobacteria) microorganisms has been recognized in the health of the root, nutrient uptake and stress tolerance. PGPR bacteria are able to produce phytohormones that regulate plant germination, development,

flowering and fruit ripening. The synthetic plant hormones and other regulatory compounds are widely used in the control of plant development. Auxins are members of juvenil hormones and have been used in plant cultivation and horticulture for over 50 years. The most of root elongation promoting bacteria can produce auxin hormone like indole-3-acetic acid (IAA) and exert the same effect as indole-acetic acid administration in the plant's environment. IAA-producing soil bacteria promote germination and result in richer roots and thus formation of a greater absorption surface. The auxins are also utilized to increase stress resistance. The drought and cold tolerance limit can be increased and the natural maturing can be scheduled for a more favorable time by auxin treatment. In the plants there are indole-3-acetic acid and other indole compounds which have more and less auxin-like effect. These materials are precursors or derivatives of indole-3-acetic acid and the reason of the auxin effect is the transformation in the plant tissue. We selected plant hormone producing bacteria from Biofil Ltd's collection of isolated strains. Thereafter we measured the production of indole-3-acetic acid (IAA) and similar type of hormones in bacterial cultures by different methods. We have grown the isolates on several types of culture media with tryptophan precursor supplementation. For preliminary studies we have used less specicfic spectrophotometric methods, screening several indole-derivative compounds. We have detected indole-3-acetic acid in addition to other indole compounds with this method. In our work the bacterial cultures were centrifuged and then the supernatant extracted into etyl-acetate. The etyl-acetate fractions were reduced by evaporation under vacuum and redissolved in absolute methanol. The extracts were chromatographed by thin-layer chromatography (TLC) and high-pressure liquid chromatography (HPLC-PDL). Besides the indole-3-acetic acid and tryptophan standards, other indole compounds could be detected in the samples undergone preliminary purification in the course of TLC. According to the spectrophotometric analysis, 21 % of the bacteria isolated from the soil could produce indole compounds. More than half of the strains producing auxin type indole compounds in the highest concentrations were enteropathogenic. These are members of the *Staphylococcus*, *Enterobacter*, *Salmonella*, *Shigella* and *Pseudomonas* genus. According to the HPLC-PDL measurement the IAA production approached 20 µg/ml while the spectrophotometric method showed that the maximum yield reached 100 µg/ml concentration.

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

POSSIBILITIES OF BIOREMEDIATION, BIOCONTROL AND PLANT GROWTH PROMOTION WITH TRICHODERMA STRAINS ISOLATED FROM VEGETABLE RHIZOSPHERE SAMPLES

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Nowadays organic farming is becoming more and more important in the agriculture. Organic farmlands are exposed to dangerous xenobiotics through distinct pollution drift effects such as wind-driven, pesticide-containing dusts and xenobiotic-containing rains. In order to achieve organic farming, there is a need for the development of new techniques which allow the bioremediation of lands previously used in common, intensive agricultural practice. Organic agriculture also faces the problem of pests including the damage caused by plant pathogenic fungi, therefore the implementation of biological control as a possible, environment-friendly solution is also of

increasing importance. In this study we examined *Trichoderma* strains isolated from vegetable rhizosphere samples for the production of laccase - an enzyme activity with potential in bioremediation of phenolic pesticide residues - on solid and liquid media supplemented with two different substrates, ABTS [2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate)] or guaiacol. In the case of the best laccase-producing *Trichoderma* strains we also determined the Biocontrol Index (BCI) values by performing dual culture tests in confrontation with the plant pathogens *Fusarium solani*, *F. oxysporum*, *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani* and *Phoma cucurbitacearum*. One of the most promising strains, a *Trichoderma asperellum* isolate was involved in pot experiments to study its effect on the growth of pepper and tomato plants in the absence or presence of the herbicide Linuron, and to study the effects of *Trichoderma* application on the level of systemic acquired resistance-related metabolites in the plants. The applied *Trichoderma* strain proved to enhance the growth of the plants and diminish the negative effects of the herbicide. Along with its biocontrol potential, this strain seems to be very promising for the development of a microbial product with multiple beneficial effects for the purposes of organic farming.

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BIOCONTROL POTENTIAL OF *PHOTORHABDUS LUMINESCENS* (ENTEROBACTERIACEAE) AGAINST PESTS IN MUSHROOM PRODUCTION

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Mushroom production is nowadays a field of agriculture with emerging economical significance. Its advantages include that it can be performed in closed systems, thereby not subjected to the effects of climate change, and that the substrates of cultivation are secondary agricultural waste materials (e.g. the straw of wheat varieties, stem residues, etc.) which are available in significant quantities in the Central European region. Furthermore, mushrooms also contain medicinally important compounds. Unfortunately the production of medicinally important mushrooms is also facing problems. Diseases caused by competitor moulds, viruses, bacteria as well as damages due to arthropod, nematode and mollusc pests may occur even during the application of elaborated cultivation technologies. The chemical control of these pathogens is polluting the environment, furthermore, residues of the applied chemical compounds are occurring in the fruiting bodies of the mushrooms, which has health-damaging effects on the consumers. The aim of this study was to work out an innovative approach for the elimination of green moulds, mushroom parasitic arthropods and nematodes. The approach is based on the application of the bacterium *Photorabdus luminescens*. Strains of the species *P. luminescens* are known to be associated with certain nematodes, which are passing into the insect larvae and adults during their life cycle. The bacteria are capable of killing the larvae and adults of insects via toxin production. *Photorhabdus* species can also be characterized by the ability of bioluminescens. Four *P. luminescens* strains from the National Collection of Agricultural and

Industrial Microoganisms (NCAIM) were included in this study. Clear inhibitory effects could be recorded during our studies on the in vitro antagonism of *P. luminescens* strains against the green mould pathogens *T. aggressivum* f. *aggressivum*, *T. aggressivum* f. *europaeum*, *T. pleurotum* and *T. pleuroticola*. The effects of the strains on oyster mushroom (*Pleurotus ostreatus*) were also evaluated. Furthermore, the effects of the bacteria on nematodes isolated from mushroom compost and on mushroom damaging arthropods and their larvae were also examined. Under the examined conditions, *P. luminescens* killed the arthropod pests and the nematodes within 5 and 6 days, respectively. Based on the results, *P. luminescens* may be a potential biocontrol agent in mushroom production enabling more economic and environment-friendly production of healthy food.

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PLANKTONIC BACTERIAL DIVERSITY OF LAKE HÉVÍZ REVEALED BY NEXT GENERATION SEQUENCING AND MOLECULAR CLONING

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Lake Hévíz is the deepest, biologically active peat bedded thermal lake of Europe, harboring particular bacterial communities. The water of the lake originates from two crater springs (26°C and 41°C) and owing to a special thermoregulatory mechanism its temperature never falls below 22°C. The water is oligotrophic, poor in nutrients mainly in forms of nitrogen, but rich in sulfur compounds, and can be characterized as calcium-magnesium-hydrocarbonate type. The aim of the present study was to gain information about the diversity of planktonic bacterial communities by simultaneously using molecular cloning and next generation sequencing methods. Water samples were collected from 14 different sampling sites in April and October 2011. In both times, composite samples from same amount of subsamples were prepared and examined. 16S rRNA gene based clone libraries contained 192-192 molecular clones. A Roche GS Junior platform was used for pyrosequencing. Following a quality filtering, denoising and removal of chimeric and singleton sequences by mothur software, 7756 (in spring) and 5705 (in autumn), approximately 500 base length sequences were aligned and identified with the SINA aligner tool using the ARB-SILVA bacterial 16S rDNA reference database. Comparing the community structures, seasonal differences were revealed. At higher taxonomic levels, similar distribution of sequences was obtained using both molecular methods. In spring the clone library was dominated by members of the Alpha- and Betaproteobacteria (44% and 49%) while in autumn Epsilon- and Gammaproteobacteria (22% and 10%) as well as Bacteroidetes (7%) related clones were also abundant. Sequences obtained by the next generation sequencing method were also mainly related to class Alpha- and Betaproteobacteria (34% and 50%) in April but members of class Epsilonproteobacteria (21%) and phylum Actinobacteria (10%) were abundant as well in October. During this study the majority of sequences showed the highest similarity to sequences derived from environmental samples, therefore species level identification was rarely possible.

DEVELOPMENT OF BIOMONITORING SYSTEMS FOR ANALYSING AFLATOXIN-B1 AND ZEARALENONE

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Mycotoxins are secondary fungal metabolites that pose serious health risks to humans and animals as well. Among the most hazardous mycotoxins are the aflatoxin-B1 (AFB1) produced by *Aspergillus* spp. due to its mutagenic, carcinogenic, teratogenic, immunomodulant and cytotoxic effects, and zearalenone (ZEA) produced by *Fusarium* sp. that causes various problems associated with reproduction thorough its estrogen effect. To eliminate mycotoxins, recently innovated biological methods came into prominence, taking advantage of the capability of certain microbes that utilize mycotoxins as an energy source. However, insufficient data present that evaluate the residual biological effect of biotransformation. In the present work biological effect of AFB1 and ZEA were investigated by prokaryotic and eukaryotic test organisms: on the one hand with the genotoxicity testing SOS-Chromotest optimized for *Escherichia coli*, and with the cytotoxicity testing method that is based on *Aliivibrio fischeri*, on the other hand with the *Saccharomyces cerevisiae* BLYES strain for testing estrogenic effect, and with the BLYR control strain for measuring toxicity. Sensitivity of these tests was determined for AFB1 and ZEA and these bio-tests were adapted for selecting the best microbes without creating harmful intermediates throughout mycotoxin degradation. Experiments to investigate sensitivity of the test-organisms for AFB1 showed that SOS-Chromotest and A. fischeri test is not suitable for direct monitoring of food and feed stuffs, as it works at a much higher concentration than the current limits, though these bio-tests are effective tools for evaluating the bio-detoxification capacity of microorganisms. For that reason SOS-Chromotest and *A. fischeri* test were adapted to mycotoxin degradation experiments. By the combination of the two modified assays, the most appropriate microbes for AFB1 degradation were selected. On the base of this combined toxicity-profiling method strains belonging to the *Rhodococcus* and the *Pseudomonas* genus proved to have effective toxin degradation without creating geno- and/or cytotoxic metabolites. Results of the estrogen tests proved the applicability of BLYES test organism for direct monitoring of food and feed stuffs, as the test is responsive for ZEA under the current limits. The yeast based bio-reporters BYES/BLYR were adapted to experiments for testing mycotoxin biodegradation; the most appropriate microorganisms with the weakest residual estrogen and/or cytotoxic effect were selected. On the base of this method the most promising strains belong to the *Rhodococcus* and the *Streptomyces* genus. Above mentioned results underline the necessity of bio-testing procedures beside conventional analytical methods, since harmful effects are revealed by these bio-tests which cannot be detected by analytical methods. Moreover, biological methods are fast, cost-effective and reliable tests, and strain collections with hundred of microbes can be screened by the use of these bio-testing procedure.

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PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF MULTIRESISTANT *ACINETOBACTER BAUMANNII*

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The incidence of multiresistant *A. baumannii* (MACI) has increased dramatically in Hungary since 2005. The aim of this study was to characterize MACI strains obtained at two large ICUs of Semmelweis University from consecutive invasive infections (n=30-30). The results were compared to the representative group of the MACI isolated and sent for molecular epidemiological investigation (n=58) to the Hungarian National Center for Epidemiology in the same year. Identification was performed with MALDI-TOF MS and phenotypic methods. Antibiotic susceptibility testing was performed with the agar dilution method for imipenem, meropenem, gentamicin, tobramycin, amikacin, and ciprofloxacin; and with microdilution for colistin and tigecycline. The genetic relationship and the carriage of blaOXA carbapenemase-genes and aminoglycoside-modifying enzymes (AME) were determined by PCR, PFGE and MLST. All isolates were resistant to amikacin, ciprofloxacin and tigecycline (MIC50 and MIC90: >256 and>256; >32 and >32; 8 and 32, respectively). All isolates were susceptible to colistin (range: 0.125-2 mg/L), although 2 % had a MIC value of 2 mg/L MIC. 28% of the isolates were susceptible to tobramycin, and 9% was susceptible to gentamicin. All isolates were resistant to imipenem, one was intermediate; 18 isolates were intermediately susceptible to meropenem, all others were resistant. 82% of the isolates contained blaOXA23, and 18% contained blaOXA58 (100% carried blaOXA51). Five frequent PFGE types were detected, of which four belonged to the EU-I clone (ST-1, ST-81), and one to the EU-II (ST-2). The OXA-23 producers all showed high grade resistance to carbapenems, intermediate susceptible strains were detected among the OXA-58 producers. Results indicated that the AME genotype was an inadequate predictor of the aminoglycoside phenotype. Aminoglycoside susceptibility was related to clonality; while carbapenem susceptibility was related to the type of enzyme produced. The significance of MACI has increased in Hungary as well. The spread of a small number of successful clones was observed. Different strains may be present at the same time at a single unit. Colistin is the only therapeutic option.

ANTIMICROBIAL RESISTANCE AND CLONAL INVESTIGATION OF CLINICAL *CORYNEBACTERIUM STRIATUM* ISOLATES

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Corynebacterium striatum is a nosocomial pathogen affecting patients mainly but not exclusively with immunosuppression. Though it often displays multiple resistance to antibiotics, definite guidelines for the determination of its antibiotic susceptibility are yet to be established. Seventeen *C. striatum* strains were isolated from various clinical samples (wound, lower respiratory tract) from patients at Semmelweis University, Budapest. Strains were identified by 16S rRNA sequencing and MALDI-TOF analyses. Genetic relatedness of the isolates was investigated by PFGE. Antimicrobial susceptibility testing was performed by disk diffusion, microdilution and E-test in line with provisional EUCAST recommendations. Tests were evaluated both after 16-20 and 44 hours of incubation. Biofilm production was quantified by the crystal violet extraction technique. PFGE established five small clusters/outbreaks of nosocomial infections caused by *C. striatum* affecting sometimes multiple facilities. Five of the strains showed multiple resistance to antibiotics. The most

effective drugs turned out to be: vancomycin, fusidic acid, tigecycline and linezolid, the least active compounds: penicillins, fluoroquinolones, macrolides, clindamycin and sulfamethoxazole/ trimethoprim. Bimodal distribution of resistance was typical for imipenem and rifampicin and tolerance could be observed towards certain β-lactam agents. Norfloxacin disk proved more reliable for the screening of fluoroquinolone resistance compared to nalidixic acid (the latter detected false resistance in 3 cases). Furthermore kanamycin disk did not turn out to be optimal for screening aminoglycoside resistance (detected false susceptibility in 6 cases). Significant difference was observed between the results of disk diffusion and microdilution for tetracyclines. Evaluation after 16-20 and 44 hours of incubation provided notable difference mostly for certain β-lactams, gentamicin, tobramycin, macrolides and tetracyclines. Three strains showed weak, nine showed moderate and five showed strong biofilm production, respectively. This is the first report of nosocomial clusters/outbreaks caused by *C. striatum* in Hungary. Five of the pathogens showed multiple resistance. Vancomycin, linezolid, tigecycline and fusidic acid proved effective against all isolates. Rifampicin can be suggested as a combination partner. The evaluation of antibiograms seems to be more reliable after an incubation of 44 hours.

CHARACTERIZATION OF THE OPPORTUNISTIC HUMAN PATHOGENIC *BIPOLARIS* ISOLATES

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Members of the genus *Bipolaris* belong to the ascomycetous order Pleosporales and can be isolated from graminiculous plants. These melanized fungi are able to produces several types of secondary metabolites, which have important roles in the infections of plants. The species *B. australiensis*, *B. hawaiiensis* and *B. spicifera* can be frequently isolated from phaeohyphomycosis, which is the summarizing name of the infections caused by melanized fungi. During the identification procedure, the morphology based method (determination of the shape, size and number of septa of the conidia) is mostly used, however, because of the similarity of the morphological characters it frequently results misidentifications,. These data are often completed with the sequence analysis of the internal transcribed spacer (ITS) region, which in the case of the clinically important *Bipolaris* species shows low sequence variability. The aim of our work was to elaborate a sequence-based method to definitive identification of the human pathogenic *Bipolaris* species. Almost 35 isolates were obtained of human keratomycosis and international strain collections. We analyzed the sequences of the ITS and intergenic spacer (IGS) regions of the nuclear ribosomal RNA gene cluster, the β-tubulin and the translational elongation factor EF-1 alpha genes. We managed to determine the positions of distinguishable characters in the sequences.

These opportunistic pathogenic species are poorly characterized from the aspects of extracellular enzyme production, which may be important in pathogenesis; therefore, their elastase, lipase, phospholipase and proteinase enzyme producing ability was also evaluated.

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MULTIDRUG-RESISTANT *E. COLI* AND COLIFORMS IN CONFISCATED FOODS OF NON-SCHENGEN ORIGIN

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Multidrug resistance (MDR) in Enterobacteriaceae is an increasing worldwide concern. The import of contaminated food may represent a food safety risk by the spread of pathogenic- and/or MDR bacteria and their determinants for antimicrobial resistance. As part of an EU collaborative research project (PROMISE), our studies aimed to identify and analyse pathogens like *Salmonella* spp., *Campylobacter* spp. and verotoxigenic *E. coli* (VTEC), and isolation of MDR *E. coli* from food samples from non-Schengen countries confiscated at Hungarian borders was also attempted. Detection of *Salmonella*, *Campylobacter* and VTEC bacteria was performed according to the appropriate ISO standards (ISO 6579:2002/*Salmonella*, ISO 10272-1:2006/*Campylobacter* and ISO 16654:2001/VTEC-O157). For the isolation of non-O157 VTEC strains the Ridascreen® method was also used. *E. coli* colonies were isolated by their phenotype on Chromocult Coliform selective agar plates (Merck) and their identity was confirmed by PCR based on the presence of *lac*Z and *uid*A genes. For the detection of MDR of *E. coli* a pre-selection was used: the isolates were tested for their resistance to ampicillin, cefotaxime and tetracycline, and those demonstrating resistance for at least one of these three antibiotics were tested by disc diffusion for 18 antimicrobials with clinical relevance. The interpretation was performed according to the CLSI recommendations, and isolates with intermediate resistance were considered as sensitive. Resistance phenotype was confirmed by the presence of the respective antimicrobial resistance genes by PCR. The integron content of the isolates was also identified. A total of 207 confiscated food samples were tested in this study. All of them proved to be negative for *Salmonella* and VTEC bacteria, and in one sample from raw chicken carcass *Campylobacter jejuni* was identified. From the 207 samples, 833 coliform bacteria were isolated (5-10 representatives/sample) according to the methods described above. There were 87 samples negative for the presence of coliforms. From the total of 833 coliform isolates 257 (31%) fulfilled the pre-selection criteria for MDR, and were subjected to the determination of the resistance phenotype. Among them 13 isolates showed resistance to at least three different antimicrobial classes thus were designated as MDR. They represented 11 different food samples: 4 porcine, 2 bovine, 2 chicken, 1 rabbit, 1 duck and 1 of sheep origin. In addition to the above 13 MDR isolates, 10 other isolates merits further analyses, being resistant to β-lactams and/or aminoglycosides. Class 1 integrons (1.0-1.5 kb) were found in 9 of the 13 MDR isolates, and majority of them showed an atypical structure lacking the sul1 gene from their conserved segments 3'CS. Identification and detailed characterization of genes underlying above resistances mechanisms is in progress, and results will be analysed in comparison with those of international and our earlier national studies on antimicrobial resistance genotypes.

PRELIMINARY RESULTS OF A MANNANE-BASED SYNBIOTIC PROJECT

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Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the intestinal tract (eg. inulin, fructooligoszacharides, mannooligosaccharides). Probiotics are live bacteria which mainly colonize the large intestine and confer health benefits to the host (eg. lactobacilli, bifidobacteria). 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. The interdisciplinary project consists of three elements: microbiology, biochemistry and animal physiology. Mouse model system was chosen for the preliminary research. In the microbiology part a series of lactobacilli were isolated on MRS medium from the cecum of mouse fed with different mannane containing additives like BioMos, Locust Bean Gum and Konjacglucomannane. The isolates were identified on species level by molecular taxonomic tools. The most frequent species were Lactobacillus reuteri and *L. johnsonii*.
The mannooligosaccharide preference of these strains was analyzed with Malthus conductance measuring instrument. In this section the selection of the most appropriate probiotic candidates were done. In the biochemistry part, mannane hydrolase enzymes were selected from the genome projects of three *Thermobifida* species, which we had earlier sequenced. His-tagged fusion proteins were created and the endomannanase enzymes were expressed in *E. coli* BLD21 host. After purification and biochemical characterization terminal hydrolysis of different cheap mannane sources (BioMos, LBG, KM) were done and the whole hydrolysate or fractionated oligomannane products were used as prebiotic fraction. In the first animal feeding trial beside the control mice DSS-treated animals with inflammatory symptoms and BioMos-fed mice were also investigated. The change of the microbiota was measured by the count of viable lactobacilli, Rt-PCR and DGGE methods. Inflammatory marker proteins (interleukins, TNF-alpha, G-CSF, MCP1) were measured by ELISA. Our results showed an opposite change in the composition of the microbiota: decrease of the viable lactobacillus count and increase of bacteroides was measured in the inflammatory model, while much lower bacteroides and higher lactobacillus count were found in BioMos-fed mouse. The level of the inflammatory marker proteins was significantly higher in DSS-treated animals than in the control and BioMos-fed mice. Interestingly the DSS-treated mice with BioMos feeding showed normal inflammatory marker protein levels.
According to our preliminary results, while BioMos has been termed as prebiotic it would more correctly be named immunosaccharide.

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ISOLATION, OVEREXPRESSION AND PURIFICATION OF THE MAJOR INTRACELLULAR BETA-GALACTOSIDASE (BGAD) FROM *PENICILLIUM CHRYSOGENUM*

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Microbial beta-D-galactosidase (beta-D-galactoside galactohydrolase; EC 3.2.1.23) is a coveted enzyme in food- and fermentation industry due to its capacity to hydrolyse lactose (milk sugar; beta-D-galactopyranosyl-(1,4)-D-glucopyranose). Fungal beta-galactosidases can be distinguished into

extracellular enzymes, that are characterised by an acidic pH optimum, and intracellular ones, that typically function optimally at neutral pH. The former generally belong to glycosyl hydrolase family 35 (GH35) while the latter usually are GH2 proteins. Although *P. chrysogenum* is amongst the most important fungi employed in biotech industry, its metabolic relationship with lactose and its monomer D-galactose remained obscure, which is surprising in light of the fact that on technical scale, penicillin was produced on lactose as a carbon source for several decades. Fungi tend to employ only one of the two principal strategies – extracellular or intracellular hydrolysis – for the catabolism of lactose. In contrast, we have shown that *P. chrysogenum* features a dual lactose assimilation scheme that likely includes an extracellular bGal (encoded by bgaA), as well as two putative lactose permeases and two intracellular bGals (encoded by *bga*D and *bga*E, respectively). *Bga*D is an ortholog of the major GH2 beta-galactosidase identified in *A. nidulans*, and we speculated that this enzyme may play a crucial role in the lactose catabolism of *P. chrysogenum* as well. After growing *P. chrysogenum* wild-type cultures on lactose as sole carbon source for 48 hours, we extracted total RNA which was then used to create a cDNA for *Bga*D with RT PCR. cDNA was then ligated into vector pET 21a which contains ampicillin resistance and 6×His-tag regions. Ligated plasmids were then transformed into the host cells (*E. coli* BL21). *E. coli* cells were grown in LB medium (200 rpm at T = 30 °C). Expression of bgaD was induced with 1 mM IPTG. Wet cells were homogenized by sonication. The homogenate was centrifuged and the supernatant was purified with column chromatography. Clean supernatant was loaded onto a pre-equilibrated Ni–IDA column which, after binding of the enzyme, was washed and the enzyme was eluted with imidazole. The eluted fraction was further purified by 4-aminophenyl-beta-D-thiogalactopyranoside-agarose (affinity matrix for beta-galactosidase). The enzyme was eluted with sodium borate. We used Mg^{2+} and Na^{+} ions to prevent degradation of the enzyme. The recombinant beta-galactosidase (BGAD) was purified to homogeneity with a 28-fold purification and a recovery of 15 %. Its molecular mass was estimated to be 120 kDa by SDS–PAGE. BGAD exhibited maximum activity at pH 6.5 and at T = 45°C. Specific activity was 163 U/mg (1 unit corresponds to the release of 1 micromol ONP per minute at 30 °C).

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MOLECULAR AND PHYSIOLOGICAL COMPARISON OF TWO HYDROCARBON DEGRADING *RHODOCOCCUS* STRAINS

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Rhodococci are Gram+ rods belonging to the Nocardiaceae family of the order Actinomycetales. These ubiquitous bacteria can be commonly found in soil and water. Rhodococci have a remarkably large set of enzymes for decomposition of various chemicals. The rhodococcal cell surface is usually hydrophobic due to the mycolic acid moieties which are attached to the arabinogalactane part of cell wall. Consequently, these bacteria can easily attach to hydrophobic compounds/surfaces. A hydrocarbon degrader bacterium strain was isolated in our lab, which was identified as *Rhodococcus* sp. MK1. The whole genome of the strain was sequenced using SolidTM next generation sequencer. The reads were mapped on the genome of *Rhodococcus erythropolis* PR4 strain (NCBI database, NC_012490.1), which was previously recognized as the closest relative of

the isolated strain. The genome sequence is 6,9Mbp long and covers 79% of the reference genome. In silico analyis of the genomes disclosed that both strains contained genes of at least 34 monooxygenases and many other enzymes which might be involved in hydrocarbon biodegradation. A comparative whole transcriptome analysis of the MK1 strain was performed using cells grown on n-hexadecane and Luria-broth. Two-fold or higher change in relative transcription level was considered as significant. There were 986 genes which had elevated expression level in the culture grown on n-hexadecane. Transcriptional regulators, ABC transporters, enzymes involved in n-alkane oxidation and fatty acid metabolism had the most potent increase in expression. Seven monooxygenases had highly elevated expression under hydrocarbon degrading conditions; two of them were alkane-1-monooxygenases. Monooxygenase expression data were confirmed by RT-qPCR experiments on both *Rhodococcus* sp. MK1 and *Rhodococcus erythropolis* PR4. Hydrocarbon contamination may occur both in soil and water. Therefore, we tested the hydrocarbon degrading efficacy of the two *Rhodococcus* strains in three different niches: potting soil, saltwater medium and minimal medium. We kept track of respiration and hydrocarbon consumption in the three medium and calculated carbon and oxygen balance. Our results suggest that both strains are equally efficient hydrocarbon degrader in potting soil, however, the PR4 strain is more potent in water based niches. High salinity condition slightly decreases the hydrocarbon degradation rate of both strains as compared to hydrocarbon consumption on minimal medium. The carbon and oxygen balance estimations suggested that the hexadecane was dominantly converted into biomass.

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CROSS-SECTIONAL SURVEY ON SEROPREVALENCE OF HEPATITIS E VIRUS IN AUSTRIA

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Hepatitis E Virus (HEV) infection is an emerging global public health problem. The seroprevalences of HEV varies considerably in different parts of the world. The aim of the present study was to assess the prevalence of antibodies against HEV in healthy adult individuals in Austria and to identify possible exposure risks. Explorative nationwide cross-sectional seroprevalence survey. Setting: Serum samples and epidemiological data were obtained from adults volunteering for international military employments between April and September 2009 at the Military Hospital Vienna. Healthy adults (from all 9 federal states) of Austria between 18-60 years were included. HEV IgG antibodies were determined by ELISA. Variables surveyed included professional soldier or civilian, previous foreign military assignments, residential area, occupational animal contact and regular outdoor activities. Overall, HEV IgG was found in 143 out of the 1003 (14.3%) samples tested. The mean age of individuals who tested positive was 35 versus 28 years for those with negative screening results ($p<0.001$). Thus, seropositivity was significantly associated with increasing age. Regarding military activities, the proportion of seropositive samples was significantly higher among professional soldiers than among civilians (17% vs. 12.4%). Likewise, seroprevalence was significantly higher in individuals who participated in military employments abroad previously (21.4% vs. 9.9%). Our data showed an unexpectedly high seroprevalence in the Austrian adult population and indicate that military activities represent a significant exposure risk.

EXOTOXIN-PROFILING OF PANTON-VALENTINE LEUKOCIDIN POSITIVE MSSA VERSUS MRSA

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The pathogenicity of *S. aureus* depends on the synergistic interaction of bacterial virulence factors. Accordingly, the emergence of virulent strains is frequently associated with the acquisition of genes encoding diverse virulence factors. As such, the recent spread of CA-MRSA was found associated with the presence of Panton Valentine (PVL) toxin. However, the exotoxin profile of PVL-positive MRSA versus MSSA is not known. We analyzed a collection of 79 clinical isolates of PVL-positive MRSA and MSSA for the presence of genes encoding 22 exotoxins, including enterotoxins (ET) (sea - see, seg - ser, seu), toxic shock syndrome toxin (TSST)-1 (tst), exfoliative toxins (EF) (eta, etb) and hemolysines (HL) (hla) using PCR. Furthermore, MRSA genes (mecA, SCCmec) and agr-locus were investigated by PCR. In total we studied 61 MRSA and 18 MSSA strains. More than 90% of mecA positive MRSA harboured the SCCmec cassette (71% SCCmec IV and 21% SCCmec V), indicating the high incidence of CA-MRSA in the investigated collection. While agr2 was present in most MRSA and MSSA, agr4 was surprisingly only found in MSSA (56%), and agr3 was detected in 52% of either MRSA or MSSA. Overall, MRSA harboured significantly fewer toxin genes as compared to MSSA. This holds true for enterotoxins, among which the genes for seg, sei, sem, sen, seo, and seu (= enterotoxin gene cluster egc) were the most prevalent ones (on average 30% of MRSA and 95% of MSSA tested positive for respective genes). Rather surprising, and in contrast to published studies from PVL-negative MRSA, we only discovered 12% of PVL-positive MRSA to express enterotoxin A (sea). This exotoxin-profiling study revealed that the majority of PVL-positive *S. aureus* are CA-MRSA. Furthermore, the absolute number of expressed exotoxin genes was higher in PVL-positive MSSA versus MRSA, while 'common' MRSA-associated toxins such as enterotoxin A, were found in relative low frequency in PVL-positive MRSA.

APPLICATION OF DNA CHIP SCANNING TECHNOLOGY FOR THE AUTOMATIC DETECTION OF *CHLAMYDIA TRACHOMATIS* AND *CHLAMYDIA PNEUMONIAE* INCLUSIONS

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Chlamydiae are obligate intracellular bacteria causing wide array of human infections. They replicate in inclusion, a specific niche inside the host cell. The standard method for quantitation of chlamydiae is the immunofluorescence staining of infected cells and counting of chlamydial inclusions under fluorescent microscope. For testing the effect of anti-chlamydial compounds and drugs that positively or negatively influence chlamydial growth high or medium throughput estimation of the reduction in chlamydial inclusions should be available, yet low-throughput manual counting is the common approach. To replace the time-consuming and subjective manual counting

we developed an automatic inclusion counting system applying a commercially available DNA chip scanner. Inclusions in cells grown and infected in chamber slides were stained with chlamydial LPS-specific monoclonal antibody and fluorescently labelled secondary antibody and were detected by the scanner. The image was processed by ChlamyCount, a custom plug-in of the ImageJ software environment. ChlamyCount was able to count the inclusions over a one log dynamic range with high correlation with the theoretical counts. ChlamyCount was successfully applied for determination of minimum inhibitory concentration of the well known antichlamydial antibiotics moxifloxacin and tetracycline and also MIC of a novel antimicrobial compound. ChlamyCount was also able to measure the chlamydial growth altering effect of drugs that influence host-bacterium interaction such as interferon-gamma, DEAE dextran and cycloheximide. ChlamyCount is an easily adaptable system with a potential for high throughput testing antichlamydial antimicrobials and other compounds influencing *Chlamydia*-host interactions.

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DIFFERENTIAL DIAGNOSTICS OF MIXED HERPETIC INFECTION IN PATIENTS SUFFERING FROM IMMUNODEFICIENCY

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Epstein-Barr virus (EBV) and Human cytomegalovirus (HCMV) are the most common amongst the viral pathogens of human. EBV and HCMV infect at least 90% of the world population and can persist in a latent form after primary infection. Reactivation of both can occur years later, mainly under conditions of immunosuppression. For EBV and HCMV, transmission via sputum (saliva) was found typical enabling to cause infectious mononucleosis characterized by fever, pharyngitis and lymphadenopathy. Furthermore, EBV infection has been associated with undifferentiated nasopharyngeal carcinoma, endemic Burkitt's lymphoma, and immunoblastic lymphoma, and primary maternal infection with HCMV during pregnancy with fetal abnormalities and neonatal diseases. Recently, mixed EBV and HCMV infection was reported to be more common than envisaged to, especially in immunocompromised patients. In this study, we applied newly developed method allowing identify mixed EBV and HCMV infection in clinical materials taken from patients suffering from various forms of immunodeficiencies. Together 8 from 12 clinical materials of 4 patients we identified as positive for both EBV and HCMV using duplex PCR. All results were confirmed by virus-specific nested PCRs allowing semi-quantification of genome copy number of viruses. New differential PCR method makes possible effective and sensitive detection of as less as 3 and 30 genome copies of EBV and HCMV in sputum and buccal swabs. It contributes cost and time benefits improving sensitive diagnostics of mixed herpetic infections and the possibility to follow the treatment of patients with antiviral and/or immunomodulating drugs.

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VP4 AND VP7 SEQUENCE ANALYSES OF HUNGARIAN ROTAVIRUS STRAINS

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New generation rotavirus vaccines proved efficient in prevention of severe acute dehydrating gastroenteritis in several countries worldwide. Monitoring of circulating rotavirus strains is essential to evaluate the impact of vaccine use on local strain diversity and virus evolution. Rotavirus positive stool samples were collected from patients with gastroenteritis in Hungary between 2007-2011. After genotyping by multiplex RT-PCR assay, sequencing and phylogenetic analysis of the genes encoding the surface antigens of selected field strains was performed to monitor the genetic relatedness of rotaviruses. Common rotavirus strains detected in different geographical regions and years were not closely related to the VP4 and VP7 genes of rotaviruses included in Rotarix® and RotaTeq® vaccines. Furthermore, amino acid changes in antigenic regions of surface proteins were determined during sequence comparison of community and vaccine strains. However, G1 and P[8] types closely related but not identical with Rotarix® parental strain were determined, their vaccine origin was unlikely. Enhanced nationwide rotavirus strain surveillance identified large antigenic diversity in Hungary after rotavirus vaccines became available for use during 2006 and 2007. Due to the low vaccine coverage in Hungary we found no evidence for an impact of vaccine use on genotype distribution. A better understanding of the long-term effect of vaccine use on epidemiology and evolutionary dynamics of co-circulating wild type strains requires continuous strain surveillance.

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THE FIRST RESULTS OF THE STUDIES ON THE BACTERIAL COMMUNITY STRUCTURES OF THE INTESTINAL TRACTS OF THE ASIAN CARPS LIVING IN LAKE BALATON

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Since the introduction of carps nearly 30 years ago, silver (*Hypophtalmichthys molitrix*) and bighead (*Hypophtalmichthys nobilis*) carps and their hybrids occur in large numbers in Lake Balaton. They are of high meat yield, and constitute an important component of fish populations owing to their consumption of zooplankton and organic detritus. However, little information is available about their nutritional habits. In the present study, bacteriological examinations of fore-, mid-and hindgut-section of the intestinal contents of three carps caught in Lake Balaton in September 2011 were performed using cultivation-based and molecular biological methods. An average of 10^{7-8}/g CFU values was determined from the intestinal contents independently of the composition of the medium used. Among the strains isolated from the silver carps and identified according to their 16S rDNA sequence analysis, members of species *Aeromonas veronii*, *Brevundimonas vesicularis*, *Micrococcus endophyticus* and *Bacillus halmapalus* were the most abundant. From the midgut section, representatives of *Aeromonas veronii*, *Micrococcus yunnanensis* and *Roseomonas mucosa*,

while in the hindgut section, *Acinetobacter lwoffii*, *Paracoccus yeei* and *Lactococcus garvieae* were identified frequently. Analysis of band patterns of 16S rRNA gene sequences, obtained from the examination of the carp intestinal contents by denaturing gradient gel electrophoresis, indicated that bacterial community structures of the fore- and midguts from the same fish showed higher similarities than those of the hindguts. Hindgut bacterial communities derived from different individuals formed a distinct similarity group.

NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* IN CHILDREN ATTENDING DAY-CARE CENTERS IN HUNGARY

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Children attending communities are well known carriers of several pathogenic bacteria. As nasopharyngeal colonisation of these bacteria can lead to the development of severe diseases in the carrier itself or in susceptible contacts (e.g. grandparents or immunocompromised persons), it is very important to explore the carriage rate. In this study we focused on *Staphylococcus aureus* and, as the emergence of community-acquired MRSA is an alarming problem all over the world, we have extended the examinations to the detection of this. We have also studied the dual carriage of *S. aureus* and *Streptococcus pneumoniae*. 879 children (aged 3-6 years) were screened in 21 DCCs between February 2009 and December 2011 for the nasal carriage of both pathogens. The identity of S. aureus isolates was confirmed by colony morphology, catalase test, Pastorex test (Bio-Rad) and nucA PCR. MRSA strains were screened by mecA PCR. The sensitivity to 9 antibiotics was determined by agar dilution or E-test, applying the EUCAST breakpoints. The genetic relatedness of the strains was examined by PFGE. Among the 879 children, 187 carried *S. aureus* (21,3%). Although 90,7% of the isolates were resistant to penicillin, luckily all of these were susceptible to oxacillin and we could not detect the mecA gene either. All the strains were sensitive to TMP/SMX and mupirocin, while the highest resistance was observed in the case of erythromycin (6,8%). The resistance rates for ciprofloxacin, clindamycin, gentamicin and tetracycline were 0,6%, 0,6%, 3,7% and 4,9%, respectively. Based on the PFGE patterns the strains showed a relatively high diversity. Among the 187 children who carried *S. aureus*, pneumococcus was also present in 53 cases (28,3%). This equals to 6,0% dual carriage in the whole population. The serotype distribution of these pneumococci was as follows (number in brackets): 19F (10), 15B (7), 38 (7), 6A (6), 14 (4), 3 (3), 6B (3), 11A (3), 13 (3), 23F (3), 22F (2), 28F (1), 34 (1). The pneumococcus colonisation rate was 37,0% (325/879 children), and 16,3% of them were also *S. aureus* carriers. The carriage rates correlate well with international data. Interestingly, at this young age, pneumococcus carriage rate exceeds *S. aureus* carriage, while the later remains high also in adulthood (29,3% in our previous study with university students). Dual carriage is typically around 3-6% according the literature. The penicillin and gentamicin resistance of the isolates is the same as observed among the clinical isolates (national surveillance data), but for the other antibiotics it is much higher among the clinical strains (e.g. erythromycin resistance is ~18% in outpatients and ~29% in inpatients). Especially ciprofloxacin resistance is low in carriage compared to disease-causing strains.

MRSA is still very rarely found in the community in Hungary. The serotypes of the pneumococci involved in dual carriage would be covered by the pneumococcal vaccines in 34,7% (Prevenar 7), 54,7% (Prevenar 13) and 66,0% (Pneumovax).

EPIDEMIOLOGICAL SURVEY AND CHARACTERIZATION OF *STREPTOCOCCUS AGALACTIAE* ISOLATES FROM THE SEMMELWEIS UNIVERSITY, BUDAPEST

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Streptococcus agalactiae (GBS, group B streptococcus) is one of the most important causes of neonatal sepsis and meningitis. The infection of newborns originates mostly from maternal colonisation, in the vagina and the lower gastrointestinal tract. Moreover, GBS has recently been more frequently associated with infections in non-pregnant adults, especially with some underlying disease such as diabetes. We have surveyed 205 GBS isolates collected between 2008-2011, 115 of these originating from pregnancy screening (mothers and/or newborns), and 90 from other adult infections. After the thorough identification of the isolates, we determined their antibiotic sensitivity with plate dilution, and in the case of macrolide resistant strains, we also detected the *erm* and *mef* resistance genes. We identified the serotypes of the strains with antisera and PCR, and we detected the presence of the major surface proteins (alpha-C, rib, alp2, alp3, and epsilon). At the genotypic level, we identified the ST-17 and ST-23 hypervirulent clones, and to refine the genetic relatedness of the first clone, we used the PFGE method. Regarding the antibiotic susceptibility, luckily all isolates were fully penicillin sensitive. On the other hand, macrolide resistance proved to be a major problem: 24,4% of the isolates showed the MLSB phenotype (i.e., resistant to both erythromycin and clindamycin), these all carried the *erm*B gene, as expected. Moreover, 31 strains carried the *erm*B gene, but was phenotypically macrolide sensitive. Seven strains had the *mef* gene. The majority of the isolates were serotype III (31,2%), followed by types V and Ia (22,5% and 20,5%). We could identify a single serotype VI strain, but no VII or VIII were found. The serotypes showed strong correlation with the presence of surface proteins: rib was associated mostly with type III, alp2/3 with type V, while epsilon with type Ia. Strikingly, the vast majority (65,4%) of the strains belonged to the ST-23 clone (mainly serotype V, but also Ia, Ib, II, III and IV), and in 31,7%, ST-17 was identified (these were always serotype III). Within the ST-17 clone, PFGE analysis could identify 4 smaller clusters. In summary, with this study we could hopefully contribute to the better understanding of the epidemiology of *S. agalactiae* in Hungary, and so, enhance the introduction of obligatory pregnancy screening. As 97,1% (199/205) of the isolates belonged to either the ST-17 or the ST-23 clone, we suggest that detection of these clones should be included in the screening. Additionally, as in case of penicillin allergy, clindamycin is given as intrapartum antibiotic prophylaxis, susceptibility testing should always be routinely performed.

VANCOMYCIN RESISTANT ENTEROCOCCI ISOLATED IN UNIVERSITY OF SZEGED ALBERT SZENT-GYÖRGYI CLINICAL CENTER DEPARTMENT OF CLINICAL MICROBIOLOGY

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Vancomycin-resistant enterococci have emerged as important nosocomial pathogens in the past two decades all over the world. The firts vancomycin resistant enterococci was reported in 1988 from

Great Britain. For this study we used the statistical datas of our laboratory. For the screening test of the vancomycin resistant enterococci we used Mueller-Hinton agar supplemented with vancomycin 2 µg/ml and teicoplanin 2 µg/ml since 2005. The antibiotic susceptibility tests were determined according to EUCAST. The strains were identificated with MALDI-TOF MS. We have found the first VRE isolate in september 2012. The second VRE was isolated in march 2013. Until 31. July 2013 we isolated VRE from 16 patients. Exept one *E. avium* isolate all of the isolate were *E. facium*. 7 times we could isolate the VRE from wound, 3 times from blood culture, 1 times from drain, 5 times from urine. The *E. avium* was isolated from blood culture. All of the strains were sent to the National Center for Epidemiology (Budapest) for further characterization. All of the strains found to be *van*A positive. The *E. faecium* isolates were resistant to all antibiotics except linezolid, quinopristin-dalfopristin and tigecyclin. In Hungary more VRE cases were reported in the last decade. These strains were isolated from different part of the country. Though we used the screening agar since 2005 we could isolate the first VRE only in september 2012. In this year until the end of July we could isolate VRE from 15 patients. The patients were treated in different departments of the clinical center. We attribute increase of VREs to the use of vancomycin to the treatment of increasing *C. difficile* infection in the clinical centre. This warns us to use antibiotics prudently.

EFFICACY STUDIES ON TARGET ANIMALS BEFORE REGISTRATING A NOVEL CLASSICAL SWINE FEVER MARKER VACCINE

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The Classical Swine Fever Virus (CSFV) is a highly virulent pathogen of domestic swine (*Sus scrofa domestica*) and wild boars (*Sus scrofa*). The Classical Swine Fever (CSF) is a severe disease; it results in great economic losses. The wild boar, as a reservoir of the virus, plays a major role in the epidemiology of the disease, since it endangers the domestic swine populations kept in the same area. In cooperation with different European research teams the Immunological Department of the National Food Chain Safety Office, Directorate of Veterinary Medicinal Products took part in the Seventh Framework Project (CSFV_goDIVA, KBBE-227003) of the European Union (EU). The aim of this project was to create a DIVA vaccine for oral immunisation of wild boars against CSF. For the authorisation, the efficacy and the safety of the immunological veterinary medicinal product shall be tested on the target species according to the relevant Monograph of the European Pharmacopoeia (Ph.Eur.). Our presentation is a detailed summary of the efficacy studies performed by our Directorate. The aim of these studies was to confirm protection provided by the vaccination against challenge with a highly virulent CSFV strain and to determine the onset of immunity. Three animal studies were carried out in our animal facility. Two studies were done on 6-week-old domestic piglets with and without maternally derived antibodies (MDAs) against CSFV. In both cases three groups were formed: two vaccinated (i.m., p.o.) and a control group. 14 days after vaccination all the animals were challenged with the highly virulent CSFV strain 'Koslov'. The studies were finished three weeks after challenge. Blood and organ samples were collected for laboratory analysis. The third study was done on pregnant domestic sows in order to examine the protection against transplacental infection. The animals were vaccinated at 40-50 days of their

gestation and were challenged 14 days later. Before farrowing the state of the foetuses of the slaughtered sows was checked. The vaccine protected the animals against challenge and proved to be effective. The dossier is applied in February 2013.

NUCLEO-CYTOPLASMIC SHUTTLING DYNAMICS OF THE TRANSCRIPTIONAL REGULATORS XYR1 AND CRE1 UNDER CONDITIONS OF CELLULASE AND XYLANASE GENE EXPRESSION IN *TRICHODERMA REESEI*

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Trichoderma reesei is a model for investigating the regulation of cellulase and hemicellulase gene expression. Cellulases are formed adaptively, and the transcriptional activator XYR1 and the carbon catabolite repressor CRE1 are the main regulators of their expression. Here we have quantified the nucleo-cytoplasmic shuttling dynamics of both transcription factors under cellulase and xylanase inducing conditions, and correlated their nuclear presence/absence with changes in gene expression. We also compared their subcellular localization patterns in conidial germlings and mature hyphae. We show that cellulase expression requires de novo synthesis and simultaneous nuclear import of XYR1, whereas carbon catabolite repression is regulated through preformed CRE1 imported from the cytoplasmic pool. Termination of induction immediately stopped cellulase transcription, but caused only very slow removal of XYR1 from nuclei. In contrast, CRE1 was rapidly exported upon glucose depletion. In mature hyphae, nuclei containing activated XYR1 were mainly present in the central colony areas, indicating that this is the main region of XYR1 synthesis and cellulase transcription. CRE1 was found to be distributed throughout the entire mycelium. Taken together, our data have revealed novel aspects of the dynamic shuttling and spatial bias of the major regulator of cellulase and hemicellulase gene expression, XYR1, in *T. reesei*.

DETERMINATION OF THE OPTIMAL CONCENTRATION OF IFN-Λ1 FOR THE INHIBITION OF HUMAN HEPRESVIRUS-1 REPLICATION IN VERO CELLS

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Interferons (IFNs) were discovered as antiviral agents during studies on virus interference by Alick Isaacs and Jean Lindemann in 1957. Interferons lambda (IFNs- λ), a new group of interferon family, were brought to light by Kotenko and Sheppard in 2003 and named IFN-λ1 (IL-29), IFN-λ2 (IL-28A) and IFN-λ3 (IL-28B). Although it is 10 years after the discovery, their therapeutic potential isn't precisely defined. The aim of this study was to examine of IFN-λ1 antiviral activity against medically important strains of human herpes virus-1 (HHV-1 K17, KOS, ANGpath and HSZP): whether it is significant and under what conditions. We examined of IFN-λ1 antiviral activity against different strains of HHV-1 by determination of decrease in virus titer during presence of different concentrations of IFN-λ1. Mammalian Vero cells were, before viral infection, preincubated with different concentrations of IFN-λ1 (10, 15, 20, 25, 30 and 35 ng/ml). At interval, 20 hours post infection (p. i.), the viral titer was compared to the control virus titer. The most significant antiviral

effect of IFN-λ1 against HHV-1 HSZP was demonstrated for MOI 0,01 at concentration of 25 ng/ml, when the titer was reduced 1,5-times. At concentrations 15 and 35 ng/ml, the titer of HHV-1 KOS was reduced 1,1-times. Using the 35 ng/ml of IFN-λ1 the titer of HHV-1 ANGpath was reduced 1,02-times. Using these concentrations of IFN-λ1 and MOI 0,01 the titer of HHV-1 K17 was not reduced. For the MOI 3, the most significant antiviral effect of IFN-λ1 against HHV-1 K17 was observed at concentration 35 ng/ml, when the titer was reduced 1,07-times. At concentration 30 ng/ml of IFN-λ1, the titer of HHV-1 HSZP was reduced 1,03-times. In case HHV-1 KOS and ANGpath, no inhibition effect of IFN-λ1 was detected. This probably means that proteins with antiviral activities which were synthesized in host cells after the interferon stimulation were not in sufficient concentration, thus were not able to inhibit large numbers of infectious viruses. Additional experiments will be aimed to examine the inhibition effect of IFN-λ1 at the determined optimal concentrations for single-stage (MOI 0,01) and multi-stage (MOI 3) replication curves of HHV-1.

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DEVELOPMENT OF A SOLID STATE FERMENTATION TECHNOLOGY ON RAPESEED CAKE AND DDGS SUBSTRATE FOR XYLANASE ENZYME PRODUCTION

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Dried distillers grains and solubles (DDGS) and rapeseed oil cake (ROP), a byproduct of biofuel production, have a relatively high fibre content, which limits its usability in animal feeding. Increasing DDGS and rapeseed cake production has entailed a rising demand for a technology that makes it digestible for monogastric animals. ROP and DDGS are potential substrates for microorganisms thus microbial conversion is an option to improve their digestibility. The fiber content can be decreased by hemicellulase-producing microorganisms. *Thermomyces lanuginosus*, a thermophilic filamentous fungus produces high levels of ß-xylanase in solid-state fermentation (SSF). In our laboratorial trials *T. lanuginosus* was cultivated on solid media containing DDGS or ROP in different ratios. During the SSF medium was supplemented with a water activity reducing substance (ALM: agroindustrial lignocellulose material) or wheat bran to enhance the microbial growth and enzyme production. The SSF process was optimized to enhance the highest xylanase, protease and phytase activity of the cultures. According to the results the *T. lanuginosus* cultures were incubated on ROP substrate for 7 days for the highest enzyme production. Samples dried on 50°C and xylanase, phytase and protease activity were measured after one week. Our results show that xylanase activity was 5200 FXU/g, protease activity was 11,2 U/G and phytase activity was 0,667 nkat/mg protein. *T. lanuginosus* cultures were incubated on DDGS substrate for 12 days for the highest enzyme production. Xylanase activity of the DDGS cultures was 10590 FXU/g and protease activity was 6,2 U/G. Our results indicate that culture of *T. lanuginosus* produce on ROP and DDGS containing medium significant enzyme activity in solid state fermentation. These enzyme preparations can effectively increase the digestibility of DDGS and ROP in animal feeding.

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PRODUCTION OF ENZYME GRANULATES TO ENHANCE THE DIGESTIBILITY OF THE BIOFUEL BYPRODUCTS DDGS AND RAPESEED CAKE

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Dried distillers grains and solubles (DDGS) and rapeseed cake, a byproduct of biofuel production, are commonly used in animal feeding. Increasing DDGS and rapeseed cake production has entailed a rising demand for a technology that makes it digestible for monogastric animals. In our experiments, enzymes were produced on media that contained different amount of DDGS or rapeseed cake. In the case of DDGS, low quality, dark brown DDGS was used for the higher enzyme production. Thermomyces lanuginosus were cultured on the byproduct containing medium for 24 hours. Highest protease activity was measured in the medium contained 4% DDGS. In the case of rapeseed cake supplementation, optimal ratio of the byproduct was 8% to achieve the highest enzyme activity. After the incubation period the liquid enzyme solutions were used for granulation. Conservation of the enzyme product was carried out via granulation process. During could extrusion and spheronization trial were developed the formulation process. Specified additives were added to the granulated materials in defined amount to achieve the proper shape. The fermentation and granulation process were optimized to enhance the enzyme activity and the efficiency of formulation. With a compression technology the concentration of the enzymes was increased in the final product. Scale-up studies were made to develop semi-industrial processes of the fermentation and granulation. The procedure was successfully adjusted to the field conditions.
According to our results, fermentation and granulation process of a protease enzyme product were developed under laboratorial and semi-industrial circumstances.

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THE EFFECT OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) SUBSTRATE COLONIZATION ON THE BACTERIAL COMMUNITY STRUCTURE INVESTIGATED IN A MODEL SYSTEM

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The importance of oyster mushroom (*Pleurotus ostreatus*) cultivation is increasing in Europe due to its high nutritional value. To maintain its permanent high-quality it is essential to get deeper knowledge about the process of cultivation and its influencing factors such as physico-chemical parameters and microbial community structure of mushroom substrate. Our earlier studies revealed a marked succession in the bacterial community during the substrate preparation by sequence-aided Terminal-Restriction Fragment Length Polymorphism (T-RFLP) based on 16S rRNA gene. Nevertheless the second part of the cultivation - in which the substrate is inoculated with *P. ostreatus* spawns, its hyphae colonize the substrate and the growth of fruiting bodies begins - is less investigated from this aspect. The aim of this study was to monitor the structural changes of the bacterial community during hyphae colonization and to identify the characteristic taxa of the mature and the colonized substrate. A laboratory model system was set up: mature substrate was filled into tubes, inoculated with oyster mushroom spawn at one end, and incubated at 26°C, 65% RH for one

month. The starting substrate material was sampled and slices were taken from the uncolonized regions, the regions of fronthyphae, the 1, 2 and 3 week-old parts and from the uninoculated control tubes. The community DNA was isolated and examined by T-RFLP and next generation sequencing techniques on the basis of 16S rRNA gene. Results of T-RFLP showed a considerable difference among the uncolonized and colonized samples. The most characteristic bacteria of the mature substrate belonged to Actinobacteria and *Thermus* spp. After 3 weeks of hyphae colonization these groups almost completely disappeared while a new taxon, *Halomonas* spp. were found. The most prominent alteration is that the members of Firmicutes (*Bacillus*, *Paenibacillus*, *Thermobacillus*, *Brevibacillus*, *Planococcus*, *Geobacillus* spp.) became dominant in the 3-week-old part. Though, this taxon was also present in the mature substrate, its relative abundance and diversity highly increased. A further aim is to test the hypothesis explaining this phenomenon.
It is presumed that the growth of the hyphae can have unfavourable effect on some of the bacteria, due to this the ratio of spore-forming species may increase.

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A STRANGE LOCALIZATION OF HYDATID CYST AND ASSOCIATION WITH UROLOGICAL PATHOLOGY

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Human cystic echinococcosis is a public health problem in many countries of Europe, like Romania and Bulgaria, but is also a reemerging problem in developed countries due to ceasing of control programs. The aim of this paper is to present the case of a hydatid cyst associated with a urological pathology and with a rare localization: in the pelvis behind the urinary bladder. We present the case of a 55 year old man who presented at the urology ward for gross hematuria. He was also known for 15 years with splenic hydatid cyst treated by open surgery. The patient did not receive preventive therapy with albendazole. At the urological and ultrasound examination we found a tumoral bladder formation localized at the base of the left bladder wall, with the dimensions of 2x1.5 cm. Under the bladder an inhomogeneous tumoral formation was described with multiple septa and transonic zone; CT was performed highlighting a large pelvic hydatid cyst. The case stated a serious issue: which tumor presented the first indication of surgical treatment? Together with the general surgeon the decision was taken to first treat the tumoral formation of the bladder by transurethral resection (TUR-V). An open surgery was performed to treat the hydatid cyst. Before surgery, the patient received treatment with Albendazol 400mg per day for one month. From the hydatid cyst an important number of daughter vesicles were extracted. Our patient presented no recurrence or side effects after the operations and the medical treatment, but follow-up continues. Despite an efficient surgical treatment for spleen hydatid disease, recurrence occurred in a strange localization. No liver hydatidosis was detected in this patient. In this case the presence of a simultaneous bladder tumor determined the gross hematuria and the pelvic hydatid disease was discovered accidentally.

IN MEMORIAM TIBOR DEÁK: YEASTS IN SCIENCE AND TECHNOLOGY

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Tibor Deák, Professor emeritus at the Department of Microbiology and Biotechnology, Corvinus University of Budapest, Hungary passed away on March 3, 2013 in his age of 77 years. Dr. Deák received his B.Sc. degree in biology and chemistry from the University of Szeged in 1957, and his M.Sc. degree in microbiology from the Eötvös University of Budapest in 1963. He received his Ph.D. and D.Sc. degrees in biological sciences in 1970 and 1989, respectively, from the Biology Section of the Hungarian Academy of Sciences, Budapest. After gaining food technological experience, he was appointed assistant professor in microbiology at the University of Horticulture and Food Science in 1967. He became associate professor in 1970 and full professor in 1980. He was the head of the Department of Microbiology and Biotechnology from 1970 to 1996, and served two terms as dean of the Faculty of Food Science (1986–1991) and one term as the rector of the university (1993–1996). He was an active member of the Hungarian Microbiological Society and has got the Manninger Award in 1991. He was a board member of numerous international scientific societies including the International Committee for Food Microbiology and Hygiene, the International Committee for Yeast, the International Committee for Food Mycology and was member of the World Federation of Culture Collection, European Culture Collection Organization. He was worked in the editorial board of the International Journal of Food Microbiology and FEMS Yeast Research. Dr. Deák is author or co-author of more than 330 research papers and 24 books and chapters, as well as 15 textbooks and manuals. His continuing research interests included the food microbial ecology, biodiversity of yeasts in the agrifood-ecosystems, yeasts as spoilage agents in foods and beverages, and yeast detection and identification. In recognition of his contributions to yeast research a new yeast species *Ogataea deakii* Peter, Dlauchy & Čadež is being named after him. This lecture will outline the role and biodiversity of yeasts in the natural and agrifood ecosystems emphasizing the unique spoiling potential of several yeast species. Special attention will be paid to the stress tolerance and stress adaptation of yeasts by introducing the signal transduction routes in the model yeast species *Saccharomyces cerevisiae*.

SNAPSHOTS ON THE MICROBIOLOGY OF DRINKING WATER PRODUCTION AND SUPPLY

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Today it is common place to discuss the strategic importance of drinking water bases. To come at drinking water is an increasing challenge at numerous places in the World. What concerns Hungary, the hydrogeographical and hydrogeological circumstances are favourable for safe drinking water production. However increasing demand, tightening official regulations, standards, and pollutant threshold limits, together with the threat of industrial and agricultural pollutions force continuous development. Concerning drinking water microbiological control, in the past more than half century the use of traditional culture based methods, and the detection of faecal indicator bacteria seemed to be adequate. The description of the „plate count anomaly", the detection of nitrification in some drinking water distribution systems, and several other phenomena (e.g. biofilm formation) warned to the weaknesses of these methods. On the other hand the use of the nucleic acids based molecular techniques in drinking water microbiology revealed a hidden biodiversity. Using the methods of the traditional culture based, and the novel molecular microbiological techniques, the water bases, raw drinking water production, water distribution and tap water quality was investigated in the case of

two Hungarian water works. One of them is based on river bank well filtration, the other on deep wells. Both water bases and technologies are characteristic in Hungary. In both cases, excepting the raw water, the culture based standard methods resulted practically no CFU values. On contrary to this, on the surface of non standard oligotrophic media 10^1-10^4 CFU/mL values were obtained. This phenomenon draws the attention to the possibility of the presence of an autochthonous microbiota in drinking water. By using different molecular techniques a broad scale of bacteria could be detected. The deep interrelation of the water chemical parameters and the metabolic types of bacteria together with characteristic shifts along the production, delivery and distribution systems proved the presence and activity of autochthonous communities. The analysis draws the attention to the deficiencies of the traditional drinking water hygienic investigations, and stresses the need for the development of novel microbiological drinking water quality control methods.
One can even ponder on the application of drinking water supply system adapted special microbiological warning methods, besides the use of novel standard techniques.

CITRININ-INDUCED UNBALANCED OXIDO-REDUCTION STATE OF *SCHIZOSACCHAROMYCES POMBE* CELLS

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In this study the citrinin-induced (CTN) accumulation of reactive oxygen species (ROS) and the regulation of the specific activities of antioxidant enzymes were investigated in the single cell eukaryotic *Schizosaccharomyces pombe* in acute (60 min) toxicity tests. The minimal inhibitory concentrations (250 μM at pH = 4.5) showed pH-dependence. The saturation of the uptake of CTN by protoplasts and vegetative cells occurred at 5 min and 20 min, respectively. Up to these time points 35% and 58% of the CTN was taken up by vegetative cells and protoplasts, respectively. In comparison to the control, 1000 μM CTN exposure of 107 cells ml-1 at pH = 4.5 induced significantly ($p<1\%$) elevated level of hydrogen peroxide and total ROS but not of superoxide, and hydroxyl radical, as well as 3-fold increase in the concentration of glutathione. In comparison to parental strain, 250 μM CTN-exposure resulted in 27.1% and 52.4% decrease in the growth yield of Δ*atf*1 and Δ*pap*1 strains, respectively suggesting the unbalanced redox state of CTN-treated cells that of regulation determiningly is done by Pap1 transcription factor moreover activation of Atf1 factor would be necessary for cells to cure the CTN-induced oxidative stress. ROS-induced adaptation processes at cell and molecular level via activation of the transcription factors resulted in significantly increased specific activities of glutathione peroxidases, glucose-6-phosphate dehydrogenase and glutathione S-transferase as well as decreased level of catalase, glutathione reductase but no detectable changes were measured in the activities in the superoxide dismutases. Significant decrease (from 85.01% to 77.82%) in the G1 phase was observed after 1 hour treatment while the percentage of the cells in the G2/M phase was increased (from 11.18% to 16.62%) significantly. Comparison of results with the well-characterized positive control (200 μM H_2O_2), CTN induced mid-level oxidative stress. Cells strived to correct the unbalanced redox state by regulation of the antioxidant system, but this was not enough to defend the cells from the cell cycle arrest, and fragmentation of the nuclei (2.2-fold and 3.1-fold increase in nuclear fragmentation of 1000 μM and 2000 μM CTN-treated cells, respectively) which is one of the markers of apoptosis.

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SIDEROPHORES PRODUCTION BY SOME SOIL MICROBIAL INHABITANTS AND THEIR IMPACTS ON *RHIZOBIUM*-BEAN SYMBIOSIS

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Microbial siderophores play an important role in the biocontrol of some soil-borne plant diseases and in plant iron (Fe) nutrition. Microorganisms elaborate a variety of low molecular-weight, high-affinity chelating agents that solubilize Fe^{3+} ion in the environment and transport it into the cell is termed as siderophore. Microbial siderophore may stimulate plant growth directly by increasing Fe availability in soil surrounding the roots or indirectly by competitively inhibiting the growth of plant pathogens with less efficient Fe-uptake system. The objective of the present study was to investigate the ability of different rhizosphere microbial inhabitants to potentially produce siderophores and their impacts on growth, nodulation and N_2-fixation of common bean. Seventy-six microbial inhabitants were isolated from common bean rhizosphere and tested for their ability to produce siderophore using four various chemical assays ($FeCl_3$ test, chrome-azurol sulphonate and chrome-azurol sulphonate agar plate test). From these inhabitants, 38 were strongly and 21 moderately positive on chrome-azurol sulphonate agar plate assay (CAS). However, they showed no reaction with catecholate (phenolates) assay. All bacterial isolates failed in citrate production except five *Rhizobium phaseoli* strains, while two strains of *Pseudomonas aeruginosa* and seven strains of *P. fluorescens* three *Bacillus subtilis*, two *B. mycoides* showed strong reaction, among positive bacterial isolates, and four strains of *Trichoderma* spp with hydroxamate assay. Two *Aspergillus niger*, two *A. flavus* and two *Penicillium chrysogenum* produced moderate and strong reactions with the hydroxamate and citrate assays, respectively. Hydroxamate siderophore production was proportional with Fe concentration up to 25 μM with *R. phaseoli* and *B. mycoides* and 50 μM with *P. fluorescens* and *B. subtilis*. However, maximum siderophore production peak was achieved by *A. nidulans* and *P. chrysogenum* at concentration of 80 μM of Fe. Glucose proved to be the most suitable carbon source for *P. aeruginosa*, *A. nidulans* and *P. chrysogenum*; whereas, *R. phaseoli* gave its highest yield when grown on mannitol. Siderophore synthesis was inhibited by 2,4-dinitrophenol, NaN_3 and $MgCl_2$ suggesting that an energized membrane and a (OH)- group are essential and required for hydroxamate synthesis. The siderophore-producing *P. chrysogenum* and *P. aeruginosa* significantly enhanced nodulation and N_2-fixation of common bean compared to plants infected with *R. phaseoli* strain alone. More siderophore was produced at 28°C than at 37°C. The ecological advantages in the synthesis of microbial siderophore encourage the use of such microbiomes as inoculants with root-nodule bacteria.

MOLECULAR EPIDEMIOLOGY OF ERTAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE* CLINICAL ISOLATES AND EXPRESSION ANALYSIS OF GENES INVOLVED IN ANTIBIOTIC RESISTANCE

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Klebsiella pneumoniae is an important human pathogen, and causative agent of nosocomial infections. Clinical strains of *K. pneumoniae* can acquire antibiotic resistance to many commonly used beta-lactam antibiotics, including carbapenems, which are considered as one of the last resort antibiotics used for the treatment of multi-drug resistant (MDR) bacteria. The spread of MDR strains is a serious threat for public health. Therefore, it is important to use molecular typing of bacteria to trace the spread of MDR strains, especially in the hospital environment. We used pulsed field gel electrophoresis (PFGE) to determine the relatedness between ertapenem-resistant clinical isolates of *K. pneumoniae*, and reverse transcription quantitative polymerase chain reaction (RT-qPCR) to determine the expression of several genes coding for efflux pump components and porin channels in order to investigate the mechanisms of resistance to carbapenems and tigecycline in *K. pneumoniae*. The total of 70 ertapenem-resistant clinical isolates of *K. pneumoniae* was collected from different departments of Clinical Hospital Rijeka, Croatia during 2 years period. Antibiotic susceptibility to imipenem, meropenem, doripenem, tigecycline, colistin, ciprofloxacin, cefotaxime, and ceftazidime was determined by disk diffusion and E-test. All isolates were analyzed by PFGE after macrorestriction with Xba I restriction enzyme. PFGE data was analyzed by BioNumerics GelCompar II software, and dendogram showing genetic relationship between different isolates was created. RNA was isolated, and reverse transcribed from ertapenem-resistant isolates that were also resistant to tigecycline, and those that showed reduced susceptibility or were resistant to other carbapenems (imipenem, meropenem or doripenem). Resulting cDNA was analyzed by RT-qPCR to determine if there is a difference in the expression of genes for efflux pump acrAB (*acr*A), outer membrane component of efflux pump tolC, and two porin channels, ompK35 and ompK36 between samples resistant to tigecycline, or other carbapenems, and control strains that were susceptible to all antibiotics tested. All ertapenem-resistant isolates showed high-level resistance to broad-spectrum cephalosporins and ciprofloxacin, and eight isolates were also resistant to tigecycline, while five isolates showed reduced susceptibility or were resistant to at least one of the other carbapenems. All isolates were susceptible to colistin. Typeabilty by PFGE was 100%. All but one out of 70 isolates (98,6 %) belonged to the same pulsogroup based on >80% similarity criterion, which implies that the isolates are the result of epidemic spread of single strain through the hospital. We did not find any statistically significant difference in the expression of *acr*A and *tol*C genes between resistant isolates and control group. However, we found significantly reduced expression of gene for ompK35 porin in isolates resistant to tigecycline compared to control strains, which implies that the downregulation of ompK35 porin might be one of the mechanisms of tigecycline resistance in *K. pneumoniae*.

COMPARING A HARVESTING METHOD TO DIFFERENT MICROALGAE STRAINS

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Microalgae are both technologically useful and problematic: they can provide several potential advantages for biofuel production when compared with traditional crops, but also can adsorb on surface of facilities resulting technological limitations for example in heat transfer or filtration fluxes. These two different characters need different approaches, but they also have connections. For studying algae-connected technological issues, we have to cultivate corresponding strains in larger amount to provide biomass in remarkable amount, then to efficiently harvest them, and finally to test their adsorptive characters. In algae production technologies, due to the dilute nature of microalgal cultures, biomass harvesting represents a major obstruction to large-scale production of algae-based biofuels. Evaluation of several harvesting methods showed that the most promising cost and energy efficient dewatering strategy will most likely utilize microalgal flocculation as an initial concentrating step. During flocculation, the dispersed microalgal cells aggregate and form larger particles with higher sedimentation rate. The purpose of this work was to adapt harvesting method developed at University of Minho to different algae species (*Chlorella* sp., *Nannochloropsis* sp.) grown at Budapest University of Technology and Economics. Since algae are not a phylogenic (taxonomic) group of organism, they are very different in their cell surfaces, sizes and other characters, so it is not obvious to apply the same harvesting method for different strains. Thus the developed method was first adapted the further investigated corresponding to the different cell lines.

PHYLOGENETIC ANALYSIS OF THE FISSION YEAST CELL SEPARATION GENE, *SEP*15/*MED*8

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The *Schizosaccharomyces pombe*, also known as fission yeast, became a popular model organism in the second half of the 20th century. It is widely used in cell biology, genetics and microbial physiology for its favorable physiological attributions. We examined the fission yeast *sep*15 gene, which has two functions. It plays a role in cytokinesis by taking part in the cell separation cascade control, and it was identified as the first Mediator homolog in *S. pombe*. The gene encodes the Med8 protein, a subunit of the mediator complex, which is required for transcription of almost all RNA polymerase II-dependent genes. While the gene is conserved in most living organisms, we focused our search on the kingdom Fungi. We used the NCBI and the Broad protein databases for homolog searching, and found orthologous proteins in several species. For later analysis we used the sequences of only 59 species. We made reciprocal BLASTp analysis (searching within the *S. pombe* genome) with the found sequences in order to prove the relation to the fission yeast protein. We used the Pfam database to analyze the protein domain structure of the putative orthologs. Multiple sequence alignments were carried out with three different algorithms: ClustalX, PRANK and MAFFT. These programs highlight areas of similarity and therefore help to identify the evolutionary relations between the species. Finally we constructed phylogenetic trees with different methods (Neighbor-joining, Maximum parsimony, Maximum likelihood and Bayesian inference). We used several different programs for this purpose, like MEGA, PHYLIP and MrBayes. The parallel use of different methods and programs made possible to draw correct conclusions. Despite the slight differences in the diverse topologies, we concluded that the phylogeny of the sep15 gene resembles

the overall phylogeny of Fungi. The shown four phyla (Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota) are grouped separately as expected, and the three subphyla of Ascomycota (Saccharomycotina, Taphrinomycotina and Pezizomycotina) also sunder distinctly.

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THE OXIDATIVE STRESS INDUCING ABILITY OF ZEARALENONE – A NON-ESTROGEN SPECIFIC EFFECT IN THE FISSION YEAST

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Mycotoxins are worldwide spreaded contaminations of many foodstuffs and animal feeds. 25% of the agricultural products in the world contain some kind of mycotoxins and cause remarkable health and economic damages. Zearalenone (ZEA) is one of the most widely disseminated oestrogenic fusarium mycotoxin found in agricultural products. In this study the ZEA-induced accumulation of reactive oxygen species (ROS) and the regulation of the specific activities of antioxidant enzymes were investigated in the single cell eukaryotic organism *Schizosaccharomyces pombe*. In comparison with the control, 500 μM ZEA treatment caused 66% decrease in the concentration of glutathione (GSH), which was a consequence, in the absence of ZEA-GSH interaction, of the GSH-consuming processes of the antioxidant system; this depletion of GSH initiated a 1.8- and 2.0-fold accumulation of the superoxide anion and hydrogen peroxide, but did not increase the concentration of the hydroxyl radical; ROS-induced adaptation processes via activation of the Pap1 transcription factor resulted in significantly increased activities of superoxide dismutases, catalase, glutathione reductase and glutathione S-transferase, and decreased activities of glutathione peroxidase, glucose-6-phosphate dehydrogenase and Mn-superoxide dismutase. This treatment altered the sterol composition of the cells by inducing decreased concentrations of ergosterol, squalene and 24-methylene-24,25-hydrolanosterol, and elevated the number of fragmented nuclei. Cells strived to correct the unbalanced redox state by regulating the antioxidant system, but this failed to defend the cells from the disturbed sterol composition, the cell cycle arrest, and the fragmentation of nuclei.

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EXPRESSION OF *CHLAMYDIA MURIDARUM* PLASMID GENES AND IMMUNOGENICITY OF PGP3 AND PGP4 IN DIFFERENT MOUSE STRAINS

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Chlamydia trachomatis is the major human etiological agent of several diseases with importance in public health including trachoma and a variety of sexually transmitted diseases which have marked risks for blindness and infertility, respectively. *Chlamydia muridarum* is a mouse-adapted species, its genome exhibits high similarity to the *C. trachomatis* genome. Infection of mice with *C. muridarum* provides a useful model of the *C. trachomatis* infection in humans. *C. muridarum* carries a cryptic plasmid (pMoPn) of 7.5 kb, which encodes seven genes. All seven genes are transcribed and translated during the developmental cycle. The strong selection to maintain the plasmid by *Chlamydia* strains implies its importance in the pathogenesis of infection or disease. Our aims were to describe the transcriptional pattern of the pMoPn genes in *C. muridarum*-infected mice and to evaluate the host immune responses against pGP3 and pGP4 proteins. BALB/c and C57BL/6N female mice were inoculated intranasally with *C. muridarum* and sacrificed at different time points, and the total RNA was extracted from the lung suspensions to determine the levels of expression of the different plasmid genes by RT qPCR. The supernatants of the lungs were subjected to the quantitation of recoverable *C. muridarum*. TCA04 and TCA05 which encode pGP3 and pGP4, respectively, were amplified by PCR and cloned into the pET vector. The proteins were overexpressed in *E. coli* HB101 and purified. Selected groups of BALB/c and C57BL/6N mice were infected with *C. muridarum* 1 to 3 times. The humoral immune responses in the sera of the mice to the proteins encoded by TCA04 and TCA05 were tested by Western blotting, and the cellular immune responses were assessed in lymphocyte proliferation assays. The proteins recognized by the mouse sera were further analysed by a LC/MSMS technique. The kinetics of *C. muridarum* growth were similar in the mouse strains used, but the pathogen burden was higher in the BALB/c mice in the late phase of infection. All the plasmid genes in the BALB/c mice showed an increased level of expression on day 7, whereas the expression of the same genes was delayed, and some of them were even down-regulated on day 7 in the C57BL/6N mice. The levels of expression of the plasmid genes were higher in the C57BL/6N mice at later time points. In Western blot assays, the sera of the singly infected C57BL/6N mice reacted with the monomeric form of pGP3, whereas the sera of the singly infected BALB/c mice reacted with the trimeric form of pGP3. The sera of the multiply infected C57BL/6N mice also recognized pGP4. Similarly to the humoral immune response, cellular immune responses to pGP3 and pGP4 were detected in the *C. muridarum*-infected C57BL/6N mice.
These results suggest that the immune response to pGP3 and pGP4 during *C. muridarum* infection depends on the genetic background of the host.

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IL-17E PRODUCTION IS ELEVATED IN THE LUNGS OF BALB/C MICE IN THE LATER STAGES OF *CHLAMYDIA MURIDARUM* INFECTION AND REINFECTION

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Chlamydiae are medically important bacteria that are responsible for a wide range of human infections and diseases. *Chlamydia muridarum* is a mouse pathogen strain commonly used to

investigate human chlamydial infections. Pathogens can influence allergic respiratory diseases. Progress in studies of IL-17A and Th17 cells has revealed important roles for IL-17A in the development of allergic and autoimmune diseases, and also in protective mechanisms against bacterial and fungal infections. IL-17A and IL-17F are involved in the development of inflammation and the host defence against infection by inducing the expression of genes encoding pro-inflammatory cytokines, chemokines, and antimicrobial peptides. IL-17E enhances Th2 cell immune responses by inducing Th2 cell cytokines such as IL-4, IL-5 and IL-13 and induces IgE production and eosinophilia, contributing to the host defence against nematodes and allergic disorders. We earlier found that multiple infections with *Chlamydophila pneumoniae* induce the production of IL-17A and IL-17E, which have roles in the pathogenesis of asthma. The present work was designed to acquire support for our hypothesis that infections with another pathogen can induce the production of IL-17A and IL-17E. At an internal of 28 days mice were infected twice with *C. muridarum*, and the kinetics of IL-17A and IL-17E expression was subsequently detected at the mRNA and protein levels. The amounts of IL-17 cytokines produced by the restimulated spleen cells were determined by ELISA methods. The presence of IL-17E in the lungs was revealed by indirect immunofluorescence test. The expression of IL-17A mRNA was highest on day 7, and then decreased. The expression of IL-17A mRNA after the reinfection displayed similar kinetics as after the primary infection, but the fold increases in transcripts were higher. The kinetics of IL-17A protein production correlated with the mRNA expression: the production increased from day 1, with the highest concentration observed on day 7. Unlike that of IL-17A mRNA, the expression of IL-17E mRNA did not demonstrate a parallel with the bacterial burden in the lungs. The expression started to increase on day 7, and the highest level was detected on day 28 after the first infection. On day 29 (one day after the reinfection), the expression of IL-17E mRNA was decreased dramatically, but after that it increased again and was highest 28 days after the reinfection. The kinetics of IL-17E protein production was similar to that of the expression of IL-17E mRNA in the lungs of the infected and the reinfected mice. In the later stages of the infection, IL-17E was produced by the epithelial cells of the bronchi. The restimulated peripheral spleen cells produced IL-17A. On the basis of our experiments we suggest that multiple infections with *C. muridarum* induce the production of a high amount of IL-17E, which takes important part in the pathogenesis of allergic pulmonary diseases.

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EXAMINATION OF INTEGRON CARRIAGE AND IDENTIFICATION OF RESISTANCE GENES IN INTEGRON-ASSOCIATED GENE CASSETTES AMONG NOSOCOMIAL *ACINETOBACTER* SPP.

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Treatment of *Acinetobacter* infections has conventionally involved the use of beta-lactams, aminoglycosides, fluoroquinolones and tetracyclines. However, in nosocomial strains multiresistance is increasingly common. According to data from the National Center For Epidemiology of Hungary, resistance to aminoglycoside antibiotics has been increased to 65-70% for the past 5 years. The aim of this study was to investigate the prevalence of integron carriage and to identify resistance genes in integron-associated gene cassettes as well as to determine the presence of potential virulence factors among *Acinetobacter* isolates, collected in the University of Debrecen between 2010-2011. Altogether 91 isolates (68 from the ICU, 23 from other wards) were tested for

aminoglycoside modifying enzyme genes *aac*(3)-IIa, *aac*(6')-Ib, *ant*(2")-Ia, *ant*(3")-Ia, *aph*(3')-Ia, *aph*(3')-VIa, rRNA methylase genes *arm*A, *rmt*A, *rmt*B and four genes encoding tetracycline efflux pumps proteins *tet*A, *tet*B, *tet*C, *tet*E by PCR. In addition, presence of fourteen virulence genes frequently found in some Gram negative bacteria, i.e. Dr fimbriae (*afa*), cytotoxic necrotizing factor (*cnf*1), curli fiber (*csg*A), colicin V (*cva*C), type 1 fimbriae, yersiniabactin (*fyu*A), invasion (*ibe*A), capsule (*kps*MT), pathogenicity-associated island (PAI), P fimbriae (papC and papG), S fimbriae (sfa and sfaS), serum resistance traT were also examined. Occurrence of resistance integrons was studied by integrase-specific PCRs; variable regions of integrons were characterised by sequencing. The distribution of aminoglycoside resistance genes were as follows; *aph* (3')-VIa 82.4 %, *ant*(3")-Ia 64.8%, *aac* (6')-Ib 48.4 %, *aph* (3')-Ia 42.9 % *ant*(2")-Ia 6.6 %, *arm*A 20.9%; *aac*(3)-IIa, *rmt*A and *rmt*B genes were not detected. Tetracycline resistance genes *tet*(A) and *tet*(B) were found in 15.4% and 24.2% of isolates, the other two tested genes *tet*C and *tet*E were not detected. Type I integrons were carried by 69.2% of isolates among which four different gene cassette arrays were identified. An integron with variable region containing aac(3)-Ia, two hypothetical proteins and ant(3")-Ia (In561) was detected in the vast majority (54/63; 85.7%) of integron-containing isolates. Four of the sixty-three (6.3%) isolates harboured an integron with the gene cassettes aac(6')-Ib and blaVIM4 (In238). Another four isolates carried an integron carrying an *aac*(6')-Ib gene, a hypothetical protein and a blaOXA-20 gene (In426). A single isolate carried and integron with a sole *ant*(3")-Ib gene (In127). Type II and III integrons were not detected.
All isolates were negative to the putative virulence factors analysed. As amikacin resistance genes *aph*(3')-VIa and *aac*(6')-Ib were carried frequently by the isolates, these results suggest that the usage of amikacin as the preferred aminoglycoside in our University might have contributed to the spread and/or maintenance of endemic *Acinetobacter* strains.

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RETROSPECTIVE STUDY FOR SCREENING USUTU VIRUS ANTIBODIES IN TWO DIFFERENT GROUPS OF HUMAN POPULATION IN HUNGARY

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In recent years Usutu virus (USUV), a member of the Flaviviridae family, *Flavivirus* genus, Japanese encephalitis virus serocomplex has been reported in Europe. This is a mosquito-borne virus which can mainly cause pathological changes in wild and captive birds. The virus is usually not associated with severe human disease. However, two human cases have been reported from Italy in 2009. Both patients were immunocompromised and developed neurological symptoms. Although, the human implications of USUV are still unrecognised, the simultaneous circulation of this virus with other human pathogen flaviviruses such as West Nile virus (WNV) and tick-borne encephalitis virus (TBEV) in Hungary has been proven. Consequently, the epidemiological patterns and the Italian case studies account for a serological survey of Hungarian healthy blood donors. USUV and WNV are spread by the same vector, thus the samples were selected especially from West Nile virus endemic areas of Hungary. We applied an in house indirect immunofluorescent assay to detect USUV specific IgG response in human serum samples. Our samples were tested parallel for WNV,

USUV and TBEV antibodies, because of the high cross-reactivity among these antibodies. We have also examined cerebrospinal fluid samples from immunocompromised patiens with neurological symptoms to detect virus specific IgG and IgM antibodies. Our results represent that presumably USUV antibodies are not presented in the Hungarian human population, even though we have evidences of the presence of cross-reactive antibodies against these flaviviruses.

APPLICATION OF CYANOBACTERIAL RESISTANCE SYSTEMS FOR CONSTRUCTION OF HEAVY METAL BIOSENSORS

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Environmental contamination of heavy metals in soil and water bodies generate health risk of increasing concern. Regardless of their natural or anthropogenic origin, the contaminations need to be correctly detected in order to set up further safety actions. The applied monitoring systems have to meet various requirements: they need to be simple to use, cheap, selective, sensitive, reproducible and quantitative, among others. Bacteria contain simple and efficient detoxification systems for coping with heavy metal ions in toxic concentrations. Each corresponding operon comprises of an inducible promoter and a specific repressor, regulating the expression of transporter, redox, or chelating proteins. These regulatory elements offer useful tools for regulating appropriately chosen reporter genes in construction of whole cell biosensors. We have chosen *Synechocystis* PCC6803, a cyanobacterium as a chassis of such biosensors, due to the available genomic sequence of the organism and the easy and cheap maintenance of the cultures. We identified and investigated in this photoautotrophic bacterium the detoxifying operons against the environmentally most important heavy metal species. The sensitivity and selectivity of the cyanobacterial promoters were tested and biosensor strains were constructed, harboring the corresponding constructs containing luminescent enzyme systems or fluorescent proteins. General stress-related genes were not induced and there were no side-effects of the heavy metal ions tested in the applied concentration ranges, which finding also points out the utility of these promoter systems in other applications in biotechnology and basic science. The tested promoter systems have strict specificities toward their correponding toxic ions investigated (Zn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, AsO_2^-). Nevertheless, the sensitivities of the constructs differ greatly, reflecting the different tolerances of the host strain against the given ions. While the nickel-responsive promoter system exerts almost three order of magnitude overexpression of the gene under its regulation and the corresponding construct is capable of detecting micromolar concentrations of the ion, the arsenic sensing system does not operate below millimolar concentrations and hence it falls behind the requirements for a sensitive environmental sensor of simple use. We also found that the chosen reporter genes have important influence on the kinetics as well as concentration dependence profile of the emergent signal. While zinc ion interferes with the activity of the bacterial luciferase system, such problem was not evident using green fluorescent protein. Beyond facilitating the development and optimization of whole cell biosensors, our results will be useful in construction of inducible expression systems in cyanobacteria.

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GENETIC AND FUNCTIONAL ANALYSIS OF A SULFIDE:QUINONE OXIDOREDUCTASE ENZYME IN *SYNECHOCYSTIS* SP. PCC6803

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Sulfidic environments persist since the early beginnings of evolution of life on Earth. Before the appearance of cyanobacteria and the accumulation of oxygen in the biosphere, microorganisms had to evolve in anaerobic, mostly sulfidic habitats. A wide variety of living organisms adapted to the presence of sulfur in this toxic redox state, either by using sulfide as hydrogen donor for their photosynthesis or oxidizing it for detoxification. Even mitochondria are known to contain enzymes that provide an alternative route of ATP production in sulfidic habitats, since sulfide is proven to hamper oxidative phosphorylation at the level of cytochrome c oxidase. Enzymes with such functions belong to the disulfide oxidoreductase flavoprotein family. One of the mostly characterized enzymes of this kind is the sulfide:quinone oxidoreductase (SQR). Although cyanobacteria gain ATP via photophosphorylation in plant-type photosynthesis, the PSII reaction center is inhibited in the presence of sulfide. Nonetheless, besides green sulfur bacteria and purple non sulfur bacteria also a few cyanobacteria contain SQR enzymes by which they can facultatively shift to anoxygenic, bacterium-type photosynthesis with sulfide as electron donor in a Photosystem I-dependent reaction, thus overcoming the deleterious effect of sulfide.

The genome of *Synechocystis* 6803 contains a gene (designated by us as *suo*S) that shows high homology with functional sulfide:quinone oxidoreductase enzymes. To investigate the expression, regulation, function and possible origin of this enzyme, RT-PCR measurements, insertional mutagenesis, enzymatic activity assays, gas-chromatography experiments and electrophoretic mobility shift assays (EMSA) were carried out. RT-PCR experiments confirmed concentration- and time dependent expression of the *suo*S gene in the presence of H_2S. To test the function of the gene, insertional mutants were constructed by homologous recombination.

We could demonstrate by gas-chromatography experiments the in vivo oxidation of the artificially supplemented H_2S in anaerobic culture media. Isolated thylakoids from wild type cells showed SQR activity compared to the mutant strain deficient in this gene. In order to clarify the regulation of the *suo*S gene, another mutant was constructed lacking the putative repressor protein of the operon containing the *sqr* gene. In the absence of the repressor constitutive expression of *suo*S was observed irrespectively of the presence or absence of H_2S in the medium. For further analysis, electrophoretic mobility shift assay was performed. Via heterologous overexpression of the gene encoding for the repressor we purified the protein and demonstrated in vitro the binding of the DNA sequence containing the promoter region of the *suo*S gene. On the pSYSM plasmid of *Synechocystis* 6803 the DNA region of the operon harboring the *arr*S gene is flanked by sequences that show the characteristic structure of IS4 type transposable genetic elements.

Therefore we assume, that the genetic region encoding for the SQR enzyme might have been acquired by *Synechocystis* 6803 thorough horizontal gene transfer. Although a lot of data is known up to now about the function, structure and phylogeny of SQR enzymes no data where found in literature about the genetic regulation of the enzyme. This is the first time to our knowledge when the exact gene regulation of an SQR enzyme is elucidated.

INVESTIGATION OF THE AMMONIA OXIDIZATION PROCESS IN FIVE SMALL DRINKING WATER SYSTEMS, ESPECIALLY THE ENUMERATION PROBLEMS OF AMMONIA-OXIDIZING BACTERIA BY THE MPN TECHNIQUE

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Microbial growth and activity taking place in drinking water distribution systems may deteriorate the water quality. In drinking water networks nitrite and nitrate could be generated from ammonia by nitrification process. In the digestive tracts nitrite and nitrate can be transformed to nitroso compounds, which cause public health problems. Aim of the study was to detect, identify and quantify microorganisms involved in the nitrification process; especially ammonia-oxidizing bacteria in five different small drinking water network systems. To quantify ammonia-oxidizing bacteria Most Probable Number (MPN) technique was applied. Further investigation was carried out to detect the selectivity of MPN test media on ammonia oxidizing and utilizing microorganisms. The total and ammonia-oxidizing bacterial community structure of the samples from drinking water networks and ammonia oxidation positive MPN test media were mapped by Terminal Restriction Fragment Length Polymorphism (T-RFLP) based on the 16S rRNA encoding, and ammonia-monooxygenase (*amo*A) genes respectively. Statistical analysis of data was performed by Principal Component Analysis (PCA). To identify the dominant bacteria clone libraries were established. The number of ammonia-oxidizing bacteria increased from wells to consumer endpoints in each drinking water network according to the MPN technique. Based on gene *amo*A, ammonia-oxidizing bacteria were detected in most of the ammonia-oxidizing positive MPN test media, but only one drinking water distribution system. *Nitrosomonas* sp. was identified in the total bacterial community of the water and the positive MPN test media as well, but proportion of the genus in the total bacterial community of drinking water networks was lower than in the MPN test media. In addition to autotrophic ammonia-oxidizing bacteria several autotrophic and heterotrophic microorganisms were also detected in the MPN test media. Interestingly the media optimized for ammonia-oxidizing bacteria was promoting the growth of the nitrite-oxidizing bacteria indirectly such as *Nitrospira defluvii*. Owing to these organisms the abundance of autotrophic ammonia-oxidizing bacteria may be overestimated. The MPN test media was characterized diverse total bacterial community which showed obvious differences from the bacterial community of the origin water samples. The growth of non ammonia-oxidizing bacteria could be facilitated by the test media.

IDENTIFICATION OF *GYR*A MUTATIONS IN QUINOLONE-RESISTANT *SALMONELLA* STRAINS ISOLATED IN ROMANIA (2006-2011)

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Quinolone-resistant *Salmonella* strains have been isolated worldwide, Romania being no exception. Several mechanisms for resistance acquisition are described, the main mechanism being linked to mutations in the quinolone-resistance determining region (QRDR) of the target genes (especially gyrA encoding DNA gyrase). During 2006-2011, 2173 strains of *Salmonella* spp. from patients with

food-borne infections have been isolated and characterized in the Cantacuzino NIRDMI. Antibiotic sensitivity was tested using Kirby-Bauer method and interpreted according to CLSI rules. Minimal inhibitory concentrations (MICs) for nalidixic acid and ciprofloxacin were performed using E-test. A number of 602 strains (27%) were resistant to nalidixic acid and 48 (0.02%) showed decreased susceptibility to ciprofloxacin. PCR targeting *gyr*A gene was performed for 42 strains belonging to several *Salmonella* serovars, randomly selected. The amplicons were sequenced in order to determine the presence of mutations in the quinolone-determining regions. Data were analyzed using the BioEdit Program. Most of the strains included in the study presented a single *gyr*A mutation (D87Y, D87G, D87N, S83Y, S83F, variable according to serovar). Four strains belonging to C2-C3 *Salmonella* group serovars which are very seldom involved in food-borne infections showed a double mutation (S83F and D87G or D87Y). Those 4 strains equally showed high MICs for ciprofloxacin (4-8 μg/ml). In two strains no mutations in *gyr*A were detected, possibly pointing to the involvement of other mechanisms for nalidixic acid resistance. The mutations encountered were already described in the literature, within 83 and 87 codons of the QRDR region of *gyr*A gene, yet most of them encountered with a decreased frequency.
The genetic mechanisms by which *Salmonella* strains develop resistance to quinolones are multiple and their further investigation is required. Studies are necessary in order to better know the epidemiology and the mechanisms of antibiotic resistance in *Salmonella* spp. Surveillance for resistant bacteria among human, animal and food sources should remain critical.

EXPLORING AND CHARACTERIZING THE FOLDING PROCESSES OF LONG-SEQUENCE TRICHOBRACHIN PEPTIDES

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Trichobrachins belonging to the peptaibol family represent a microheterogeneous mixture of fungal peptides, and this group of peptaibols covers short-sequence molecules, as well as long-sequence ones. In a previous study, four distinct long-sequence trichobrachin peptides were identified, which were composed of 20 amino acid residues. The structural properties and folding features of these trichobrachins have not been investigated either by experimental or by theoretical methods, so far. In this study, molecular dynamics (MD) calculations were performed on four long-sequence trichobrachin peptides, in order to explore their folding processes in detail, as well as to determine their structural and folding features. For each trichobrachin, starting from the same conformation characterized by an extended peptide backbone, 100 individual 100-ns MD simulations were carried out, using random initial velocities in the case of every single calculation. Based on the MD trajectories, the alterations of different conformational features were studied as a function of time, as well as it was determined how these structural properties change during the simulations. For the trichobrachin peptides, the helix and H-bond formations were investigated comprehensively as a function of simulation time. The evolution of helical structures was examined taking into account not only the entire sequence of peptides, but also each amino acid residue. The evolution of various intramolecular H-bonds evolved between the backbone NH donor and CO acceptor groups were also studied. The so-called local H-bonds (i.e. the i←i+4 H-bonds between a NH group of i+4th and a CO group of ith amino acids; as well as the i←i+3 H-bonds between a NH group of i+3th and a CO group of ith residues) play a relevant role in the structural stabilization of different helical conformations. Nevertheless, the occurrence of so-called non-local H-bonds (i.e. the i←i+n H-bonds between the backbone CO and NH groups, where $n > 4$; as well as all types of i→i+n H-bonds between the backbone NH and CO groups) was examined along the entire sequence of peptides. The

evolution of all above-mentioned types of intramolecular H-bonds was investigated considering the average numbers of various H-bonds. Furthermore, the evolution of i←i+4 and i←i+3 H-bonds was studied as a function of time, taking into account each different intramolecular H-bond, respectively. On the basis of multiple MD simulations, the folding processes were explored in detail, as well as the structural and folding features were identified for the long-sequence trichobrachin peptides.

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CONFORMATIONAL ANALYSIS OF THE SHORT-SEQUENCE PEPTAIBOLS, HYPOMUROCINS BY MOLECULAR DYNAMICS METHODS

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As the members of peptaibol family, hypomurocins are constituted by two distinct groups of peptides, which were isolated from the fungus *Hypocrea muroiana*. The first group (i.e. the hypomurocin A molecules) comprises six peptides consisting of 11 amino acids, while the second group (i.e. the hypomurocin B molecules) includes also six peptides composed of 18 amino acid residues. The peptides belonging to the hypomurocin A series are the representative members of short-sequence peptaibols, which are characterized by a sequence pattern of Ac-Xaa1-Gln2-Xaa3-Xaa4-Aib5-Pro6-Leu7-Xaa8-Aib9-Pro10-Leuol11. In this theoretical study, a detailed characterization of three-dimensional structure of the short-sequence hypomurocin peptides was performed applying both simulated annealing (SA) and molecular dynamics (MD) methods. In the case of six hypomurocins, the backbone conformations were examined, and the presence of various secondary structures, such as different types of beta-turns, as well as helical structures, was studied along the entire sequence of peptides. Additionally, the side-chain conformations of amino acids were also investigated, and the proportions of different rotamer states, as well as the preferred rotamers were determined. For the hypomurocin molecules, two types of distances were measured, as follows: (1) the end-to-end distance between the N atom of the backbone NH group of Xaa1 residue and the C atom of the backbone CH_2 group of Leuol11 residue; (2) the residue-residue distances between all possible residue pairs of peptides. In order to describe the flexibility of molecules, the average fluctuations of the heavy atoms of backbone were studied, and root mean square fluctuation (RMSF) values were calculated. Based on the distribution plots and average values of end-to-end distances, and on the residue-residue distances represented in contact maps, as well as on the RMSF vs. backbone atom number plots, the six hypomurocins were characterized in detail and they were compared to one another. On the basis of conformational states derived from the SA and MD calculations, cluster analyses were carried out, in order to identify the conformationally related subfamilies, and to determine their representative structures. The comprehensive structural investigation on the hypomurocin peptides supplied a characterization of the various structural and conformational properties of these short-sequence peptaibols.

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BACTERIAL PHYLOGENETIC DIVERSITY OF HUNGARIAN DEEP SUBSURFACE GEOTHERMAL WELL WATERS STUDIED BY MOLECULAR CLONING

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Geothermal wells characterized with higher than 30°C thermal water can be found in more than 65% of the territory of Hungary. These waters can be utilized as spa water, drinking water and also as geothermal energy source. The water of the thermal wells located nearby Szarvas is only used for heating industrial facilities because of their high hydrocarbon content. The thermal waters of South Plains were found mainly during crude oil drilling exploratory wells, this is why these waters are rich in benzene and phenol. The water of the studied wells has high salt concentrations (3980 mg/l sodium bicarbonate), and also contains benzene (28.1 µg/l), phenol (9.33 mg/l), dissolved organic materials (COD 808 mg/l) and phenol derivatives. Their base depth is nearly 1900-2100 m, the average rate of flow is 1400 l/minute; therefore annually 150-270 000 m^3 of slightly alkaline (pH 8.41) and high-temperature (90-95°C) anoxic thermal water can be extracted. The aim of our study was to reveal the bacterial community structure of the water of K-81(Szr-18) and K-87 geothermal wells by construction of clone libraries. The sampling of the well waters was conducted in March 2012. Following the DNA isolation from the filtered water samples, clone libraries with nearly 100 members per sample were created. Universal bacterial primers (27f, 1401r) were used for the PCR. In order to group the molecular clones ARDRA method using restriction enzymes of BsuRI and MspI were applied. The 16S rRNA coding DNA regions of the clones were sequenced.
Although the phylogenetic distribution of molecular clones detected was very diverse for the two well water samples, in both cases the closest sequence similarities were found with uncultured environmental clones from similar thermal waters. From the water of K-81(Szr-18) well, members of the genera *Fictibacillus* and *Alicyclobacillus* (Firmicutes) were only revealed. The phylogenetic diversity of the water bacterial community of the K-87 well was much higher. Here members of the phyla Thermodesulfobacteria, Proteobacteria and the OP1 were also detected besides Firmicutes.

NEW PRIMYCIN PRODUCING STRAIN(S)

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Primycin is almost a Hungaricum, since its first discovery was realized in Hungary in the early '50s. Since then several pharmaceutical form with primycin basis reached the market and are used for efficient treatments of dermatitis and eye contaminations. There are different patents on primycin production (both fermentation, separation and purification), among them Juhasz et al. [1] described and patented several commercial microbial strains for primycin production. Since then some new species are also commercially available. Three of them were tested for primycin production, and found two strains providing similar cleaning zone around the colony, that we found for the reference strain (*Saccharomonospora azurea*). According to our knowledge, this is the first report of these strains in term of primycin production, thus a new (patent) priority application is under submission.

[1] Juhász, G. et al. (2011) WO201105174.

REPRODUCIBILITY OF LIQUID EGG HHP TREATMENT

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In both economic and scientific circles, significant efforts have been made to weaken the undesirable effects on foodstuffs that are caused by heat. This is how non-thermal technologies have been developed. Many „gentle" technologies have emerged, for example: ultraviolet rays, ultrasound, pulsing electric field (PEF), UV sterilization, high intensity laser, and high voltage discharge. However, none is more promising than the technology of high hydrostatic pressure (HHP). We conducted experiments on whole liquid egg (pressure treatment, determination of spore count, and measurement of color changes). The results were then compared with our earlier measurements. The current experiments occurred in a Resato FPU-100-2000 machine, where we observed the adiabatic increase in temperature and its effect, as opposed to the measurements obtained previously on a Stansted Food Lab 900. We intended to find the similarities and differences between the two sets of measurements. A close correlation was found between measurements obtained with liquid egg using varying times and equipment, but identical parameters of treatment. Therefore, it can be stated that the effect of high pressure treatment to reduce spore count is reproducible. Further, I have proved by my measurements that barely noticeable organic changes in liquid egg will occur with hydrostatic pressure less than 250 MPa. Also these conditions produce a significant magnitude 5 reduction of Salmonella Enteritidis live spore count. In summary, it has been proved that the positive results achieved with HHP technology are reproducible.

GENOMIC DIFFERENCES AMONG VARIOUS ISOLATES OF THE PATHOGENIC YEAST *CANDIDA PARAPSILOSIS*

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Based on recent data candidiasis has become the most commonly diagnosed yeast-related infection worldwide. Where, *Candida parapsilosis* is the third most frequent cause of candidemia. Despite its clinical importance, little is known about the genetic basis of fungal virulence traits that enable *C. parapsilosis* to cause disease. In the last decade, whole genome analysis became important in study of pathogen microorganisms. Thanks to comparative genomics, many correlation were found between genetic differences and virulence of various *C. albicans* strains. Having had the highest clinical relevance *C. albicans* has turned into the best-characterised species of the genus. However, much less attention was paid to the "non-albicans *Candida* species" (NAC) like *C. glabrata*, *C. parapsilosis* and *C. tropicalis*. The importance of these NACs has increased in the last decade. *C. parapsilsosis* seems particularly special because its incidence among young children (especially under the age of 2) is much higher, than in adults. Recently, the genome sequence of the *C. parapsilosis* CDC317 reference strain was published, that facilated the molecular investigation of this organism. Since then some authors reported differences in virulence and genetic background in various *C. parapsilosis* strains. These observations were supported by our results by producing knock-out mutants and by the chromosomal analysis of various *C. parapsilosis* isolates by using Pulsed-field gel electrophoresis. Even so the genome wide analysis and genomic comparison of different *C. parapsilsosis* isoltes have never been performed. To fill the gap, the whole genome of

C. parapsilosis GA1 clinical isolate and two environmental strains were sequenced and a genome wide comparison was performed. In silico analysis identified several genomic variabilities. To validate these findings we developed strategies using PCR, Sanger sequencing and Southern blotting techniques and veryfied gene fusions, inversions, large homozygous and heterozygous deletions. Notably, rearding the distribution of gene families among different isolates of *C. parapsilosis*, we found the complete loss of 3 members of the ALS-like gene family (important virulence factors in *C. albicans*) in GA1 compared to CDC317.

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LACTOSE INDUCTION OF STERIGMATOCYSTIN FORMATION IN *ASPERGILLUS NIDULANS*

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Seed contamination with polyketide mycotoxins such as aflatoxin (AF) and sterigmatocystin (ST) produced by *Aspergillus* spp., is an agricultural, economic, and medical issue worldwide. Sterigmatocystin is the penultimate intermediate in the biosynthesis of AT; moreover, in several fungi including the model fungus *A. nidulans*, it is the end product of the AT pathway. The AT/ST biosynthetic pathway is well-characterized in *A. nidulans* with all the structural genes and intermediate described, but many of the regulatory aspects including those related to the carbon source available for the fungus are still enigmatic. For example, *A. nidulans* mutants unable to produce ST on glucose medium have been observed to produce ST on medium using lactose, (and, to a smaller extent, D-galactose), as a carbon source. One of the genes important for ST production is the so-called "velvet" gene (*ve*A). *Ve*A is involved in the regulation of a variety of cellular processes such as asexual and sexual development as well as secondary metabolism. The wild type allele is *ve*A while *ve*A1 is the mutation and one can miss important metabolic connections and identify artifacts using mutants with *ve*A1 background. In *A. nidulans*, VeA has been shown to control the AF/ST regulatory gene *afl*R and, subsequently, ST production. Similarly VeA regulates AF production in those fungi producing AF. To address the relative importance of VeA and its interaction with carbon source, we initiated a project of lactose and D-galactose metabolism vs. ST production in *A. nidulans* in both a *ve*A and *ve*A1 background. Since environmental factors are known to influence ST formation, we employed well-controlled submerged cultivation methods where temperature, pH and dissolved oxygen levels could be kept at preset values, and light intrusion could be prevented completely. *Aspergillus* minimal medium with ammonium ions as a sole nitrogen source was used. Under these conditions, independently of the carbon source available, *A. nidulans* was not able to produce any ST in a *ve*A1 background. In contrast, *ve*A strain was capable of producing ST on both glucose and lactose in a final overall concentration of up to 0.5 mg per liter. Time-profiles of ST formation were markedly different, however: on D-glucose, ST could be detected only after glucose was completely depleted from the medium, while on lactose, ST appeared in the early stages of the rapid growth phase. ST-formation in *A. nidulans* may either be mediated by a carbon catabolite regulatory mechanism prominent on D-glucose, or induced by the low specific growth rate attainable on lactose. We are currently testing this hypothesis.

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A NOVEL HPLC-UV METHOD TO DETECT STERIGMATOCYSTIN FORMATION FROM SUBMERGED CULTURES OF *ASPERGILLUS NIDULANS*

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Sterigmatocystin (ST) is a toxic metabolite structurally closely related to aflatoxin (AT), and both molecules are members of a large, diverse class of compounds known as polyketides. Molecular formula of ST is C18H12O6, and is produced by several species of Aspergilli. These fungi can contaminate food and feed leading to huge economic losses worldwide. Therefore, the aim of many studies has been to develop a suitable quantative method to measure ST. The easiest (and thus most widespread) method to detect ST is thin layer chromatography, but in many labs HPLC-MS has become the method of choice. In this study we present a rapid, easy and reliable HPLC–UV method recently developed in our lab to isolate and detect ST from submerged fungal cultures. First, ST was concentrated in an organic phase due to the relatively low (a few mg per L) level of this compound even in complex fermentation media. Several organic solvents have been tested with ethyl-acetate being found as the most suitable one (partition coefficient of ST in water-ethyl-acetate mixture is q=5.94 while in e.g. water-chloroform mixture, it is only q=2.58). The sample has been extracted thrice with one and a half volume of ethyl-acetate, followed by the organic phase being collected and evaporated. Samples were then resuspended in 1 ml of aceto-nitrile. This method is suitable to extract ST with an efficiency of 98.5% from water and an efficiency of up to 70 % from fungal cultures. The 30 percent loss could likely be attributed to the fact that ST binds strongly to the cell wall; however, the extraction efficiency could be successfully reproduced, thereby generating a standard error only. Reverse phase high performance liquid chromatography coupled to ultra-violet detection (RP-HPLC-UV) has been employed to determine ST concentrations. The mobile phase applied was water : acetonitrile (4 : 6) mixture buffered with acetic-acid and Na-acetate, at a flow rate of 0.5 ml/min with isocratic elution. Temperature of the column was kept at T = 55 C°, detection occurred at λ = 245nm. Since ST is a relatively stable molecule (pKa= 9.58), pH of the mobile phase (pH 4.76) did not modify structure and thus elution properties, but the acidic solvent was necessary to separate ST from by-products of fungal metabolism such as proteins and nucleotides. Under these conditions the retention time of ST is 11.9 minutes and the detection limit is 0.1 mg/L, which is safely over the concentration range A. nidulans can produce under submerged conditions, even in minimal medium. Accordingly, concentration of ST was measured as being between 0.4-0.7 mg/L in minimal medium and up to 2.5 mg/L in complex medium. However, actual final ST-concentrations could be some 30 percent higher.

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HYDROGEN PRODUCTION FROM LACTATE BY A PURPLE SULFUR PHOTOTROPHIC BACTERIUM

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Hydrogen might be regarded as the cleanest energy carrier of the future, since its combustion/oxidation does not emit any pollution. Hydrogen can be produced by biological systems, such as photosynthetic and/or fermentative microbes. In the dark processes, biomass - deriving from energy plants or many kinds of organic wastes - is fermented under anaerobic conditions yielding hydrogen as well as volatile organic acids. However, photosynthetic microorganisms can utilize organic acids and - under certain conditions - produce hydrogen. The dark and photofermentation processes can be consecutively combined leading to higher conversion rates and better yields. Each biohydrogen production approach is based on the hydrogen evolving enzymes, which can be either nitrogenase or hydrogenase. Purple (sulfur) photosynthetic bacteria are able to utilize small organic acids and possess enzymes for hydrogen production. *Thiocapsa roseopersicina* is an anaerobic, purple sulfur phototrophic bacterium, which is known to synthesize 4 active [NiFe] hydrogenases (HupSL, HynSL, Hox1, Hox2) and contains a nitrogenase enzyme. Hydrogen can be evolved by the Hyn, Hox1,2 hydrogenases or nitrogenases. Thus, the hydrogenase- and the nitrogenase-mediated hydrogen production can be compared in the same organism. The aim of this study was to investigate the possibility of utilizing lactate generated from dark fermentation for hydrogen production through photofermentation using *T. roseopersicina*. For this purpose, the culture medium was supplemented with lactate and various amount of thiosulfate, which is the primary electron donor of this strain. Experimental data showed that the HynSL membrane-bound hydrogenases were not able to produce hydrogen from lactate; however the soluble hydrogenase Hox1 was capable of lactate driven hydrogen production but only at lower thiosulfate concentrations. It was concluded, that lactate could promote the Hox catalyzed hydrogen production but the sulfur metabolism might interfere with it. Under nitrogen fixing conditions, nitrogenase reduces nitrogen in the concomitant formation of hydrogen. It was demonstrated that *T. roseopersicina* was able to produce considerable amount of hydrogen by nitrogenase enzyme from the usage of lactate even at high thiosulfate concentration. In contrast to the Hox1 based system, lactate serves as additional reducing power for the nitrogenase at any thiosulfate concentration.

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INVESTIGATION OF THE Ω-6 AND Ω-3 FATTY ACID PRODUCTION OF DIFFERENT *MORTIERELLA* AND RELATED SPECIES

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Polyunsaturated fatty acids (PUFAs) are elemental structural components of biological membranes and precursors of a wide variety of metabolites regulating critical biological functions, such as prostaglandins, leukotrienes and hydroxy-fatty acids. Considerable attention has been paid to them due to their beneficial effects on human health: ω-3 PUFAs have significant role in the prevention of the development of cardiovascular diseases, whilst ω-6 PUFAs are important in inflammation processes. *Mortierella* species belonging to Zygomycetes are particularly active in PUFA synthesis.

M. alpina is one of the most important industrial PUFA producers, its lipid production and the background of PUFA biosynthesis is extensively examined, but little information is available about the fatty acid production of other *Mortierella* species. So the aim of our work was to investigate the PUFA production and profile of several *Mortierella* and related strains in order to identify new, efficient ω-6 and ω-3 PUFA-producers and at the same time investigate the diversity of PUFA production in the order Mortierellales. In our study 105 *Mortierella* and related strains were screened. The strains were incubated in submerged cultures, and then the lipids were saponified and extracted with a potassium hydroxide-methanol-chloroform treatment. To improve the detection the halogenoalkyl type 3-Bromomethyl-6,7-dimethoxy-1-methyl-1,2-dihydroquinoxaline-2-one (Br-DMEQ) derivatisation reagent was used to convert the PUFAs into the corresponding fluorescence derivatives. After derivatisation the PUFAs were separated with HPLC. All strains produced ω-3 and ω-6 PUFAs, but significant differences were observed in their lipid yields. Several promising PUFA producers belonging to different phylogenetic groups were identified as some *Mortierella* and *Micromucor* species produced PUFAs in high amount. According to this PUFA production seems to be a general among these fungi. There were differences in their lipid profile: *Mortierella* species produced mainly arachidonic acid, whilst the main products of *Umbelopsis/Micromucor* species were γ-linolenic-, oleic-, linoleic- and palmitic acid. Interestingly, some *Mortierella* strains produced mainly linoleic acid instead of arachidonic acid; moreover *M. tuberosa* also produced α-linolenic acid in high amount, which was unique among *Mortierella* species.

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VIRO-IMMUNOLOGICAL BACKGROUND AND CLINICAL CONSEQUENCES OF ROSEOLOVIRUS-ASSOCIATED ENCEPHALITIS

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Both species of human herpesvirus 6 (HHV-6A, HHV-6B) and HHV-7 infect majority of the population worldwide, establishes latent infection in CD4 immune cells and neuronal cells. Their partial or complete reactivation might contribute to serious immunological and neurogical disorders. We described their transactivating role role in HIV-1 infection, lymphomagenesis, skin disorders, and facilitating the progression of multiple sclerosis. Recent epidemiological studies suggest that both HHV-6A and HHV-6B, as well as HHV-7 contribute to further disorders with combined immunological and neurological background. All these three viruses might elicit sporadic form of encephalitis occassionally in immunocompetent children, but at higher frequency in immunocompromised persons. The scale of contribution by Roseoloviruses has not been appreciated in the past, but such cases must now be fully explored by both clinicans and virologists! Primary HHV-6B infection with or without exanthem subitum might be followed by central nervous system (CNS) involvement with occassional sequele. HHV-6A might be involved in rhomboencephalitis. Primary HHV-7 infection might induce serious encephalitis with generalized symptoms. Major risk factors for single or simultaneous reactivation of HHV-6 and/or HHV-7 followed by encephalitis and frequently lethal sequele are such as unmatched cord blood cell transplantation and repeated

hematopoietic stem cell transplantation. Roseolovirus-associated encephalitis after solid organ transplantation is rare. Additional risk factors are immunosuppressive or cytotoxic drug treatment, young age, and chromosomally integrated HHV-6 in either donor or recipient. HHV-6B has been shown to enter the brain via the olfactory route. HHV-6 damages the brain stem, hippocampus, limbic tissue. The most severe manifestation is the syndrome of post-transplantation acute limbic encephalitis (PALE) with extremely high morbidity. Posterior reversible encephalopathy syndrome (PRES) might follow medical interventions applied in underlying diseases. Shift in cytokine pattern mediates inflammation. Careful clinical observation, sophisticated neuroimaging procedures and laboratory findings together help set up proper diagnosis. Continuous monitoring of high load of HHV-6 or HHV-7 in the cerebrospinal fluid and blood is pathognomic.
Ganciclovir, valganciclovir, foscarnet or cidofovir therapy of pre-emptive approach have been shown a variable value. Restricted use of cytotoxic drugs or commonly used drugs with known Roseolovirus activating potential in underlying conditions would be desirable, which is in sharp contrast to recently accepted guidelines of other viral encephalitis.

HUMAN PAPILLOMAVIRUS 16 ONCOPROTEINS ALTER THE EXPRESSION OF GENES IMPORTANT IN KERATINOCYTE DIFFERENTIATION

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The life cycle of human papillomavirus (HPV) is closely linked to the differentiation of their natural host cells. The oncoproteins of high-risk HPV (such HPV 16) are responsible for the transforming activity of the virus. The expression of HPV 16 E6 and E7 oncogenes increases in differentiating keratinocytes, which results in the inactivation of p53 and pBb tumour suppressors. The HPV infection can delay the normal differentiation program of keratinocytes, however, the underlying mechanisms responsible for this phenomenon are not yet clarified. The goal of our study was to investigate the effects of HPV16 oncoproteins on the expression of some selected genes important in keratinocyte differentiation. Primary human keratinocytes were transduced by LXSN (control) retrovirus or virus vectors expressing HPV16 E6, E7 or E6/E7 genes and induced to differentiate in medium containing increased calcium for 5 days. Global gene expression analysis was used to study the mRNA expression pattern of keratinocytes expressing HPV oncoproteins or vector control. The results of microarray analysis were confirmed with real-time RT-PCR in transduced differentiating and non-differentiating cells. Primary keratinocytes were co-transfected by reporter plasmids carrying the regulatory region of some selected genes involved in differentiation along with vectors expressing HPV 16 E6, E7 oncogenes. 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. In order to validate our data, we used quantitative real-time PCR analysis and investigated the mRNA level of some selected genes important in keratinocyte differentiation. The E6 and E7 oncogenes of HPV 16 together caused down-regulation or had a little effect on the genes both in proliferating and differentiating cells.
Transient transfection analysis was performed to see whether the down-regulation of the expression of some selected genes (keratin 4, 5, cornifin-A, S100 small calcium binding protein A8, desmocollin 1) was caused by inhibition of promoter activity by the HPV oncogenes.

THE ULTIMATE TEST: DOES NADPH-AVAILABILITY INDEED CONTROL THE OXIDO-REDUCTIVE PATHWAY OF D-GALACTOSE CATABOLISM IN *ASPERGILLUS NIDULANS*?

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D-galactose, being the other monomer of lactose next to D-glucose and a crucial building block of plant hemicelluloses and the substrate for galactosyl transferase-catalyzed reactions, is a prominent hexose sugar in fungal physiology and biotechnology. D-galactose metabolism via the Leloir pathway is a ubiquitous trait in pro- and eukaryotic cells. In the absence of an external D-galactose source it represents the only means to provide this carbohydrate by biological interconversion from D-glucose to D-galactose. It involves an ATP-dependent galactokinase to form D-galactose 1-phosphate, which is then transferred to UDP-glucose in exchange with D-glucose 1-phosphate by D-galactose 1-phosphate-uridyltransferase. The resulting UDP-galactose is a substrate for the reaction catalyzed by UDP-galactose 4-epimerase, resulting in UDP-glucose. Galactokinase (*gal*E) defective mutants of the fungus could grow on D-galactose in the presence of ammonium, providing evidence for the presence of an alternative pathway of D-galactose breakdown in *A. nidulans* [1]. This oxido-reductive pathway involves the NADPH-dependent reduction of D-galactose to galactitol as a first step [1]. However, the pathway is not operating when ammonium is replaced with nitrate ions as a sole nitrogen source. Nitrate assimilation is a heavily and strictly NADPH-dependent process, thus we speculated that availability of the intracellular NADPH may be a limiting factor upon growth on D-galactose plus nitrate in a galE background (e.g. when the Leloir pathway is blocked). To test this hypothesis, we created NADH-kinase (AN8837.2; *ndk*A) overexpressing strains in wild-type and *gal*E backgrounds. NADPH formation and thus NADPH availability is profoundly increased in these mutants [2]. Constructs were created with either inducible or constitutive promoter – the former being based on the TET on/off system that requires doxycycline supplementation to initiate transcription. Both strains lack non-homologous end-joining activity to increase transformation efficiency and are uridine-negative to enable mutant selection. We used a vector set based on the USER (uracil-specific excision reagent) cloning technique, which allows rapid and easy generation of constructs for targeted integration and heterologous expression of a gene of interest in A. nidulans. Growth of the *A. nidulans gal*E mutants on D-galactose plus nitrate as sole carbon and nitrogen sources, respectively, was restored in an *ndk*A overexpression background, and was quantitatively similar to the growth of the *gal*E mutants on D-galactose plus ammonium ions. Evidence was therefore provided that availability of NADPH is indeed a crucial physiological point of control in the regulation of the oxido-reductive D-galactose catabolic pathway.

[1] Fekete, E. et al. (2004) Arch Microbiol 18: 35-44.
[2] Panagiotou, G. et al. (2009) Metabol Engin 11: 31-39.
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IDENTIFICATION OF THE TRUE INDUCER OF THE BGAD (BETA-GALACTOSIDASE-ENCODING) GENE IN *ASPERGILLUS NIDULANS* UPON GROWTH ON D-GALACTOSE

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Fungal beta-D-galactosidase is an elementary enzyme in biotech industry on account of its ability to hydrolyse lactose to produce galactose and glucose. Fungal beta-galactosidases can be distinguished into extracellular enzymes characterised by an acidic pH optimum, and intracellular ones, that function optimally at neutral pH. In *Aspergillus nidulans*, only a lactose- and D-galactose inducible intracellular activity with a neutral pH optimum has been described. Functionally characterised intracellular (pH-neutral) beta-galactosidases belong to the Glycosyl Hydrolase family 2 (GH2). A number of mutations resulting in beta-galactosidase negative phenotype have been described in *A. nidulans*, the large majority of which mapped to two loci on linkage group (chromosome) VI. Genome annotation predicts nine genes for GH2 enzymes, out of which four were mapped to linkage group (chromosome) VI. From these four potential candidate loci, only one (AN3201) appeared to specify a gene big enough to produce a peptide of 120 kDal, the experimentally estimated molecular weight of the beta-galactosidase subunit. This gene was assigned the abbreviation *bga*D, to differentiate it from the three bga loci described earlier [1]. In this study we report on the expression of the *A. nidulans bga*D gene upon growth on D-galactose. In principle, either intermediate of the two D-galactose catabolic pathways [2] would be able to induce *bga*D. The potential inductors can be identified by testing loss-of-function mutants defective in a single defined step of D-galactose catabolism. In case of a galactokinase (*gal*E) mutant, *bga*D is strongly induced by D-galactose, indicating that the intermediates of the Leloir pathway are dispensible for the induction. The expression profile of *bga*D was similar in L-arabitol dehydrogenase (*ara*A1) and hexose kinase (*fr*A1) negative backgrounds, indicating that intermediates of the oxido-reductive pathway downstream of galactitol are not necessary for bgaD induction either. An *fr*A1/*gal*E double mutant (which cannot grow on D-galactose) still produced *bga*D transcript upon transfer onto D-galactose. Taken together, these results suggested that the true inducer of *bga*D upon D-galactose induction is either D-galactose itself or its poliol form (galactitol). To test this hypothesis, we grew the wild-type as well as the *gal*E mutants on galactitol as a sole carbon source. No *bga*D induction was observed at any time-points tested, e.g. galactitol is not an inducer of the major intracellular beta-galactosidase in *A. nidulans*. The true inducer of *bga*D in *A. nidulans* upon growth on D-galactose is the sugar itself, and its catabolism is not needed to achieve sufficient level of transcript formation.

[1] Fekete, E. et al. (2012) Fungal Genetics and Biology 49: 415–425.
[2] Fekete, E. et al. (2004) Arch Microbiol 18: 35-44.
Supported by the EU, co-financed by the European Social Fund, project ENVIKUT (TÁMOP-4.2.2.A-11/1/KONV-2012-0043), and by the Hungarian Scientific Research Fund (OTKA Grant K1006600 to Dr. Erzsébet Fekete).

CHARACTERIZATION OF BACTERIOPHAGES INFECTING *CRONOBACTER* SPP.

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Cronobacter spp. (previously known as *Enterobacter sakazakii*) is a gram-negative, facultative anaerobic opportunistic bacterial pathogen affecting all age groups, with particularly severe clinical complications in neonates and infants. Symptoms include bacteraemia, necrotizing enterocolitis and meningitis, with fatality rates as high as 80%. Although the microorganism is widely distributed in the environment, powdered infant milk formula has been implicated as the vehicle of transmission in many clinical manifestations. Bacteriophages can be perspective used in phage therapy of *Cronobacter* infections and also in the protection of infant formula from this pathogen. The aim of

this work was to isolate and characterize bacteriophages specific for *Cronobacter* and to find suitable candidates for application in food control. Bacteriophages were isolated from sewage water by cultivation on indicator strains. Phages were isolated from single plaques and purified by centrifugation in CsCl gradient. Host specificity was determined on double agar plates. Phages were identified by PCR and by whole genome sequencing. Several bacteriophages were isolated from sewage water. The phages differed in their host specificity; they were able to infect 2 – 39 from 50 tested *Cronobacter* strains. The Pet-CM-3 bacteriophage isolated on *C. malonaticus* strain possessed the broadest host specificity as it was able to lyse 39 from 50 tested strains belonging to six species. Based on whole genome sequencing this phage belonged to the T4-like group of Myoviridae family. The whole DNA was 172 kbp long and showed highest similarity to CC31 enterophage throughout the whole length. The Dev2 bacteriophage was specific only for two *C. turicensis* strains. Its genome contained 39 kbp DNA and showed the highest similarity to EcoDS1 and K1F phages belonging to T7-like group of Podoviridae family. The ability of the Dev2 phage to kill the host bacteria in reconstituted infant milk formula was confirmed. The Dev-CS-11 and Dev-CM-15 bacteriophages were isolated from the same sewage on two indicator strains and they infected 17 and 25 tested strains. These phages were closely related to *Cronobacter* lysogenic phages ENT47670 and ES2 belonging to Siphoviridae family. The results obtained in the study will be used for selection phages with the most suitable properties for food control applications.

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ASPERGILLOSIS DIAGNOSTICS IN HUNGARY, STATE OF THE ART AND FURTHER CHALLENGES WORLDWIDE

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Despite the rapid development in antifungal therapy during the past decade invasive aspergillosis is the main cause of infection-associated mortality in patients being treated with haematological malignancies and is an emerging disease in solid organ transplant recipients. Mayor causative agents of the highly devastating systematic mycoses are mainly the opportunistic filamentous fungi of the *Aspergillus* genus, such as *Aspergillus fumigatus*, *A. terreus*. The saprophytic *Aspergillus* species are ubiquitous in our environment. The exposure to *Aspergillus* spores or conidia is therefore almost constant. Due to the immunocompromised state of these individuals aspergillosis can become invasive and cause systemic infections. In spite of the fact that in the status of the primary disease improvement may appear, the secondarily evolved infections lead to death. The only means of the survival is the antifungal therapy initiated early enough. For an improved patient outcome early, sensitive and reliable diagnosis is mandatory. The most reliable microbiological and histopathological methods are time consuming, signs and symptoms of systemic diseases caused by *Aspergillus* species are non-specific and patients are often unable to undergo invasive diagnostic procedures. Real-time PCR technique is a highly sensitive method detecting small amount of fungal DNAs in biological samples and supporting diagnosis.

We have developed species specific, highly sensitive quantitative real-time PCR diagnostic assays for detecting and identifying *Aspergillus fumigatus* and *A. terreus* species in different biological samples. The assays are based on the detection of the fungal orthologs of the *Streptomyces fac*C gene. Due to the fact that *fac*C orthologs are almost exclusively found in *Aspergillus fumigatus* and *A. terreus*, the high rate of false positive results that is given from the presence of other pathogen species obtained by using other multi copy target genes in DNA based assays will be reduced to

zero. Our real-time assays are able to detect 1-5 GE in biological samples both in manual and in automated DNA extraction systems. Routine clinical tests are in progress.

EVALUATION OF A SIMPLE MICROTITER PLATE VARIANT OF AMES TEST FOR THE GENOTOXICITY ASSAY OF ENVIRONMENTAL SAMPLES

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The Ames genotoxicity test employs several histidine auxotrophic strains of *Salmonella typhimurium*, which have been selected based on their sensitivity to distinct types of mutagens. This reverse mutation tests are performed by mixing the test substance solution and the tester strain together in a reach liquid medium, which contains only small amounts of histidine. The histidine permits the inoculated test organism to undergo a limited number of divisions, but is insufficient to permit normal growth. If, however, the strain undergoes a reverse mutation, the organism no longer requires histidine to grow and can produce visible revertant colonies after spreading the treated cell suspension on the surface of minimal medium. In our investigations we worked out and evaluated a simple Ames test system for the genetoxicological measurement of water and air samples. The tester *S. typhimurium* strains employed were TA98 and TA1535. TA98 detects mutagens that cause frameshift mutations and TA1535 detect base substitution mutations. In our new approach of Ames test assay we washed the cells in minimal liquid medium, resuspended the cells in the same medium and from this suspension 107 cells were pipetted in 150 µl volume to the wells of a sterile microtiter plate (with lid). To the cell suspensions 50-50 µl sterile filtered environmental samples were added. The optical density of the mini-cultures was measured at 620 nm immediately and after 24 hours of incubation at 35 °C. The increase in absorbance at 620 nm reflects the mutagenecity of the samples. Between the nitrite contents of the Maros water samples and the relative genotoxicity values determined by our method showed positive correlation with all the two used tester strains.

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COMBINATION OF FISH, STABLE ISOTOPE LABELING AND RAMAN MICROSPECTROSCOPY TO ISOLATE SPECIFIC SINGLE CELLS FROM MICROBIAL COMMUNITIES

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RAMAN microspectroscopy is a non-destructive analytical tool providing a chemical fingerprint on the single-cell level. Its ability to detect chemical bonds of stable isotopes incorporated to the cell

biomass allows activity-based identification of specific cells from a microbial community. RAMAN microspectroscopy can be directly combined with FISH-signal detection for the phylogenetic identification of microorganisms. With the addition of an optical tweezer, cells or microcolonies of interest can be isolated from the sample for further processing (culturing, PCR, single-cell genomics), or the process can be performed on a microfluidic chip, leading to high-throughput analysis of samples. Hereby we report on the proof-of-principle experiments of ^{13}C and deuterium-based activity labeling and detection combined with FISH, and successful isolation of specific microcolonies from activated sludge with subsequent MDA amplification.

DIAGNOSTIC CAPABILITY OF THE HIGHLY PATHOGENIC MICROORGANISM AT THE HUNGARIAN NATIONAL BIOSAFETY LABORATORY AT NATIONAL CENTER FOR EPIDEMIOLOGY – RESULTS OF INTERNATIONAL PROFICIENCY TESTS

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The continuous threat of emerging and reemerging infections and the appearance of highly pathogenic microorganisms require considerable attention. The Hungarian National Biosafety Laboratory (HNBL) at the National Center for Epidemiology (NCE) as the only functioning high containment (BSL3/4) public health biosafety laboratory in Central Europe is responsible for the diagnostic of the risk group 3 and 4 (RG3/4) pathogens of public health importance and bioterrorism. For the accurate diagnosis of these pathogens, the participation in international cooperation such as the Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens (QUANDHIP) project is crucial. QUANDHIP's main purpose is to create a stabilized permanent consortium to support a joint European response to outbreaks of highly infectious agents by organizing external quality assurance exercises. For the accurate detection of viable and non viable highly pathogenic microorganisms in different matrices, using more than one microbiological technique is necessary. In the frame of QUANDHIP bacteriology proficiency tests (PT) the goal was to detect *B. anthracis*, *F. tularensis* ssp., *Yersinia* spp., *Brucella* spp., *C. burnetti* and *Burkholderia* spp. among target like (for instance *Ochrobactrum antropii*) and contaminant (for instance *Pasteurella aerogenes*) bacteria. In the QUANDHIP virology PT the main goal was to determine the limit of detection of RG4 agents such as Ebolavirus, Marburg virus and Lassa virus, using protocols standardized in the previous PT. The Reference Laboratories reached excellent results using broad spectra of microbiological tests (cultivation, Biolog, rapid test, conventional and real time PCR, MALDI-TOF). Nowadays, the *Hyalomma* spp. tick, which is responsible for the spread of the Crimean-Congo hemorrhagic fever virus (CCHFV) has appeared in Hungary due to the climate change. To test the most sensitive virus isolation in vitro system, different cell cultures were compared using different CCHF strains. The participation of HNBL in networks has high priority in order to contribute to and take part in EU harmonization processes, standardization of biosafety/biosecurity practices and diagnostic procedures, to share methodologies, to access international databases, to consult experts and to strengthen biosafety/biosecurity awareness.

PURIFICATION OF TWO ISOFORMS OF NATIVE RECOMBINANT M3 PROTEIN OF MURID HERPESVIRUS 4 AND CHARACTERIZATION OF ITS CHEMOKINE-BINDING PROPERTIES

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Murid herpesvirus 4 (MuHV-4) is currently widely used animal model for research of oncogenic human gammaherpesviruses Epstein-Barr virus and Human herpesvirus 8. MuHV-4 genome encodes unique M3 protein capable of binding a broad spectrum of chemokines inhibiting their immunological activities, thus facilitating the virus spread. Several scientific works have been published about the M3 protein of the prototypic strain MHV-68 and its potential use in the treatment of diseases associated with chemokine´s deregulation using animal models. However, much less is known how the change in the sequence of the M3 protein affects its biological activity. Formerly, we identified Asp307Gly mutation near the chemokine-binding site of M3 protein of MHV-72, another strain of MuHV-4 exhibiting different pathogenesis in immunocompetent mice. The aim of this work was to prepare new bacterial expression system for production of biologically active recombinant M3 protein. We produced M3 proteins of both MHV-68 and MHV-72 viruses in two *E.coli* strains (BL21 (DE3) and Rosetta-gami 2 (DE3)) using recombinant plasmids utilizing pET-26b(+) expression vector. In the case of E.coli BL21 (DE3) cells we also co-expressed chaperone molecules GroEL-GroES and TF to facilitate correct folding of recombinant M3 proteins, while the correct tertiary structure of M3 protein expressed in *E.coli* Rosetta-gami 2 (DE3) cells was achieved only by an oxidizing cytoplasmic environment. We optimised cultivation conditions of bacterial cells and the expression and purification procedures to preserve biological activities of M3 protein. We purified M3 proteins by immobilized-metal affinity chromatography (Ni-NTA) via the polyhistidine anchor fused to the C-terminus. Both recombinant M3 isoforms from either E.coli BL21 (DE3) or Rosetta-gami 2 (DE3) strain had binding activity against human chemokines of two distinct chemokine families exemplified by CCL5 and CXCL8. Overall binding activity of recombinant M3 MHV-72 isoform against both chemokines was lower than the binding activity of M3 MHV-68 isoform due to Asp307Gly mutation and the binding activity of all purified M3 proteins was higher against CCL5 than CXCL8 (up to 50-times in the case of M3 MHV-68). These results were in correlation with the binding activities of the same M3 protein isoforms secreted into the culture medium of BHK-21 permissive cell line infected with MHV-68 or MHV-72 viruses but the increased purity of recombinant M3 protein allowed detection of its biological activity in much lower amounts. Most biological active recombinant M3 MHV-72 protein against CXCL8 was purified from Rosetta-gami 2 (DE3) strain which bound 64,8% of chemokine. On the other hand, most of CXCL8 bound M3 MHV-68 protein purified from BL21 (DE3) strain (98,4%), thus its activity could be also affected by the way the protein is being folded. The work done showed that *E.coli* expression system is suitable to produce biologically active M3 protein of MuHV-4.

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UNUSUAL BEHAVIOR OF *CANDIDA PARAPSILOSIS* CDR1-2 DOUBLE DELETION MUTANT AGAINST IMMUNE CELLS

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The incidence of invasive fungal infections has significantly increased over the past 30 years, with the *Candida* genus representing the most common cause of disease. *Candida parapsilosis* is now the second or third most common cause of bloodstream infections in intensive care units. Triazoles are commonly used to combat diverse forms of candidiasis. The prolonged and frequent use of azoles drugs has led to the development of multidrug resistance (MDR) mechanisms in *Candida*. Several mechanisms have been characterized that contribute to MDR in these yeasts. One of these mechanisms is the overexpression of efflux pump proteins that function as ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters. Major *C. albicans* ABC-transporters involved in azole resistance are candida drug resistance protein 1 and 2 (Cdr1p, Cdr2p). In contrast of the well studied *C. albicans* ABC-transporters, little is known about the role of CDR genes in *Candida parapsilosis*. To clarify the significance of CpCDR, we have generated *C. parapsilosis* CDR1-CDR2 (CpCDR1-CDR2) double deletion mutant and determined the minimal inhibitory concentration (MIC) for amphotericin B, fluconazole and caspofungin. Our results showed decreased MIC for fluconazole and caspofungin, respectively. Flow cytometry experiments using calcein dyeing was performed to determine the structure specificity of Cdr1p and Cdr2p beside the ABC-transporter superfamily. Our data demonstrated that the double deletion mutatns were stained on a higher level than the wild tipe, respectively. This staining feature is typical to a mammal ABC-transporter called MDR1. The knock out mutants showed similar sensitivity against osmotic and oxidative stress to that of the wild type. Interestingly, the killing of CDR1-2 deletion mutant yeasts by murine monocyte-like cells (J774.2) significantly decreased relative to WT. CDR1-2 deletion yeast cells were more resistant against the host killing mechanisms during infection of A/J mice, suggesting involvement of these transporters in the pathobiology of the fungus.

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SYNERGISTIC INTERACTIVE EFFECTS OF PATULIN AND CITRININ MYCOTOXINS IN FISSION YEAST

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Contamination of food and feeds by different mycotoxins pose a major risk to human and animal health and are also detrimental to the economy. Although numerous studies investigate the environmental, toxicological and epidemiological aspects of the different fungal metabolites separately, in most cases more than one mycotoxin is found in the same contaminated commodities, which represents a cumulatively hazardous risk. To date, only little information is available on the interaction of simultaneously produced fungal secondary metabolites. The present study focuses on the interaction and accumulation of reactive oxygen species (ROS) produced by two, potentially co-

occurring mycotoxins in foodstuffs, patulin (PAT) and citrinin (CTN) in the fission yeast *S. pombe* model system. To determine the possible interactive effects of the mycotoxin mixtures, checkerboard titration was performed from 0-1000 μM concentration range. In most of the cases, in the presence of high CTN or PAT concentrations (1000 μM) antagonistic interactions were observed; however, lower concentration combinations mainly resulted in synergistic effects. Equimolar synergistic combinations were tested by the determination of the survival rates after a 1 h treatment. 250 μM PAT and 250 μM CTN treating concentrations were chosen for further investigations, where 80% of cell survival was observed. 1.5-fold increased total ROS concentration was observed in the presence of both toxins after the exposure of the 1 h treatment of exponentially growing 10^7 cells ml-1 that was supported by the 3.5-fold elevated level of peroxides. Although PAT (50, 500 and 1000 μM) increased the superoxide level alone; nevertheless, higher CTN concentration (1000 μM) alone did not change it significantly. In comparison the combination of the two mycotoxins did not demonstrate significant alteration in superoxide level. Compared to the results of our previous experiments, PAT (500 μM) certainly decreases the concentration of glutathione by 41.6%, but CTN (1000 μM) elevates it by 202%. The combination resulted in a 400% increase in the main intracellular antioxidant agent of the cell. Based on these results, the effect of the examined mycotoxins on each other could change their original impact and the altered ROS generating profile may result in a higher degree of toxicity.

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CAROTENE BIOSYNTHESIS GENES OF A *MUCOR RACEMOSUS* STRAIN ISOLATED FROM CHEESE PROCESSING

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Carotenoids are used in the food, pharmaceutical and cosmetic industries. Due to their beneficial effects on human health, there is an increasing interest for the discovery and development of new carotenoid sources from microbial origin. Species belonging to the fungal group Mucoromycotina are considered as good beta-carotene producers and some of them, such as *Phycomyces blakesleeanus*, *Blakeslea trispora* and *Mucor circinelloides*, have been involved in the study of the microbial carotenoid biosynthesis. Several Mucor species are used in different food fermentation processes, where production of carotenoid compounds can be a favourable feature. Unfortunately, mucoral fungi used in food processing are poorly characterized. Recently the genome sequence of a *Mucor racemosus* strain isolated from cheese ripening processes was determined. In the present study, carotenoid content and composition of this strain was analysed by HPLC. The fungus produces beta-carotene as the main carotenoid compound in significant amount. Based on the genome database of this fungus, sequences of several genes participating in the carotenoid biosynthesis, such as those of isopenthenyl pyrophosphate isomerase, farnesyl and geranylgeranyl pyrophosphate synthases, HMG-CoA reductase, phytoene dehydrogenase (*car*B) and phytoene synthase/lycopene cyclase (*car*RP), were analysed and compared with the corresponding genes of *P. blakesleeanus* and *M. circinelloides*. Transcription of certain genes under different cultivation conditions were also examined by quantitative real-time PCR analysis. Knowing the genetic background of the carotenogenesis could open the way for further strain improvement studies.

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IMPLICATION OF SINGLE STRANDED DNA BINDING PROTEIN IN CHROMOSOME SEGREGATION DURING REPRODUCTIVE GROWTH OF *STREPTOMYCES COELICOLOR*

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Single stranded DNA binding proteins (SSB) proteins are essential for cell survival. SSB proteins, identified in all domains of life, exhibit their role through binding and protecting single stranded DNA. During DNA replication, recombination and repair SSB proteins interact and modulate the functions of various proteins involved in all aspects of DNA metabolism. Comprehensive analysis of SSB interactions in *E. coli* and *B. subtilis* showed its prominent role in the complex protein network responsible for genome maintenance. Our recent bioinformatics analysis of all available bacterial genomes revealed presence of the ssb parologues in many bacteria. In addition, these results indicated that evolution of SSB proteins in Eubacteria is highly dynamic. However the role of duplicated ssb genes is poorly studied. Therefore, in this study we selected *Streptomyces coelicolor*, bacterium with complex life style that also possesses two SSBs, as a good model system to study biological role(s) of paralogous SSB proteins. We demonstrated that two *ssb* genes in *S. coelicolor* are differentially regulated. Expression of *ssb*A is constant and high during life cycle, decreasing towards late stationary phase, while the expression of *ssb*B is at all-time low. In minimal medium the *ssb*B is significantly up regulated. Promoters for both genes are determined. Surprisingly, promoter of *ssb*B possesses unusual features and is active in taxonomically distant bacteria, *Escherichia coli*. Both genes were disrupted on the genome and our results showed that SsbA is essential for survival while SsbB is important during sporulation process. The 3D structure of SsbB was solved, showing an interesting variation in comparison to quaternary structure of the previously published *S. coelicolor* SsbA protein. To the best of our knowledge this is the first example of solved crystal structures of two paralogous SSB proteins from the same organism.

MICROARRAY ANALYSIS OF A FORKHEAD TRANSCRIPTION FACTOR OF *SCHIZOSACCHAROMYCES POMBE*

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The forkhead proteins are a family of the transcription factors that play important roles in regulating the expression of genes, involved in cell growth, cell division, sexual differentiation or in embryonic development. [1] [2] The forkhead family of proteins contain a highly conserved Fork Head domain that are widespread in eukaryotes, found throughout yeast and human.
To understand the process of regulating of forkhead transcription factors, researchers use model organisms, such as Saccharomyces cerevisiae or the fission yeast *Schizosaccharomyces pombe*. Earlier we started to characterize the *Fhl*1 forkhead gene of *Sch. pombe*. It revealed that deletion of this gene lead to slow cell growth, delayed G2-to-M transition and modify the response of environmental stresses[1]. Our aim was to carry on these experiments and gain more information about function of the *Sch. pombe Fhl*1 gene. A microarray analysis of the Fhl1 deleted strain was carried out. Our results suggest that *Fhl*1 gene display a remarkable function in meiosis as regulates the *ste*11 gene. It also influences the transport mechanisms and kinases. Its target genes can be found at all chromosomes. Both down- and up regulated genes appeared among its targets.

[1] Szilágyi, Z. et al. (2004) Gene 28: 101-109.
[2] Carlsson, P., Mahlapuu, M. (2002) Dev Biol 250: 1-23.

*RSV*1 GENE HAS FUNCTIONAL HOMOLOGY IN THE *SCHIZOSACCHAROMYCES* SPECIES

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Transcriptional regulators are involved in the regulation of gene expression. They are usually bound to the special sites of DNA sequence and hereby allow for a sophisticated response to multiple conditions in the environment. One of the transcriptional regulatory proteins is the rsv1, which is partly characterised in *Sch. pombe*. It is a negative regulator of cellular carbohydrate metabolic process. It encodes a zinc finger protein, which is required for cell viability in stationary phase induced by glucose exhaustion. Cells lacking rsv1+ are viable and the gene is regulated by pka1. [1]
The four fission yeast species form an early-branching clade among the Ascomycete. One of them is the *Sch. pombe*, which is a well-known model organism. Its relatives, such as *Sch. japonicus*, *Sch. octosporus* or *Sch. cryophilus* can also be attractive models of the genetic studies. We wanted to get information about the role of *Sch. octosporus rsv*1 gene. As there is not *rsv*1 mutant strain at this moment in this species, its characterisation is impossible. Therefore, we used the quantification of sequence similarity and interspecific complementation analysis to prove the function of *Sch. octosporus rsv*1 gene. The BLASTp analysis suggested, that SOCG_03341 gene must be the *rsv*1 gene. However, sequence-based functional predictions are reliable, not all homologs have the same function. Therefore, we cloned the *Sch. octosporus rsv*1 gene into a *Sch. pombe* vector, and this DNA construction was transformed into the *Sch. pombe rsv*1 mutant strain.
The characterisation of the transformants proved the functional homology between the *Sch. pombe rsv*1 and *Sch. octosporus* SOCG_03341 gene.

[1] Zhonglin Hao, et al. (1997) J Cell Sci 110: 2557-2566

ANALYSIS OF THE PHYSIOLOGICAL ROLE OF *cab*B, A CA^{2+}-BINDING PROTEIN IN *STREPTOMYCES COELICOLOR*

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Streptomycetes are Gram-positive, obligate aerobe, soil bacteria well known for their complex morphological differentiation process which is coupled to the production of a wide variety of secondary metabolites with medical importance. On solid medium spores of *Streptomyces coelicolor* A3(2) germinate to produce a complex network of apically growing filamentous hyphae. Subsequently this substrate mycelium give rise to aerial hyphae, which undergo septation to form chains of monogenomic, thick walled spores. Complete understanding of the regulation of differentiation and antibiotic production would require the characterization of regulatory mechanisms and signaling pathways present in *Streptomyces*. Ca^{2+} is the most common signal transduction element in pro- and eukaryotic cells. Cytosolic free [Ca^{2+}] is tightly controlled and its effect is often mediated by EF-hand type Ca-binding proteins (EFCaBP), such as the ubiquitous calmodulin. Ca^{2+} was reported to be involved in the regulation of spore germination and aerial hypha formation in streptomycetes; however, the underlying mechanism of this regulation is not known. At least four genes coding for EFCaBP with unknown biological functions are present in the *S. coelicolor* genome (*cab*B-D), previously it was suggested that they act as Ca^{2+}-buffer systems. We have found that spore germination, growth and sporulation was also sensitive to calmodulin antagonists in *S. coelicolor*, suggesting that EFCaBP mediated regulation might be involved in these processes. In order to analyze its physiological role the *cab*B gene, coding for the calmodulin homologue CabB, it was disrupted with an apramycin cassette by PCR-targeted mutagenesis to produce the *S. coelicolor Δcab*B::*apr*a mutant. Its phenotype was analyzed by different microscopic techniques (SEM, CLSM, AFM). Disruption of the *cab*B gene resulted in a conditional pleiotropic phenotype. Sporulation was delayed and significantly inhibited in the mutant compared to that of the wild type. The mutant phenotype was more pronounced on rich medium, especially under stress conditions. Spore morphology was also affected, the mutant spore compartments were frequently deformed and smaller in size, especially in rich and osmotically enhanced medium. More importantly, the distribution of the genetic material was also affected, mutant spore compartments were frequently in lack of DNA. The *Δcab*B::*apr*a mutant produced lower amount of actinorhodin in liquid cultures, however, this phenomenon was conditional. Using calmodulin-agarose affinity chromatography, 2D-SDS-PAGE, in-gel digestion and MALDI-TOF analysis, we have identified the DnaK and EshA proteins as possible downstream effectors of calmodulin-like EFCaBPs connecting Ca^{2+} signaling to the stress response and morphological differentiation in *S. coelicolor*. We have also found that cellular crude extract of S. coelicolor was able to substitute calmodulin in eukaryotic systems, providing indirect evidence that prokaryotic EFCaBP might have more specific biological role, than buffering intracellular [Ca^{2+}]. Our results demonstrate that CabB is involved in Ca^{2+} mediated regulation of morphological differentiation and antibiotic production in *S. coelicolor*.

BIOLOGY AND APPLICATION OF *CAMPYLOBACTER* BACTERIOPHAGES

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Campylobacters are leading cause of foodborne diseases in EU. Main source of infection is considered poultry meat contaminated by *Campylobacter* that colonizes their intestine to high level. It has been calculated that a 100-fold decrease of *Campylobacter* load on farm level would result in 30-fold reduction in the incidence of campylobacteriosis. Bacteriophages and their derivates have a clearly demonstrated antibacterial applicability. Treatment of broiler chicken with bacteriophages reduces number of Campylobacters for 1-4 log10. However, for successful implementation of bacteriophage application, a deeper understanding of *Campylobacter* bacteriophage biology and ecology is needed. At the same time *Campylobacter* bacteriophage production issues remain to be addressed. Presentation will discuss basic biology properties of *Campylobacter* bacteriophages and efforts to resolve certain bottlenecks for their application. Isolation and deep characterization of bacteriophages is a prerequisite for their application as therapeutic or biocontrol agents. The best sources for bacteriophage isolation are environments where the host is highly prevalent. We will compare different strategies and protocols for *Campylobacter* bacteriophage isolation. Characterization of bacteriophages in terms of lytic spectra, protein profile, genome organization, and morphology will be described for specific bacteriophages and importance of characteristics for bacteriophage application will be presented. We will describe phage-host cell interaction, emphasise development of bacterial resistance and its significance for bacteriophage application. Bacteriophage production challenges such us propagation, concentration and formulation will be presented and discussed. We compare and discuss application strategies for bacteriophages and their derivates.

STOCHASTIC CHARACTER MAPPING OF CARBON SOURCE UTILIZATION DATA ON A MULTIGENE PHYLOGENETIC TREE OF THE MORTIERELLALES

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Members of the Mortierellales belong to one of the largest groups of the former phylum Zygomycota. Most of the species can be isolated from soil where – like many other organisms – they act as decomposers of various organic materials. Some of them are also known as opportunistic animal pathogens (such as *Mortierella wolfii*) by causing severe diseases of the respiratory tract or fetal abortion. On the other hand, several species are used by the biotechnological industry as producers of polyunsaturated fatty acids (such as *M. alpina*).

Unfortunately, little is known about the phylogeny and evolution of this interesting and early group of terrestrial fungi. In this study, a phylogeny of the order was inferred from a combined data set of two protein coding genes (tef and RPB1) and three ribosomal sequences (the nrSSU and nrLSU genes and the complete ITS region). A study focusing on the carbon source utilization and the morphological patterns (e.g. shape of the colony, colour, appearance of sporangia) of these fungi was also performed. Carbon sources had been separated into two groups according to their effects on the isolates: inhibiting (0) or utilizable (1). This binary data matrix was documented. We tested 67 different carbon sources from which 39 showed larger differences in their effects. The phylogenetic data and the results of the carbon source utilization tests were combined by mapping the binary characters on the tree using the softwares Mesquite and SIMMAP. Our results show that utilization capabilities appeared independently in many cases. We also found that members of the closely related Mucorales show differences in their carbon source utilization patterns.

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IN VITRO EFFECT OF NON-ANTIFUNGAL COMPOUNDS ON *CRYPTOCOCCUS NEOFORMANS*

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The opportunistic pathogenic yeast *Cryptococcus neoformans* is the causative agent of a serious and potentially lethal infection, cryptococcosis. The most common manifestation of the illness is meningitis. The infection develops in immuno-compromised patients. The prevalence of the disease has been increasing during the last years along with the growing number of patients with defective cell-mediated immunity. The mortality rate of cryptococcosis is still very high despite extensive antifungal therapy. Nowadays, the increasing number of antibiotic resistant fungal species raises the importance to search for alternative treatments against fungal infections. In this study the in vitro inhibitory activity of amantadine hydrochloride, R-deprenyl hydrochloride, valproic acid sodium salt, chlorpromazine and trifluoperazine was tested against *Cryptococcus neoformans* isolates with broth dilution method. Amantadine hydrochloride and R-deprenyl hydrochloride had only slight effect on the growth of *C. neoformans* in the applied concentrations but chlorpromazine and trifluoperazine exerted complete inhibition at 100 μg/ml while valproic acid at 125 μg/ml concentration. The combination of valproic acid, chlorpromazine and trifluoperazine with acetyl salicyl acid was tested with checkerboard titration method; additive interaction was demonstrated.

GENOMIC AND BIOINFORMATICS ANALYSIS OF SIMIAN ADENOVIRUS 19 CONFIRMS THE NEED TO ESTABLISH A NEW ADENOVIRUS SPECIES

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Adenoviruses (AdVs) are icosahedral, non-enveloped viruses with double stranded, linear DNA genome. Because of their widespread occurrence in a variety of vertebrate hosts, AdVs make an ideal model for the study of viral evolution. They also keep gaining popularity as gene delivery vectors to be used as recombinant vaccines or therapeutic agents in genetic diseases and tumors. However, preexisting antibodies in the human population significantly limit the medical use of human AdVs (HAdVs). In the framework of an EU-financed project, we seek animal AdVs that could serve as appropriate alternatives. Our research group is also interested in the taxonomy of the Adenoviridae family. More than 50 HAdV types are classified into seven HAdV species (HAdV-A to HAdV-G) within the genus Mastadenovirus. Certain HAdV species do also contain chimpanzee or other ape AdVs; HAdV-G contains HAdV-52 and several monkey AdVs.

While HAdVs are among the best-studied viruses, the AdVs of the more ancient simian lineages especially those of the New World monkeys are hardly known. Species Simian adenovirus A (SAdV-A) is so far the only species officially approved for monkey AdVs exclusively.
The purpose of the present work was to examine the genetic content and phylogenetic relationships of Old World monkey AdV (SAdV-19) isolated from yellow baboon (*Papio cynocephalus*). To this end, full genome sequencing of the prototype strain (VR-275) of SAdV-19 was performed. Consensus and specific PCR primers were applied to amplify conserved genome fragments and to connect them, respectively. The SAdV-19 genome was found to consist of 34,063 bp with an average G+C content of 52%. Every gene characteristic of the genus Mastadenovirus, including that of the hypothetic agnoprotein, could be identified.
Among the 38 putative genes, we found a single VA-RNA gene, and two genes of different lengths predicted to code for the adenoviral cellular attachment protein, the fiber. For the first time in SAdVs, the two other exons belonging to the so-called U exon were also identified. Since the initiation of this project, genomic study of several novel AdVs from Old World monkeys has been published by others. These included two new baboon AdV strains (BaAdV-2 and BaAdV-3), and some others proposed to form novel species Simian adenovirus C (SAdV-C) and Simian adenovirus B (SAdV-B), respectively. Phylogenetic calculations based on the major capsid protein, the hexon, implied that SAdV-19, BaAdV-2 and BaAdV-3 represent three different (sero)types within the proposed species SAdV-C. Phylogeny inference based on the viral DNA-dependent DNA polymerase protein further supported this species classification. However, significant divergence was found between the shorter fiber proteins (fiber1) of SAdV-19 and BaAdV-2 or BaAdV-3. The closest relative of fiber1 of SAdV-19 in the GenBank was that of SAdV-1, sharing ~47% amino acid sequence identity. This finding may reflect that a usually rare, inter-species homologous recombination event took place between the two viruses (or their ancestors or relatives) in the past.

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EFFICIENT *DE NOVO* SEQUENCING AND TYPING OF MICROBIAL GENOMES WITH NEXT-GENERATION SEMICONDUCTOR SEQUENCING

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Next-generation sequencing technologies have tremendously facilitated in the last years to access full genome sequence information of smaller genomes such as bacteria or virus. The long-read length capabilities of the Ion Torrent semiconductor sequencing platforms are ideally suited for simple and economic *de novo* sequencing of small genomes or for metagenomic analysis. An review of technology and workflows for rapid *de novo* sequencing, microbial typing and 6S rRNA metagenomic analysis using the Ion PGM™ next-generation sequencing platform will be presented.

EMERGENCE OF SANDFLIES (PHLEBOTOMINAE) IN AUSTRIA, A CENTRAL EUROPEAN COUNTRY

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The possible existence of autochthonous sandfly populations in Central Europe north of the Alps has long been excluded. In the last decade the occurrence of sandflies has been documented in Germany, Belgium and recently also in Austria close to the border to Slovenia indicating spreading tendencies of this vector towards Northern Europe. Meanwhile autochthonous human *Leishmania* and Phlebovirus infections have been reported in Central Europe, particularly in Germany. From 2010 to 2012, sandfly trapping was performed at 53 different capture sites in Austria, in the federal states Styria, Burgenland, and Lower Austria (740 trap nights). Collection bags from battery-operated CDC miniature light traps were collected and inspected daily in the morning. Sandfly specimens found were transferred to 70% ethanol for conservation. Identification was based on morphological characters of the male genitalia and the female spermathecae, respectively. Altogether, 24 specimens, all identified as *Phlebotomus* (*Transphlebotomus*) *mascittii* Grassi, 1908, were found at 6 different sampling sites in all three federal states investigated. During the entire study period, the earliest capture was made on July 3rd and the last on August 28th. Sandflies are apparently much more widely distributed in Austria than previously assumed and the period of sandfly activity in Austria is long enough to allow *Leishmania* transmission to vertebrate hosts.
These findings are of high relevance for the emergence of sandfly-borne diseases in Central Europe, not only with respect to transmission of *Leishmania* spp., for which reservoir animals exist in Austria, but also of other pathogens, in particular phleboviruses.

PLANT VIRUS AND HOST PROTEIN INTERACTIONS IN RELATION TO DISEASE SYMPTOM DEVELOPMENT

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Cucumber mosaic virus (CMV) is member of the Cucumovirus genus and has extremely wide host range. CMV particle has an isometric shape and its genetic material consists of 3 positive stranded RNA molecules. The genomic RNAs encode five proteins. Based on our previous results significant differences were found in induced disease symptoms on *Nicotiana clevelandii* test plants when the R-CMV isolate and a mutant virus strain (R3E79R), containing a single amino acid mutation in the coat protein (CP) sequence was used. Replacement of Glu79 to Arg79 has caused electrostatic potential change on the virion surface. These amino acid side chains are located in the middle of the outer BC loop of the CP. As long as R-CMV strain causes mosaic disease and malformation, the mutant causes chlorotic lesions on the inoculated leaves and strong stunting and necrosis on the systemic leaves leading to complete destruction of the entire plants. Proteomics analysis of virus-

host plant protein interactions have been performed in order to get possible explanation for the differences in symptom development. *Nicotiana clevelandii* plants have been inoculated with the wild and the mutant type of CMV. Purified proteins from the partially purified virion preparations were separated by isoelectric focusing in range pH3-10 and on SDS-PAGE by molecular masses seven days after the inoculation in three biological and three technical replicates. Protein patterns resulted in significant differences in protein extracts of plants infected by the wild-type and the mutant virus isolates. Several protein spots visualised in different amount on the gels were isolated and have been identified using Edman sequencing and LC-MS-MS. Numbers and significant intensity of protein spots showed remarkable differences between the two isolates in the 50-75 kDa molecular mass range, resulting in more proteins of the mutant type of R-CMV. Edman sequencing is resulted mainly putative movement protein, hairpin inducing protein, RUBISCO and pathogen-inducible alpha-dioxygenase. Results obtained by Edman sequencing suggest that mutation of the coat protein affects virus-host protein interactions. Principally the mutation of coat protein induces hypersensitive response (HR) and strong necrotic symptoms on the systemic leaves which presumably is connected to the different electrostatic potential of the virions by affecting their interactions with cellular proteins. The proteins identified by mass spectrometry in most cases represented the ATP synthase alpha- and ATP synthase beta subunits. The ATP synthase is one of the most important housekeeping enzyme of eucaryotic cells. On the basis of the 2D gel electrophoresis results we can conclude that the mutant virus completely blocks the ATP synthase function in the infected plant cell. This strong and invasive protein-protein interaction finally leads to cell death suggesting that the plant cell cannot express enough new ATP synthase subunits to replace the CMV blocked ATP synthase complexes.

EFFICACY OF A NOVEL EBV PEPTIDE VACCINE

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The goal of an EBV (Epstein Barr virus or HHV 4) vaccine may be either prevention of infectious mononucleosis, or modification of the immune status in order of treating chronic fatigue syndrome and/or prevention of EBV-related oncogenesis. We had already reported the design of pathogen-mimicking Stealth MicroparticlesTM- PMM. The PMMs are microbeads coupled with pattern recognizing receptor agonists (non-specific stimulants) in combination with the specific EBV peptides. In comparison to 87 epitopes stimulating the B and T lymphocytes which were described so far, for this study 67 were carefully selected by a computer program in way that some of them were coming from EBV polypeptides not tested by others. Chemokine production in dendritic cells G106 was followed for 7 interleukins in vitro. Then the rabbit model of in vivo infection was elaborated in 21 rabbits. The results (which had been reported previously) confirmed that despite of the absence of a typical IM syndrome, at least 4 reliable signs of productive infection could be identified. These were: l. Early-antibody production. 2. Presence of antibodies against EBV proteins as detected by immunoblot. 3. Presence of EBV DNA within the white blood cells (as detected by PCR). 4. Expression of LMP1 antigen in correlation with EBV DNA (as seen by indirect IF). Here we report the efficacy results based on statistical analysis of the positive rates of above mentioned signs found in the immunized rabbits following virus challenge as compared to controls. Together 19 animal groups (3 rabbits each) and a small control group (3 rabbits) were included into the

protection test (total of 60 animals). At least sampling 4 intervals were examined (1 sample taken before and 3 samples taken after challenge). Using the four selected methods, altogether 960 data were obtained at the challenge experiment. These data were also compared with those coming from the large control group (336 data previously reported). Statistical analysis of the positive rates within individual animal groups showed that at least 6 encrypted epitope combinations (from immunization groups 1, 6, 12, 15, 16 and 18) revealed convincing protection (total positive rate from all results was lower than 10 %, difference to the controls at P value lower than 0.001). One epitope combination (from the immunization group 13) showed a less convincing protection effect since the IB results did not differ significantly. In contrast, 4 immunization groups (2, 5, 7, 11) revealed no protection (in any signs compared), while the rest of epitope combinations (8 immunization groups numbered 3, 4, 8, 9, 10, 14, 17, 19) had a minimal effect only (but still differing at P = 0.05). The 7 most efficient epitopes included into the 6 animals groups showing the most convincing protection, were coming from 3 structural and 4 non-structural polypeptides. When comparing them with those described in the literature, 1 non-structural lytic protein and 1 envelope glycoprotein were found possessing a novel immunogenic epitope not yet described by others.

BIODETOXIFICATION OF ZEARALENONE BY EXTRACELLULAR EXTRACTS OF *RHODOCOCCUS SPECIES*

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Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by species of *Fusarium*, which colonize maize, barley, oats, wheat and sorghum and have been implicated in numerous mycotoxicoses, induces mainly reproductive disorders in farm animals or even in humans. Elimination of mycotoxins from food and feedstuffs is an urgent issue, and biodetoxification is an appropriate way for this purpose. During this processes microorganism and enzymes are involved in mycotoxin degradation. In a previous study bioluminescent bioreporters (*Saccharomyces cerevisiae* BLYES and BLYR) were successfully used to monitor toxin degradation; the results of zearalenone biodegradation experiments were confirmed by parallel chemical analysis (HPLC-FLD) and immunoanalytical (ELISA) tests. The most promising strains belong to *Rhodococcus* genera. The Qualified Presumption of Safety (QPS) concept provides a generic assessment system for use within EFSA (European Food Safety Authority) that in principle can be applied to all requests received for the safety assessments of microorganisms deliberately introduced into the food chain. Thus EFSA maintains a list of the biological agents to which the QPS assessment can be applied. In case of *Rhodococcus* species the bacteria are not allowed to apply as feed additives, thus enzyme-based formulation would be a successful solution for mycotoxin elimination. In the present study further researches were carried out with extracellular extracts of *Rhodococcus* species to reveal enzymatic processes. For that reason after growing cultures supernatant and pellet were separated and 1 ml supernatants were contaminated with ZEA to reach 1 mg/l final concentration. Extracellular matrixes with the ZEA were incubated at 28°C for 7 days, and samples were taken in every 24 hours for monitoring ZEA degradation. To reveal induced and constitutive enzymes, cultures were grown with or without ZEA in the first step before separating supernatant and pellet. To determine whether enzymes taking part in the degradation are extra- or intracellular, samples were treated in different ways: to get extracellular enzymes supernatant was applied, to get intracellular enzymes cells were destroyed by sonication. On the base of our experiments culture did not need pre-incubation with ZEA for the degradation, since within five days extracellular matrix of three strains

could cease the estrogen effect of ZEA when cells were destroyed by sonication. Results indicated that the degradation is enzymatic and that the enzymes responsible for the degradation of ZEA are constitutively produced. However, the biodetoxification was not manifested when pure supernatants was applied, thus intracellular enzymes may be involved in the biodegradation.

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CHICKEN INNATE IMMUNE RESPONSE TO ORAL INFECTION WITH *SALMONELLA ENTERICA* SEROVAR

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The characterization of the immune response of chickens to *Salmonella* infection is usually limited to the quantification of expression of genes coding for cytokines, chemokines or antimicrobial peptides. However, processes occurring in the cecum of infected chickens are likely to be much more diverse. In this study we have therefore characterized the transcriptome and proteome in the chicken cecum after infection with *Salmonella Enteritidis*. Using a combination of 454 pyrosequencing, protein mass spectrometry and quantitative real-time PCR, we identified 48 down- and 56 up-regulated chicken genes after *Salmonella Enteritidis* infection. The most inducible gene was that coding for MMP7, exhibiting a 5952 fold induction 9 days post-infection. An induction of greater than 100 fold was observed for IgG, IRG1, SAA, ExFABP, IL-22, TRAP6, MRP126, IFNγ, iNOS, ES1, IL-1β, LYG2, IFIT5, IL-17, AVD, AH221 and SERPIN B. Since prostaglandin D2 synthase was upregulated and degrading hydroxyprostaglandin dehydrogenase was downregulated after the infection, prostaglandin must accumulate in the cecum of chickens infected with *Salmonella Enteritidis*. Finally, above mentioned signaling was dependent on the presence of a SPI1-encoded type III secretion system in *Salmonella Enteritidis*. The inflammation lasted for 2 weeks after which time the expression of the "inflammatory" genes returned back to basal levels and, instead, the expression of IgA and IgG increased. This points to an important role for immunoglobulins in the restoration of homeostasis in the cecum after infection.

DEGRADATION OF ANILINE AND PHENOL DERIVATIVES IN SOIL BY A CRUDE *GANODERMA* LACCASE PREPARATE

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Laccases are copper containing oxidoreductases, able to oxidise a wide range of distinct aniline and phenol derivatives. This offers an environment-friendly enzymatic method for remediation of soil and water habitats, polluted with these types of xenobiotics. In our study, fruiting bodies of the white rot fungus *Ganoderma* were collected from decaying woods at different areas of Csongrád County and their laccase production were investigated. Previously, the strains were identified with molecular methods. The laccase production of the isolates was tested under different conditions. After optimizing the enzyme production, the detoxification abilities of the laccase containing

ferment broths against aniline and phenol compounds were studied. Crude enzyme extracts were prepared by using filtration, freezing and lyophilisation. Degradation of different aniline and phenol derivates (2,4-dichlorophenol, 2-methyl-4-chlorophenol, aniline, 3-chloroaniline, 3,4-dichloroaniline, 3-chloro-4-methylaniline and 4-isopropylaniline) were investigated in three distinct soil types with the crude enzyme preparate. The amount of the xenebiotics in the soil was determined with UHPLC method. In some cases (2,4-dichlorophenol, 3-chloroaniline, and 4-isopropylaniline) the crude preparate accelerated the degradation of the xenobiotics in meadow soil and in sandy soil, but in forest soil it disturbed the soil own degradation systems except of the degradation of 2-methyl-4-chlorophenol and 3,4-dichloroaniline.

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DIVERSITY OF THE CYTOCHROME B GENE COINCIDES WITH CHANGES IN THE QUINOL OXIDATION INHIBITOR RESISTANCE OF HUNGARIAN *BOTRYTIS CINEREA* ISOLATES

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Each eukaryotic cell contain lots of mitochondria. In case of heteroplasmy, both wild-type and mutant mitochondrial DNA can be detected within one cell or organism. Genes of the mitochondrial DNA primarily encode the enzymes of cellular respiration. Fungicides belonging to the family of quoinon oxidase inhibitors (QoIs) 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. These fungicides therefore inhibit the mitochondrial respiration and consequently they inhibit ATP synthesis. The QoI resistance operates via at least two mechanisms. One is the point mutation of the cytochrome b gene (cyt b), e.g. the substitution of a single glycine by alanine at position 143 results in high-resistance. The other is the cyanide-resistant alternative respiration sustained by the alternative oxidase. Whether the mutation in the mitochondria causes phenotypic diversity or not depends on the dose, i.e. it depends on the percentage mitochondrial DNA that suffered mutation upon treatment. We investigated QoI resistance and related cytochrome b gene genotypes in Hungarian group I and II B. cinerea populations. Allele-specific PCR reaction and PCR-RFLP method were used to confirm the presence of the G143A mutation. The development of the QoI resistance was induced by using mediums with increasing concentrations of azoxystrobin. Using real time PCR we developed a method to track heteroplasmy. Single copy gene located in the mitochondrial genome (cox1) was used as a standard. The existence of an alternative group I-type intron in the cyt b gene immediately after the codon 143 was suggested by PCR fragment length analysis. All isolates, where the G143A mutation could be detected with both PCR-RFLP and allele-specific PCR, showed high resistance against azoxystrobin. In a few cases, the mutation could only be detected with allele-specific PCR i.e., not with PCR-RFLP, and these strains were 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 could induce resistance toward

azoxystrobin. We related the change in the ratio of the mitochondrial DNA containing the G143A point mutation compared to the sensitive, "wild" sequence.
Botrytis cinerea can easily develop resistance against single-target fungicides, like QoIs. The reason for this is the extreme genetic variability and the short life cycle of the fungus. In most cases the high resistance against QoIs comes along with one particular G-to-C point mutation in the mitochondrial cyt b gene. As expected, many Hungarian *Botrytis cinerea* field isolates carried the G143A mutation which causes intense resistance. The level of the heteroplasmy was able to vary in short period of time which led to changes in the phenotype.

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FIRST TAXONOMICAL SURVEY OF FUNGI ISOLATED FROM GRAPEVINE TRUNK DISEASES IN THE TOKAJ WINE REGION, HUNGARY

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Grapevine Trunk Diseases (GTD) are amongst the most important diseases in vineyards. GTD have been characterized only recently because of a worrying increase of the frequency of their symptom expression worldwide. Esca-, Petri- and black-foot disease, *Eutypa*- and *Botryosphaeria*-dieback are considered as major GTDs, are caused by a variety of pathogens which attack the woody perennial organs of the vine and ultimately lead to the death of the plant. Control of GTD is problematic, no grapevine cultivar is known to be resistant to GTD and there are no highly effective treatments available. The present knowledge of epidemiology, host-pathogen interaction and microbial ecology is still poor. The objective of this study was to isolate and identify fungi associated with symptoms of GTD and from canes of healthy grapevines in vineyards from the Tokaj wine region. The plantation studied is 21 years old of grapevine variety called Furmint. Four rows of grapevine were chosen for the collection of samples. Pure fungal cultures were used for morphological and molecular identifications of fungal species. Chips were cut, disinfected and incubated on potato dextrose agar medium at room temperature. Fungal DNA was obtained from pure fungal colonies after 3 days of growth on 50 ml potato dextrose broth on a rotary shaker (125 rpm). Mycelia were harvested by vacuum filtration. Total genomic DNA was extracted from freeze-dried mycelium and isolated using NucleoSpin Plant II (Macherey-Nagel) according to the protocol, followed the manufacturer's instructions. DNA concentrations were measured by NanoDrop (Thermo Scientific) and used in PCR amplification of the internal transcribed spacer region (ITS) and the 5.8S ribosomal subunit with primers ITS4 and ITS5 [1]. The PCR products were purified by using Nucleospin Gel and PCR Clean-up (Macherey-Nagel). Purified amplification products were sequenced by MWG Biotech Company in Germany. Sequences obtained were assembled and manually corrected. Reference sequences for the Botryosphaeriaceae were obtained from GenBank. Phylogenetic analyses were performed with MEGA. One thousand boostrap analyses were run to determine confidence levels at the branching points. The principal aim of this work is to assess the GTD types and their occurrence in the Tokaj wine region as well as the identification of the pathogenic fungi (isolated from symptomatic and asymptomatic grapevine trunks) on morphological and genetic basis. In this study, 101 fungal isolates were identified from grapevine woody tissues.

The majority of them (72.3%) were determined as member of Botryosphaeriaceae, 66 sample were determined as *Diplodia seriata*, 6 *Botryosphaeria stevensii*. Other fungi, like *Alternaria*, *Mucor*, *Phoma*, *Phomopsis*, *Diaporthe* and *Xylaria* also were identified from grapevine samples.

[1] White, T.J. et al. (1990) In: Innis, M.A. et al. (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322.
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SYMPATRIC DIFFERENTIATION OF *BOTRYTIS CINEREA* POPULATIONS ON TWO HOST PLANTS

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The necrotrophic phytopathogenic ascomycetous fungus *Botryotinia fuckeliana* (de Bary) Whetzel – anamorphic stage, *Botrytis cinerea* Pers. Fr. – causes grey mould disease worldwide. *B. cinerea* was previously considered a generalist fungal plant pathogen, infecting more than 220 host plants worldwide. In phylogenetical-taxonomical terms, the pathogen is considered a complex of two cryptic species, group I (*B. pseudocinerea*) and group II (*B. cinerea* sensu stricto). Recently, significantly structured populations specialized on the host plants were described, suggesting sympatric specialization [1]. We studied populations of *B. cinerea* on sympatric strawberry and raspberry cultivars in the North-East of Hungary for three years during flowering and the harvest period. Altogether, 490 group II *B. cinerea* isolates were analyzed. Three different data sets were generated: (i) PCR-RFLP patterns of the ADP-ATP translocase and nitrate reductase genes, (ii) MSB1 minisatellite sequence data, and (iii) the fragment sizes of five microsatellite loci. The structures of the different populations were similar as indicated by Nei's gene diversity and haplotype diversity. The F statistics (Fst, Gst), and the gene flow indicated ongoing differentiation within sympatric populations. Population genetic parameters were influenced by the extent of polymorphism within the data sets. Nevertheless, the F statistics and the gene flow strongly suggested differentiation within the sympatric populations on strawberry and raspberry. The Bayesian analysis of the microsatellite data set highlighted the basis of differentiation. The sudden change of fungal population observed following fungicide treatment of infected plants supports the hypothesis that a change of the *B. cinerea* population present in the air – in the form of vegetative spores – could result in an abrupt change of the populations living on the hosts. However, eventual host preferences of *B. cinerea* variants may also play a role.

[1] Asadollahi, M. et al. (2013) Microbiol Res 168: 379-388.
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COMPARISON OF DIFFERENT STATIC SOIL RESPIRATION METHODS IN AN INCUBATION EXPERIMENT SETTING UP ON CHERNOZEM SOIL

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The measurement of carbon dioxide production, -as an important parameter of the C-cycle in the soil- could characterise the connection among the atmosphere, biosphere and pedosphere. The CO_2-production is the microbial respiration results from degradation processes and it can show the state of the various agricultural soils because the living microorganisms react sensitively to the various anthropogenic effects and other external influences. So, this parameter can give information about the consequences of the soil pollution together with other microbiological parameters, e.g. microbiological biomass, number of bacteria colony, various enzymes activity, etc. Besides the direct methods several remote sensor and modelling tools are available with their advantages and disadvantages. In the experiment three relatively simple methods were chosen to compare the reliability and reproducibility of soil respiration and substrate induced respiration through the measurement of CO_2-production. Among the three chosen methods there were two classical methods, where the CO_2 produced is absorbed in sodium hydroxide and quantified by titration with diluted hydrochloric acid. The differences between the two methods is that in the first method the soil was taken to the bottom of the laboratory bottles and sodium hydroxide solution was taken on the top of the soil in a glass with large surface, this is the modified Minderman method. In the second method – it is used wide range internationally- the sodium hydroxide solution was taken to the bottom of the incubation bottles and the soil was taken to the fine mesh bag insuring for gas exchange, above the sodium hydroxide solution. The OxiTop measuring system was applied as third method for standardised determination of the respiration. This method is developed to determine the oxygen uptake rate, or respiration rate of organic matter. Along the measurement the developed CO_2 is absorbed on an agent, so it does not appear as a free gas in the system. The changes in pressure due solely to the decrease in oxygen consumption it is measured by manometric method. A chernozem soil from Debrecen-Látókép was chosen for the experiment as a biological active soil, the incubation time was seven days in all cases. Large differences were found between the results of various methods, as long as the standard deviation was negligible between each repetition. The measured amount of carbon dioxide by the OxiTop device shows almost the same values as it was in the modified Minderman method where the soil was taken to the bottom of the incubation bottle. When the sodium hydroxide solution was taken to the bottom of the incubation bottles and the soil was taken to the fine mesh bag to ensure the free gas flow the values was higher by an order. We would like to involve the gas chromatographic and dynamic measurement of CO_2 method to get more accurate results for measuring the soil respiration.

HEAT-INACTIVATED, INTRADERMALLY ADMINISTERED VARICELLA-ZOSTER VACCINE AS A POSSIBLE STRATEGY TO PREVENT VARICELLA AND HERPES ZOSTER IN IMMUNOCOMPROMISED INDIVIDUALS

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Varicella-zoster virus causes chickenpox usually in children, establishes latency in the dorsal root ganglia, may reactivate later in life and cause herpes zoster. A vaccine (OKA strain, live, attenuated,

given s.c.) to prevent varicella was developed and approved for use in many countries (1995 USA, Europe, earlier in Japan). The decline of the cell-mediated immunity (CMI) in the elderly or immunocompromized individuals may have a major role in the reactivation of VZV. A zoster vaccine was approved for use in the USA in 2006. The titer of the virus in the zoster vaccine is about 14-fold higher than in the varicella vaccine. The production of zoster vaccine on human diploid cells is difficult and expensive. An alternative delivery system may reduce the dose of the vaccine and improve the stimulation of the CMI of the elderly to prevent the reactivation of VZV. One of the alternative delivery systems is the liquid jet injection which is a needle free method, that pressures liquid or powder into the skin. In our animal studies we applied this system for intradermal vaccination. We vaccinated guinea pigs with the live attenuated VZV vaccine (Varilrix, GSK), 1/5 of the 103.3 PFU pediatric dose in live or in heat inactivated forms. Skin biopsies were obtained on days 1, 3, 5 and 8 and processed for analysis of VZV –DNA content by real-time PCR. The results showed that the amount of VZV-DNA was very similar in the biopsy samples obtained at the same time (days 1, 3, 5, 8) from the guinea pigs, indicating that very similar dose of the vaccine was administered by the device in the skin. Vaccination of immunocompromised subjects with the live attenuated VZV vaccine is problematic and various strategies need to be explored. One of the possible strategies is the use of the heat inactivated form of the vaccine. To test this vaccine, twenty-one guinea pigs were immunized in four groups with live or heat inactivated vaccine administered subcutaneously or intradermally, 2 times in a 4 weeks interval. Four animals were kept unimmunized and used as controls. At the end of the immunization period the animals were bled for antibody testing and sacrificed for measuring CMI responses using VZV- or control-antigen stimulated splenocytes. The mRNA expression of IFN-γ and perforin was determined by real-time PCR. All animals immunized with live vaccine responded with IFN-γ mRNA expression. The number of responder animals was lower in the groups inoculated with heat-inactivated vaccine, but the i.d. administered vaccine seemed to be more effective than the s.c. administered vaccine. Expression of perforin mRNA was very similar in all groups. VZV antibodies as determined by IF test showed that the i.d. administration of either the live or inactivated vaccine induced higher antibody titers than s.c. administration of the same vaccine. Our results are important for the development and use of the varicella and zoster vaccines possible in heat-inactivated form and administered i.d. in immunocompromised children and adults.

GENES IN MICROORGANISMS: FROM THE CISTRON TO THE PAN-GENOME

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When I started my Ph. D. in 1963, the theoretical framework of microbial genetics was dominated by the cistron concept; that is the gene, as a unit of function, divisible by recombination, changeable by mutation and equivalent to a non-determinable DNA sequence. The operon theory (1961) extended and somewhat undermined this elegant concept, as cis-acting mutations in the operon belonged formally to more that one gene. In 1968 Gunther Stent announced the death of molecular biology. Of course he was wildly off the mark. What had happened is, that while the first revolution in molecular biology was fuelled by techniques coming from outside the field (ultracentrifugation,

X-ray crystallography), new technologies arose within Molecular Biology that allowed a direct access to the genetic information, leading to the cloning revolution. In 1977 Fred Sanger published the sequence of phage ΦX174, all 5386 bases of it. I think no futurologist could have predicted what followed. In January 2013 there were 3762 bacterial genomes recorded as finished in a biochemist's blog. No doubt there are quite a few more now. The number of fungal genomes available is surely in the hundreds, and the genomes of protists of all kingdoms complete the picture. This deluge of data is changing our conception of the microbial world. Metagenomics tell us that the microbes we know are only a fraction of those that are out there. The concept of the Pan-genome was proposed for the first time in an inaccessible article in Russian. Today the sequence of many "strains" of the same microbial species had led to a conception of the species genome as possessing a core of common sequences surrounded by a changing cloud of subsidiary sequences. I think, however, that the most drastic impact of the genomic revolution will be in evolutionary biology. The pan-genome implies horizontal transmission within different closely related strains. But horizontal transmission goes far beyond that. The availability of a plethora of genomes shows interchanges of genes and gene clusters between members of species widely separated in the evolutionary tree, which is really becoming, especially in the microbial world, an evolutionary network.

SENSITIVITY OF TWO THERMOPHILIC FUNGAL SPECIES TO DIFFERENT TOXIC COMPOUNDS

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Thermophilic fungi can colonize organic substrates of high temperature in natural ecosystems. Especially, composting materials in the thermophil phase are the most common habitats for them in communal environment. Their cells of eukaryotic type combined with extremely fast growth offer a good opportunity for the application as ecotoxicological test organisms. We tested the sensitivity of two strains belonging to the species *Rasamsonia emersonii* and four strains belonging to the species *Thermomyces lanuginosus* to four toxic heavy metal ions and two organic toxic compounds with the method of growing on artificially contaminated media. The sensitivity of the tested strains of the same species proved to be remarkably similar to cadmium and mercury ions but slightly different to copper and selenium ions. The EC_{50} values concerning the species *R. emersonii* appeared to be 0.05-0.1 ppm to cadmium and 0.02-0.05 ppm to mercury. As for the sensitivity of the strains of species *T. lanuginosus* to cadmium and to mercury, the EC_{50} values ranged between 0.01-0.05 ppm and 0.01-0.02 ppm, respectively. The herbicide substance glyphosate began to inhibit colony growth of two *T. lanuginosus* strains already at a dose of 0.25 ppm. However, total growth inhibition was found only over 50 ppm of the drug. Surprisingly, the sensitivity of *R. emersonii* strains to the herbicide compound atrazine seemed relatively similar, but they could influence the ascocarp development even at a low concentration (3 ppm) that did not inhibit colony growth.

Sensitivity of thermophilic fungi both to inorganic and organic toxic compounds varies by strains within the tested species. The application of highly sensitive representatives of them can contribute to the improvement of the ecotoxicological strategy.

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ADVERSE EFFECTS OF EXPOSURE TO AIRBORNE FUNGI IN INDOOR ENVIRONMENTS

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Airborne fungi have been incriminated to cause several respiratory disorders based on different immunological and toxic pathways including hypersensitivity pneumonitis, asthma, rhinitis, chronic bronchitis and organic dust toxic syndrome. Mycotoxins in fungal spores have been proposed to cause adverse health effects particularly after chronic exposure to high levels of airborne fungi in indoor environments. Recent study (January 2012-November 2012) taken in grain mill (GM), apartments, and basements (Zagreb, Croatia) showed that average concentration of airborne fungi in GM was about 30 times higher (up to 40000 cfu/m^3) than in other locations (up to 1244 cfu/m^3). At GM species of Aspergillus, Penicillium and Eurotium dominated over other detected airborne fungi. Among *Aspergilli* and *Eurotia*, *A. ochraceus* and *E. herbariorum* were constantly present in the samples taken at GM. *Aspergillus ochraceus* (AO) is well-known producer of ochratoxin A (OTA) and *Eurotium herbariorum* (EH) produces a wide range of metabolites with poorly investigated toxicity. In the current investigation OTA was detected (HPLC, immunoaffinity columns) in spore extracts (0.3-28 μg/mL) of 5/6 AO strains isolated in November 2012, while none of tested EH strains were able to produce OTA. Taking into account maximum detected concentration of OTA in the spores, daily intakes of OTA by inhalation were calculated to be 0.08 and 3 ng/kg b.w., which is below the tolerable daily intake of OTA (17 ng/kg b.w.). Cytotoxicity of pure OTA, OTA-positive (AO-OTA[+]) and OTA-negative (AO-OTA[-]) spore extracts, as well as spore extract of EH, was tested on human lung adenocarcinoma cells A549, individually and in combination, using colorimetric MTT test (570 nm). Concentrations that decreased the cell viability by 50% (IC_{50}) were as it follows: pure OTA (53 μg/mL); AO-OTA[+] (mass concentration 934 μg/mL corresponds to 10.5 μg/mL of OTA in spore extract); and 2126 μg/mL for EH. Highest applied concentration of AO-OTA[-] spore extract (4940 μg/mL) decreased cell viability by 30% and IC_{50} for the extract could not be determined. Combinations EH + AO-OTA[+] and EH + AO-OTA[-] applied in subtoxic concentrations showed dominant additive interactions. Also, synergistic effect was obtained for one combination of EH and AO-OTA[+]. Despite the low calculated daily intake of OTA by inhalation, chronic exposure to high levels of OTA-producing airborne fungi in combination with other moulds pose significant threat to human health due to their possible additive and/or synergistic interactions.

FIRST RECORD OF WINTER PLANKTONIC MICROBIAL COMMUNITIES IN SOME SPECIFIC AQUATIC HABITATS

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Information on the composition of winter microbial communities from specific or extreme aquatic habitats are relatively scarce compared to the earlier literature data related to warmer seasons, since sampling sites are not easily accessible or this period of the year was supposed to have low

microbial activity. However, it has been proved that extreme aquatic habitats may also harbour abundant microbial communities even in the coldest period of the year. We report the first description of the winter planktonic microbial communities from various sites in Romania: from a peat bog, a crater lake, saline lakes and sulphuric fumaroles. The abundance of microorganisms was assessed by epifluorescence microscopy, while the taxonomic composition of these communities (Bacteria and Archaea) was investigated by denaturing gradient gel electrophoresis (DGGE) coupled with the partial sequence analysis of the 16S rRNA gene. A detailed physico-chemical analysis of the water samples was also performed with a multi-parameter submersible sonde and with laboratory measurements. Although many of the studied environments were extreme habitats regarding some or several parameters (low pH, high salinity, low light intensity etc.), microbial communities in many cases were abundant and/or diverse with the presence of previously unidentified taxa. The characteristic detected genera were: *Ferroplasma*, *Acidithiobacillus* and *Metallibacterium* in the sulphuric fumaroles; *Methanosaeta*, *Acidomonas* and *Polynucleobacter* in the peat bog; and *Prosthecochloris*, *Marinobacter*, *Polaromonas* and *Albidiferax* in the salt lakes. We also found that, even phototrophic microbes may form dense populations under the ice, as it was observed in the case of the green sulphur bacterium *Prosthecochloris* in the stratified saline lake.

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DISTRIBUTION OF ADHESION FACTORS AND THEIR IMPACT ON THE PATHOGENICITY OF BOVINE *PASTEURELLA MULTOCIDA* STRAINS IN BOVINE RESPIRATORY DISEASE

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Bovine Respiratory Disease (BRD) is the most common and costly disease affecting beef and dairy cattle industry all over the world. The economic losses are either direct due to lethal cases, or indirect through additional expenses such as the cost of treatment and lower weight gain and decreased carcass value. For those cattle that survive, the presence of pulmonary lesions at slaughter has been associated with significant reduction in daily weight gain. The pathogenesis of BRD is complex, involving a number of viruses, bacteria and stress factors. The stressors, combined with the impact on the immune system and viral infections, allow bacteria to invade the lungs. One of the most frequently isolated bacteriological agents is *Pasteurella multocida* in these cases. This organism commonly inhabits the pharynx or upper respiratory tract of most cattle. However, it is not considered as normal flora of the lungs. The inhaled bacteria that attached on the mucus of lower respiratory tract successfully using a wide-range of adhesins, under conditions of impaired pulmonary defences, replicate rapidly and cause pneumonia. The aim of our study was to detect and characterise the known putative adhesive factors within the bovine *P. multocida* population in Hungary. The studied 39 strains were isolated from diseased animals in different cattle herds and they were earlier characterized with traditional microbiological and general molecular methods and divided into subpopulation considered to contain strains of different virulence. The presence of *ptf*A (subunit of type IV fimbriae), *fim*A (fimbriae), *hsf*-1, 2 (autotransporter adhesins), *tad*D (putative nonspecific tight adherence protein D) and *pfh*A (filamentous haemagglutinin) genes were detected

by PCR. The distribution of virulence factors was compared with the strains' recorded diagnostic data. The prevalence of the different genes was various. Whereas the *fim*A was present in all and *hsf*-2, *tad*D, *ptf*A genes in most strains, the occurrence of *hsf*-1 and *pfh*A showed variability. Based on these results, seven combinations of the genes could be detected. These profiles well characterized the delineated main subpopulations. Two of them were dominant. The strains with these latter ones varied in possession of gene encoding filamentous haemagglutinin. The presence of *pfh*A showed significant correlation with diagnostic data. It was associated with pneumonia. For the discrepancy of virulence, the changes in structures of the virulence factors could also be responsible. Thus detailed study of them is recommended. For the additional characterization of the strains we carried out the sequencing of extended genome region included *tad*D gene. The sequence analysis showed low per cent but characteristic differences between the representative strains. The effect of the changing to the protein structure or function required further investigations.

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MOLECULAR EPIDEMIOLOGY OF GRAPEVINE PHYTOPLASMOSES IN CROATIA: MULTIGENE SEQUENCE ANALYSIS APPROACH

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Phytoplasmas (genus '*Candidatus Phytoplasma*') are wall-less bacteria belonging to the class Mollicutes, along with mycoplasmas, spiroplasmas and acholeplasmas which are all believed to share a common *Bacillus*/*Clostridium*-like ancestor. Their genomes are relatively small and reduced with many basic metabolic pathways missing, yet many predicted genes are present in multiple copies. *Phytoplasma* axenic cultivation is still challenging and not common. However, four phytoplasma genomes have been completely sequenced and annotated so far which started a new era in functional and comparative genomics research. These endocellular pathogens have a unique life-style as they have hosts from two kingdoms – Plantae (plants) and Animalia (insects), and need both for their survival and dispersal in nature. Numerous plant species worldwide are affected by phytoplasmoses resulting in serious yield losses, including a range of economically important crops such as wheat, maize, rice, potato, sugarcane, oilseed rape, coconut, and grapevine as well as many fruit trees. The most important etiological agents of grapevine yellows (GY) diseases in Europe are also associated with phytoplasma species: '*Ca. P. solani*' and '*Ca. P. vitis*', also referred to as Bois Noir (BN; ribosomal group 16SrXII-A) and Flavescence Dorée (FD; ribosomal group 16SrV), respectively. In Croatia, surveys of GY molecular epidemiology have been continuously conducted since 1997, with recently discovered FD phytoplasmas being restricted to the areas of the country bordering Slovenia and Hungary and BN phytoplasmas being widespread and diverse. Three locations have been chosen for a case study of grapevine phytoplasma pathosystems: Vukanovec, as a BN focus, and Brckovština and Jagnjić dol, representing FD phytoplasma foci. Grapevine and weed samples were collected together with samples of BN and FD phytoplasma insect vectors Hyalesthes obsoletus Signoret and Scaphoideus titanus Ball. Triplex real-time PCR assay was performed in order to simultaneously detect the presence of both BN and FD phytoplasmas, as well

as the PCR/RFLP analysis of phytoplasma 16S rDNA. Samples that tested positive for phytoplasma presence were subjected to the multigene sequence analysis in order to further elucidate epidemiology of the pathosystems studied. Following genes were amplified and analyzed: *tuf*B and *sec*Y, together with stamp and *vmp*1 genes that are BN-specific and potentially involved in phytoplasma-insect interactions (for BN phytoplasma positive samples). *Sec*Y, *map*, *uvr*B-*deg*V and *vmp*A genes were analyzed for FD phytoplasma positive samples. Multigene sequence analysis has revealed a strong relatedness between phytoplasmas detected in grapevine and insect samples, showing their affiliation to the same pathosystem at the same location. However, variability was found in some of the analyzed gene regions. BN phytoplasma found in weed *Convolvulus arvensis* has shown a considerable genetic variability in all of the analyzed genes. The latter result, together with the finding of FD phytoplasmas at the same location in both grapevine and insect samples, suggests a co-existence of different phytoplasma pathosystems.

METHYLENE BLUE IMMOBILIZED ON ZEOLITE: AN IN VITRO STUDY ON PROKARYOTIC AND EUKARYOTIC CELLS

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A goal of this study was to form an attempt to convert the starting an inert carrier (ZSM-5 zeolite) and active methylene blue sensitizer (MB) into a heterogeneous photoactive system and study its visible light (LED light) induced effects on microorganisms. The composite system was prepared by incorporation of MB into the zeolite channels and by MB adsorption on the zeolite surface. Immobilization could have control the aggregation process and self-quenching of the photosensitizer to increase its photostability and allow control more effective the generation of reactive oxygen species. These species could have more efficiency photoinactive of tested microorganisms. The composite photosensitizer effectively absorbs the red LED light and upon illumination with visible light effectively generates reactive singlet oxygen ($1O_2$) in aqueous solution, which was proved by EPR spectroscopy using a spin trapping agent. The efficiency of complex was evaluated on Gram–negative bacteria *Escherichia coli* CCM 3988 and Gram–positive bacteria *Staphylococcus aureus* CCM 3953 and yeast *Candida albicans* CCM 8186. It was found out that after the microorganisms have been adsorbed at the surface of such modified zeolite, the photogenerated $1O_2$ quickly penetrates their cell walls and bringing about their effective photoinactivation. Cells biosorption of microorganisms to heterogeneous system was demonstrated by fluorescence microscopy and SEM. Ultrastructural changes in bacterial and fungal cells were analyzed by using TEM. In order to elucidate the effectiveness of microorganism's inactivation by photogenerated $1O_2$, we compared the amount of MB in the medium and MB immobilized by the zeolite material, causative 1.3 log10 decreased of CFU bacterial and fungal cells count. The comparison revealed that the largest difference in sensitivity was observed in the combination of MB and zeolite system for *S. aureus*. The amount required for 1.3 log10 decreased in the CFU count in *S. aureus* was order of magnitude lower for a zeolite-incorporated MB than for a free dissolved dye.

EXAMINATION OF THE EXPRESSION AND COPY NUMBERS OF THE *cfx*A CEFOXITINASE GENE AS DETERMINANTS FOR CEFOXITIN RESISTANCE OF *BACTEROIDES*

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One of the most effective antibiotics for the treatment of anaerobic infections is cefoxitin. For *Bacteroides* spp. a serious resistance rate has been observed and the main resistance mechanisms are changes in the affinity of the penicillin-binding proteins and a cefoxitinase produced by the *cfx*A gene. However the extent of the "division of labor" between these two resistance mechanisms has not been clarified yet. In this study we targeted to examine the factors contributing to the cefoxitinase expression levels among *Bacteroides* strains.

First we optimized the usual cefoxitinase assay regarding the buffer, pH, temperature, and ionic strength in an enzymatic assay. The roles of the copy numbers and promoter activities were also examined by RT-PCR in case of 10 *cfx*A-positive *Bacteroides* isolates, and we determined the *cfx*A nucleotide sequences in case of some isolates. The own promoter of the *cfx*A gene displayed considerable active in reporter assays. The cefoxitinase activities of the 10 examined strains correlated well with the cefoxitin MIC values ($r>0.95$). Moreover, another significant correlation was experienced between the mRNA levels and cefoxitinase activities of cells growing exponentially. The behavior of two "outlyer" strains could be explained well in light of the nucleotide sequences of the *cfx*A genes (stop codon, amino acid change). The detected copy numbers of the *cfx*A genes did not correlate with the cefoxitinase activities and with the mRNA levels.

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CLINICAL COURSE OF *CLOSTRIDIUM DIFFICILE* INFECTION AT THE VIENNA UNIVERSITY HOSPITAL IN 2012: A RETROSPECTIVE CHART ANALYSIS

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The incidence of *C. difficile* infection (CDI), has been increasing over the last 5 years in Canada, the US and Europe. CDI has only become a reportable disease in Austria since 2010. However, based on official hospital discharge data according to the International Classification of Diseases (10th revision), enterocolitis due to *C. difficile* given as primary diagnosis increased four-fold in Austria from 2002 until 2009, from 278 to 1153 cases. In order to determine the number of severe cases of CDI, to assess the risk factors for a severe CDI and to evaluate possible nosocomial transmissions, data of the patients admitted at the Vienna University Hospital from January to December 2012 were retrospectively analysed. All patients with a positive toxigenic *Clostridium difficile* laboratory result and diarrhoea were included and their medical records retrospectively reviewed for: (a) demographic data, (b) duration of hospital stay, (c) clinical course of CDI, (d) co-morbidity, (e) fatal outcome, (f) ward of admission (g) use of antibiotics before onset of CDI and (h) possible nosocomial transmission. Severe CDI was defined according to the European Society of Clinical

Microbiology and Infectious Diseases (ESCMID) as an episode of CDI with either a marked leukocytosis (leukocyte count > 15 x109/L) or a rise in serum creatinine (>50% above the baseline). The Charlson Co-morbidity index was used as a prognostic index for classifying comorbid conditions which might alter the risk of mortality in our patient collective. Out of 278 CDI cases 46 (16, 5%) were classified as severe CDI. Overall 42 patients with CDI died during their hospital stay, among them 21 (50%) were severe CDI cases. Mortality was significantly higher ($P<0.0001$) in severe CDI cases (21/46) compared to not severe cases (21/232). Multivariate analysis showed that only a Charlson comorbidity index of ≥ 3 (adjusted odd ratio = 2.87; 95% CI: 1.36 – 6.07; $P=0.0043$) remained independently associated with severe outcome. No significant difference in gender, age and length of stay or in antibiotic therapy before onset of CDI could be found in the severe compared to the not severe CDI cases. No major CDI clusters or outbreak were observed. According to our findings CDI is a contributing factor but not the direct cause of death in severely ill patients. The restrictive use of antimicrobials is essential in reducing overall CDI rates, to avoid bowel dysbiosis and severe CDI in this patient group. Additionally, strict adherence to infection control measures in all CD patients is crucial to prevent nosocomial CD transmission.

IN VITRO IL-10 INDUCTION BY HHV-7 AND ITS CLINICAL RELEVANCE

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Human herpesvirus 7 (HHV-7) is an ubiquitous opportunistic human pathogen. It belongs to the Betaherpesvirinae subfamily. HHV-7 causes roseola, pityriasis rosea and could be frequently reactivated in immunocompromised patients. The virus infects the CD4+ cells and establishes latency in these cell types. Their pathogenicity can be attributed to indirect ways via mediators produced by infected immune cells. IL-10 is an anti-inflammatory cytokine produced by monocytes/macrophages and also by CD4+ T- and B-cells. This Th2 type cytokine has a pleiotropic effect in the immune regulation. It suppresses the synthesis of the pro-inflammatory cytokines (e.g. IFN-gamma, IL-2 and TNF-alpha) and stimulates the humoral immune response via enhancing the survival and maturation of B cells. IL-10 can be induced by TNF-alfa, bacteria, bacterial products (e.g. lypopolisaccharide) and also by viruses. We studied the IL-10 inducing properties of HHV-7 in human peripheral blood mononuclear cells (PBMC) of seronegative and seropositive donors. PBMCs were treated also with infectious, heat-inactivated and UV-irradiated HHV-7 in the presence or absence of mitogen (phytohemagglutinin, PHA). Up to 72h post infection, cytokine secretion was monitored by ELISA in the supernatant of the cell cultures. We examined IL-10 induction at the level of transcription in PBMCs and SupT1 cell culture. Samples at different time intervals post infection were analysed by RT-PCR and Northern blot. Results were extrapolated to HHV-7 induced clinical entities. HHV-7 significantly augmented the IL-10 secretion of PBMCs, especially the infectious virus preparation upon primary infection. PHA also elicited the production of this cytokine, not only alone, but synergistically in parallel treatment with HHV-7. In contrast to the rapid effect in the seronegative cases, IL-10 production was delayed in seropositive cases. Not only the infectious, but the inactivated viral preparations were able to augment the IL-10 protein output. IL-10 mRNA was not detectable by RT-PCR 4 hours post infection, but it comes well 12 hours post infection in PBMC of both seronegative and seropositive persons. The IL-10 transcription starts earlier in the SupT1 lymphoid cell line after infection with HHV-7, as compared to PBMC. Bacterial endotoxin (LPS) treatment, as a model for bacterial superinfection, could induce the

accumulation of IL-10 mRNA in this cell line. The Northern blot analysis showed that HHV-7 modulates the IL-10 production independently from the TNF-alpha.
These results show that HHV-7 can induce IL-10 production during an infection, and this can contribute to the moderate immunosuppressive effect of the virus. This phenomenon can contribute to prolonged diseases course of pityriasis rosea. In immunocompetent persons secondary HHV-7 infection usually does not elicit clinical manifestation. Simultaneous bacterial and HHV-7 infections predispose to impaired cellular immunity, while the stimulating effect of IL-10 on humoral immunity results in relatively rare bacterial superinfection during the HHV-7 infections.

ANAEROBIC BIODEGRADATION OF CELLULOSE RICH SUBSTRATES

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Biogas is one of most important renewable energy carrier. Various kind of biomass, including plants and plants residues can be converted into biogas via anaerobic fermentation. Plant tissues consist of cellulose as the major component. My aim was to examine if it is possible to adapt the biogas producing microbe consortium to cellulose-rich substrate. The experiments were carried out at thermophilic (55°C) temperature because at higher temperature the degradation is more efficient and the biogas yield is higher. For the cellulose adaptation α-cellulose was used as substrate meanwhile the controls were fed with glucose. To monitor proper operation, the VFA concentration and pH was measured weekly along with the β-glucosidase enzyme activity, which refer to the cellulose hydrolysis. To examine if the adaptation was successful new fermentations were started with the adapted and non-adapted sludge. *Caldicellulosiruptor saccharolyticus* is a thermophilic, hydrogen producing bacterium, which can also degrade cellulose. Earlier results suggested that this bacterium is able to improve biogas production. *C. saccharolyticus* enhanced the biogas yield by 12% in the non-adapted reactors and the adapted sludge provided 14% more biogas than the non-adapted one. The results also imply that the adaptation to cellulose was successful. From the fermenters adapted to cellulose, samples were taken and inoculated onto cellulose containing media. Two mixed culture was obtained from the parallel fermenters. These cellulose degrading microbe communities were marked as AD1-I and AD2-I. In subsequent experiments corn stover was used as substrate. The cellulolytic cultures were added as inoculum to sludge from an operating biogas plant. The added cultures had positive effects, increased the biogas yield and methane content as well. Identification of the members of the cellulolytic consortia could contribute to the development of an efficient cellulose degradation community in biogas fermentations.

EFFECT OF SUBSTRATE CONCENTRATION ON SYNTHESIS ACTIVITIES OF PECTINEX ULTRA SP-L

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Carbohydrates play an important role in biological systems such as energy source, cell-cell communication, prebiotics etc. It is well known that these biological functions depend not only on themself, but also on their seqence, the type of glycosidic linkages, configuration and branching etc. Even many studies are published in literature regarding with saccharides, numerous functions,

mechanisms as well as properties are still unknown. Pectinex Ultra SP-L is reported to be had fructofuranosidase and ß-galatosidase activities in synthesis of fructooligosaccharides and galactooligosaccharides, respectively. In this work, screening of synthesis activities as well as effects of substrate concentration were investigated. Eight different substrates (glucose, mannose, maltulose, melibiose, cellobiose, maltose, turanose, palatinose) were monitored to produce carbohydrates. The reactions were carried out at 60 °C and pH6.5. The effects of substrate concentration were determined in range of 10-80 g/100ml (it was depend on the solubility of substrate). Carbohydrates were analysed by HPLC and TLC methods. The enzyme preparation was able to do reverse hydrolysis reaction on glucose and mannose producing glucose-disaccharide and glucose-oligosaccharide (DP3) and mannose-disaccharide, respectively. Moreover, Pectinex ultra is able to catalyze glucosyl transfer reactions on cellobiose, maltose, maltulose, palatinose and turanose. Galactosyl transfer activity was also detected on melibiose substrate. In the case of the mannose, the highest reverse hydrolytic activity (90,5 ug DP2/min/ ml enzyme) was measured at 60 g/100 (in range of 10-60 g/100ml). On glucose the enzyme shows the best activity at 80 g/100ml carbohydrate content (331 ug DP2/min/ml enzyme). The highest glucosyl transfer activity was measured on 60 g/100ml palatinose substrate. In this case Pectinex has 1081 ug DP3/min/ml enzyme activity. The optimal melibiose concentration of galactosyl transfer reaction was 70 g/100ml, in this case 620 ug DP3/min/ml enzyme activity was detected. More studies are needed to clear the mechanisms of transfer and reverse hydrolytic activities of Pectinex, but these results are promising for development technology for synthesis of certain carbohydrates.

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DRAFT GENOME SEQUENCE OF A BOVINE *ESCHERICHIA COLI* O157:H43 STRAIN REPRESENTING A NOVEL GENOTYPE

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Enterohaemorrhagic *Escherichia coli* (EHEC) strains are significant food-borne pathogens. Typical EHEC O157:H7/NM strains produce Shiga-toxin (Stx), and carry the locus of enterocyte effacement (LEE) pathogenicity island. Because of their clinical and epidemiological significance, whole genome sequences of several *E. coli* O157:H7/NM strains were determined. There are, however, considerably less data about the genomes of stx-negative O157 strains producing non-H7/NM antigens. In this study we aimed to determine the draft genome sequence of a stx- and eae-negative *E. coli* O157:H43 strain (T22), isolated from healthy cattle. According to our knowledge this is the first draft genome of an O157 strain other than O157:H7/NM. The size of the chromosome is 4,959,535 bp and the strain also carries a 80,112 bp plasmid. T22 belongs to sequence type (ST)155, containing several human and animal pathogenic and commensal *E. coli*. As the known integration sites of key EHEC virulence genes are intact in the genome of this *E. coli* O157:H43 strain, it is possible that T22 represents a so far unknown stage in the evolution of O157 strains.

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NEXT GENERATION AMPLICON SEQUENCING OF THE MICROBIAL COMMUNITY OF A CRATER LAKE (LAKE SAINT ANA, ROMANIA)

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High mountain lakes have been in the focus of the scientific research for the past decades, because the anthropogenic influences in these environments are limited, and their hydrodynamics are mostly related to natural conditions. Due to their oligotrophic character pelagic food webs are less complex, which makes them good model systems to environmental changes. The Lake Saint Ana (in Romanian: Lacul Sfânta Ana) is situated in the Eastern Carpathian Mountains, formed by a Holocene volcanic eruption. In our study, the physico-chemical parameters of the water body were measured monthly from January to July 2012. Samples for molecular biological analyses were collected in July 2012 from four different depths, and in different time of the day. The T-RFLP method was performed to reveal differences within the composition of the communities. Although remarkable diurnal changes were not found, there was a significant variation among the communities from the surface water bodies and from the bottom zone. Next generation amplicon sequencing was performed for two selected samples (from 0.4 m and 5.5 m depth) for the V1-V3 region of the 16S rRNA gene on a Roche GS Junior platform. The mothur software was used for quality filtering, denoising, and for the detection and removal of chimeric and singleton sequences from the datasets. The alignment was performed with the SINA aligner tool using the ARB-SILVA bacterial 16S rDNA reference database. The results showed a more diverse community close to the sludge, and several taxa were identified from both samples. The most dominant genera were the *Koribacter*, *Solibacter*, *Geothrix*, and the *Holophaga*.

EXAMINATION OF THE CHANGES IN A BACTERIAL DIVERSITY CAUSED BY HYDROCARBON CONTAMINATIONS IN POLLUTED GROUNDWATER SAMPLES FROM HUNGARIAN SITES

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The main goal that we focused was a statistical examination of the changes in a bacterial community and its diversity caused by hydrocarbon contaminations in groundwater samples from Hungarian polluted sites. The bacterial diversity of groundwater samples from three hydrocarbon contaminated sites was examined. On these sites (with a minimum of three times) biological and geochemical monitoring was performed to determine the changes caused by the examined contaminants on the bacterial community and diversity. During this study not only contaminated but background (by the results of pollutant concentration and groundwater flow) groundwater monitoring wells were sampled on the same sites and examined similarly. Aliivibrio fischeri based toxicological (ToxAlert100™, Merck Inc.) and accredited analytical methods were performed and compared. Significant and directly proportional coherency was verified between the based toxicological (EC50) and TPH and BTEX analytical results of contaminated and background groundwater samples of the

examined sites. Based on this close coherency between the toxicological and analytical results, it was thought that the bacterial diversity or the composition of the community in the samples was highly influenced by the contamination concentration. According to this hypothesis, the contaminated and background samples (taken at the same time as for the toxicological examinations) were evaluated by T-RFLP method (like a genetic fingerprint). It was found by the results of statistical analyses (Man Whitney U test, cluster analysis) that there was no significant coherency between the TRFs (as OTUs) based bacterial diversity and the concentration of contaminations. Moreover in most cases the T-RFLP profiles of same, but taken at different time samples were substantially variant of each other. According to the analysis of contaminated and background groundwater samples from these three, Hungarian, hydrocarbon-polluted sites, it can be said that measured concentrations of the BTEX and TPH contaminants are not causing significant changes in the bacterial diversity or the composition of the community. Clone libraries were made and examined around 100 clones per samples. At the time of writing, two samples from the site #1, two from site #2 and three from site #3 have been prepared.
Based on 16S rDNA clone library analysis of groundwater samples from these hydrocarbon polluted sites, Betaproteobacteria dominated the communities.

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ANTIMICROBIAL EFFECT OF APPLE POMACE EXTRACTS

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Food industry is searching for natural preservatives because consumers demand natural, safe and environmentally sound food preservatives. Using different plant extracts, which have antimicrobial effect as natural preservative has increasing tendency, for instance grape seed-and grape pomace extracts, cranberry extracts, different citric extracts. In the latest years many research deal with investigation of the antimicrobial effect of apple pomace extracts. Apple pomace, a residue from apple juice production, contains high amount of polyphenols which are known to have antioxidant effect. In this present study antimicrobial effect of apple pomace extracts, variety 'Idared' and 'Cordelia' was evaluated. Polyphenol compounds in apple pomace extract were determined by HPLC method. In both of the apple pomace extract was determined rutin, hydroxy-cinnamic acid and phloridzin. In addition 'Idared' contained chlorogenic acid and syringic acid, too. Antibacterial effect of apple pomace was investigated by agar diffusion method. Apple pomace extracts (APE) showed inhibitory effect on the growth of *E. coli* O157:H7, *E. coli* 8739, *L. monocytogenes* 4ab, Eb. cloacae and *Ec. faecalis*. *Asp. niger*, *Kluyv. marxianus*, *Kluyv. thermotolerans* és *S. cerevisiae* were resistant against the APEs. Minimal Inhibitory Concentration (MIC) was the following: in the case of 'Cordelia' 1.28 mg.ml-1; in the case of 'Idared' 1.0 mg.ml-1polyphenol content samples. MIC was also evaluated by using culture medium. 'Cordelia' pomace extract inhibited the growing of test-microorganisms at the concentration 0.96 mg/ml. Regarding 'Idared' pomace extract MIC were the follows: *Eb. cloacae* and *L. monocytogenes* 1.39 mg.ml-1; *E. coli* O157:H7 and *Ec. faecalis* 1.54 mg.ml-1; *E. coli* 8739 1.67 mg.ml-1 . 'Cordelia' pomace extract had more powerful effect against the investigated test-microorganisms. Inhibitory effect of 'Cordelia' APE was analysed in model-system where the aim was to investigate the utilisation of the apple pomace extract as natural preservative. For the investigation apple juice (100% fruit content) and water were used and 3 different APE concentrations were performed. The model-system was inoculated by *L.*

monocytogenes 4ab. Changes of cell viable cell number of the test-microorganism were followed up and results were given as CFU.ml-1. Results indicated that both of the model-system apple pomace extract had inhibitory effect, and there was no considerable difference between the water and apple juice media. Using the highest apple pomace extract concentration after 24 hours living cell was not detected. According to the results, apple pomace, an inedible by-product of juice manufacture, might be a potential source of antioxidants and could have potential use for food preservative purposes.

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GENOTYPIC AND PHENOTYPIC ANALYSIS OF THE INFLUENZA STRAINS FROM THE 2012/2013 INFLUENZA SEASON IN HUNGARY

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Influenza viruses cause annual epidemics and occasional pandemics in humans, resulting in high mortality primarily in the elderly and other high risk population. One of the main defenses against influenza virus infection – besides the vaccination - is the use of antiviral drugs, such as oseltamivir. Oseltamivir is a neuraminidase (NA) enzyme inhibitor, which binds to the active site of the viral NA molecule and prevents the virus budding. The active site's characteristic amino acid (AA) in the position 275 is Histidin (H), changing it to Tyrosine (Y) is responsible mainly for the resistance of the virus against oseltamivir. Different NA mutations within the enzyme site result in different levels of NA functional loss. NA inhibitor-resistant variants have been shown to differ in their fitness in vitro and in vivo. However, one or more secondary mutation may support the protein level properties damaged by the functional mutation but by itself may confer no major adaptive benefit. During the Influenza surveillance (2012/2013) several H1N1pdm strains were isolated. None of them had the H275Y major mutation but the A/Hungary/16/2013 (H1N1pdm) has several mutations compared to the reference strains; the most interesting is the H275P (P) mutation detected by sequence analysis. In the Neuraminidase Inhibition Assay (Peanut Agglutinin lectin ELISA) the IC_{50} (the half maximal inhibitory concentration) was 65.8nM which is one lg higher than the IC_{50} of the wild type H275, but 2 lgs lower than the IC_{50} of mutant 275Y reference strain. Using the most advanced method - 4-MUNANA-based Neuraminidase Assay - gave similar results. However, compared to wild type's haemagglutination titer (1215 HA), the H275P mutant's titer was similar to the 275Y mutant's titer (405 HA). The multiple mutations in the A/Hungary/16/2013 (H1N1pdm) may be responsible for the minor change in oseltamivir sensitivity and the decreased virus load. On the phylogenetic tree our strain belongs to the group 6, but represents a different node distinct from other isolates from Europe in the influenza season 2012/2013. The results highlight the importance of functional drug resistance assaying when reporting novel mutations. Resistance cannot be assumed only on the basis of data from different amino acid substitutions at the same position.

CHARACTERIZING THE INTERACTION BETWEEN THE HUMAN PAPILLOMAVIRUS TYPE 16 E7 ONCOPROTEIN AND THE SRC KINASE

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Src family cytoplasmic tyrosine kinases are key signal transducers regulating cell survival, proliferation, and migration; therefore, they can exhibit oncogenic activity upon upregulation. Elevated activity of Src kinase has been associated with increased cell proliferation and invasivity also in human papillomavirus (HPV)-associated cervical cancer. We recently discovered that high-risk HPV 16 E7 oncoprotein upregulates the protein expression of certain Src family kinases in transduced human keratinocytes via post-transcriptional mechanisms. Furthermore, we found the presence of HPV 16 E7 associated with enhanced activating phosphorylation of all expressed Src kinases in keratinocytes. Here, we have further characterized the effect of HPV 16 E7 on Src kinase upregulation. Mutagenesis data indicate that the ability of HPV 16 E7 to upregulate Src protein expression is dependent on its ability to bind the retinoblastoma protein.

Support: Hungarian Scientific Research Fund (OTKA K81422) and TÁMOP-4.2.2/B-10/1-2010-0024 project.

TEMPO AND MODE OF EVOLUTION AMONG MUSHROOMS: ANALYSIS OF DIVERSIFICATION RATES IN THE AGARICALES

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The order Agaricales is the most species-rich group of the Basidiomycota, numbering ca. 480 genera and 14,000 described species. Highly diverse fungi belong to this order, bearing distinct morphologies and displaying life history traits which show considerable diversity. The driving forces of speciation and evolutionary diversification in these mushrooms are poorly known. Understanding why and how certain lineages became extremely species-rich, while others are only represented by a few species and determining the timing of major lineage expansions, or the impact of fruiting body morphologies on speciation rates may have an effect on fungal conservation and mycotaxonomy, however, theoretical and experimental proof so far remained spurious. A recently launched 4-year project, ADiv (short for Analyzing Diversification in the Agaricales), aims to investigate patterns of speciation, extinction and variations in the rate of evolution in the Agaricales. To address these questions, we will use statistical models of lineage diversification in a phylogenetic framework. Modeling of diversification will rely on a new two-gene dataset (referred to as diversity dataset) for ca. 3,000 species accepted in the Agaricales. For molecular studies we selected the nLSU and RPB2 loci, which are known to provide sufficient phylogenetic information for relationships at the infrageneric level. In addition to the diversity dataset, a phylogenomic dataset is also being produced from the accessible whole genomes of agaricalean fungi, meaning about 60 to 100 species from the Agaricales, which will provide robust support for the backbone of the Agaricales to reinforce phylogenetic inference from the diversity dataset. These two datasets will be used to examine general patterns of speciation and extinction, in the identification of shifts in diversification rates and whether transitions between different fruiting body types have an effect on rates of speciation and extinction in the Agaricales. In this lecture, we report an experimental workflow designed specifically for the Agaricales, progress in generating sequence data and preliminary results about adaptive radiations in the Agaricales.

Support: Hungarian Scientific Research Fund (OTKA NN 106394) and TÁMOP-4.1.1.C-12/1/KONV-2012-0012.

DIVERSIFICATION AMONG MUSHROOMS: ANALYZING RATES OF EVOLUTION IN THE AGARICALES

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The order Agaricales is the most species-rich group of the Basidiomycota, numbering ca. 480 genera and 14,000 described species. Extremely diverse fungi belong to this order, bearing highly distinct morphologies and displaying life history traits which show considerable diversity, yet the driving forces of evolutionary diversification in these mushrooms are poorly known. Understanding why and how certain lineages became extremely species-rich, while others are only represented by a few species, determining the timing of major lineage expansions, or the impact of fruiting body morphologies on speciation rates may have an effect on fungal conservation and taxonomy, however, theoretical and experimental evidence so far remained spurious. A recently launched 4-year project, ADiv, aims to investigate patterns of speciation, extinction and variations in the rate of evolution in the Agaricales. To address these questions, we will use statistical models of lineage diversification in a phylogenetic framework. Modeling of diversification will rely on a new two-gene dataset (referred to as diversity dataset) for ca. 3,000 species accepted in the Agaricales. For molecular investigations we have selected the nLSU and RPB2 loci, which are known to provide sufficient phylogenetic information for relationships at the intrageneric level. In addition to the diversity dataset, a phylogenomic dataset is also being produced, which will provide robust support for the backbone of the Agaricales to reinforce phylogenetic inference from the diversity dataset. These two datasets will allow us to examine general patterns of speciation and extinction, to identify shifts in diversification rates and whether transitions between different fruiting body types have an effect on rates of speciation and extinction in the Agaricales. In this contribution, we report an experimental workflow designed specifically for the Agaricales, progress in generating sequence data and preliminary results about adaptive radiations in the Agaricales.

REVIEW OF PEPTAIBOLS PRODUCED BY *TRICHODERMA* STRAINS RELATED TO THEIR PURIFICATIONS, STRUCTURAL ELUCIDATIONS AND BIOLOGICAL ACTIVITIES

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The efficiency of antimicrobial chemotherapy is increasingly challenged by the emergence of pathogenic strains exhibiting high levels of antibiotic resistance. Therefore, it is very important to search for novel compounds produced by living organisms, which are undergoing intensive investigations. The filamentous fungi are valuable sources of new biologically active compounds and their application in different medicines has a long history. The soil borne *Trichoderma* species (Ascomycota) are known producers of numerous secondary metabolites such as different antibiotics and peptaibols as well as several plant promoting compounds, which could enable their use in the green-agriculture as biocontrol agents. Peptaibols are linear, amphipathic polypeptides forming a family of peptide antibiotics of fungal origin, whose number is constantly growing since the first member, alamethicin was reported in 1966. A considerable part of these compounds was isolated

from the soilborne filamentous fungal genus *Trichoderma*. These peptaibols are composed of several amino acids (7-20) containing characteristic nonproteinogenic amino acid residues. Their purification from the ferment broth and mycelia contained usually comparable isolation and separation steps regarding their similar structures and chemical properties. Furthermore, the elucidations of the peptaibol structures were generally undertaken using mass spectrometric methods using ESI ionization techniques, where the peptide fragmentation pattern enables the de novo peptide sequencing. These types of antibiotics show interesting physico-chemical and biological properties, such as the formation of pores in bilayer lipid membranes, as well as antibacterial, antifungal, and occasionally antiviral activities, and may elicit plant resistance. The aim of this review is to summarize the significant amount of information about the purifications, structural elucidations and biological activities of peptaibols.

This study forms part of the project TÁMOP-4.2.2.A-11/1/KONV-2012-0035, which is supported by the European Union and co-financed by the European Social Fund. This research was also realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund. The research work was also supported by grant OTKA K-105972 from the Hungarian Scientific Research Fund.

SCREENING METHOD FOR THE RAPID DETECTION OF PEPTAIBOLS PRODUCED BY *TRICHODERMA* STRAINS

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Species of the imperfect filamentous fungal genus *Trichoderma* with teleomorphs belonging to the Hypocreales order of Ascomycota are of great economic importance as sources of antibiotics, enzymes, as plant growth promoters, decomposers of xenobiotics, and as commercial biofungicides. The peptaibols and related peptaibiotics (PrPs) are secondary metabolites constituting a family of fungal peptide antibiotics, which is constantly growing since alamethicin was isolated from cultures of strain NRRL 3199 of *Trichoderma arundinaceum* (initially identified as *T. viride*). These compounds are linear, amphipathic oligopeptides composed of 5-20 amino acids which usually contain several nonproteinogenic amino acid residues representing characteristic building blocks of the structure. The major part of the already described peptaibols is also produced by members of the genus *Trichoderma* including the species *T. aggressivum*, *T. asperellum*, *T. atroviride*, *T. aureoviride*, *T. brevicompactum*, *T. citrinoviride*, *T. harzianum, T. inhamatum, T. koningii, T. longibrachiatum, T. parareesei, T. parceramosum, T. polysporum, T. pseudokoningii, T. pubescens, T. reesei, T. saturnisporum, T. strigosum, T. stromaticum, T. virens* and *T. viride*. In our present work, a rapid bacterium-based screening method was developed and optimized for the detection of peptaibol production using the commercially available alamethicin as a peptaibol reference compound. Initially, a sensitive bacterium strain able to indicate the presence of alamethicin (*Micrococcus luteus*) was selected from the Szeged Microbiological Collection using plate assays. In the final method, the agar overlay technique was applied, which resulted in a homogeneous lawn of bacteria within a thin layer of agar across the surface of the plate. The inhibitory effects of solved compounds were investigated in standard-sized holes bored in the plates. During the experiments the pre-cultivation time and the concentration of the inoculated suspension of the selected *M. luteus* strain were optimized and standardized. The limit of detection and reproducibility of the assay were also determined. The developed method is able to detect the selected peptaibol molecule at relatively low concentration. Thus, it seems to be suitable for our peptaibol screening programme.

This study forms part of the project TÁMOP-4.2.2.A-11/1/KONV-2012-0035, which is supported by the European Union and co-financed by the European Social Fund. This research was also realized in the frames of TÁMOP 4.2.4. A/2-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system convergence program". The project was subsidized by the European Union and co-financed by the European Social Fund. Furthermore, the research work was supported by grant OTKA K-105972 from the Hungarian Scientific Research Fund.

MONITORING OF WEST NILE VIRUS IN MOSQUITOES DURING 2011-2012, HUNGARY

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West Nile virus (WNV) is a widely distributed mosquito-borne flavivirus. WNV strains are classified into several genetic lineages based on phylogenetic differences. While lineage 1 viruses are distributed worldwide, lineage 2 WNV has been first detected outside of Africa in 2004 in Hungary. Since then WNV-associated diseases and mortality in animal and human have been documented periodically in Hungary. Despite the isolation of lineage 2 viruses from animals and wild birds, infected mosquitoes have not been detected until 2010. After the first isolation of WNV in a *Culex pipiens* pool of mosquitoes, a two-year-long monitoring program was implemented in the country. The collection areas were located in the Southern-Transdanubium, in North-East Hungary, in East-Hungary and in South-East Hungary. During the two years, 25279 mosquitoes in 645 pools were screened for WNV virus presence with RT-PCR. In 2011 three pools (one pool of *Ochlerotatus annulipes* collected in Fényeslitke in June, one pool of *Coquillettidia richiardii* collected in Debrecen, Fancsika-tó in July and one pool of *Culex pipiens* captured near red-footed falcon colonies in Kardoskút in September) were found positive for WNV showing a minimal infection rate (MIR) of 0.22 regarding all mosquito pools, 2.02 in *Ochlerotatus annulipes*, 0.63 in *Cochlettidia richiardii* and 2.70 in *Culex pipiens*. Molecular data have demonstrated that the same, lineage 2 WNV strain circulates in wild birds, horses, humans and mosquitoes in Hungary since 2004. Mosquito based surveillance system is a useful tool to assess the risk of infection in animals and humans. Furthermore, the control of mosquitoes promotes lower hazard, protection against emerging and expanding viral diseases is much more complex challenge for animal and public health.

TAM RECEPTOR TYROSINE KINASES ACTIVATION IN ORAL CARCINOMA SURFACES COLONIZED BY *CANDIDA* SPECIES

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In the oral cavity, candidiasis is the most frequent opportunistic fungal infection. Interestingly, there is growing evidence for *Candida* species being implicated in various epithelial cancers, which leads to our hypothesis that alteration in immune regulatory receptors may contribute to increased interactions of oral carcinomas and *Candida*. Hence, we investigated the relationship between Tyro3/Axl/Mer (TAM) expression in oral squamous cell carcinoma (OSCC) and fungal colonization. Mammalian TAM receptors are a family of receptor tyrosine kinases that have been shown to down-regulate TLR signaling. Binding of TAM ligands induces an anti-inflammatory signaling pathway through activation of the suppressor of cytokine signaling-1 (SOCS1) protein and SOCS3. In addition to their roles in limiting TLR and cytokine signaling, TAM receptors play critical role in mediating cancer cell proliferation, invasiveness, and survival. To define the association of *Candida* with OSCC, samples from patients with newly diagnosed disease were obtained from the central surface of the lesions and from contiguous healthy mucosa and processed to define the fungal tissue burdens. The median number of colony forming units (CFU)/mL at carcinoma sites was significantly higher than that of the healthy mucosa in the same patient. Similar results were obtained when we compared the CFU in patients with OSCC to the CFUs on samples of healthy volunteers. *C. albicans* was the predominant species in the oral cavity, followed by *C. glabrata* and *C. parapsilosis*. Afterwards, we examined the TAM receptor expression in in vitro and in vivo samples. First we compared the TAM receptor activation in the OSCC cell lines (SAT, HO-1-N-1, HEP2, HSC2) with that of healthy primer human gingival epithelium cells, with or without *Candida* co-culture. We show that OSCC cells significantly overexpress TAM receptors, especially TYRO 3 and MER, compared to healthy control cells. Additionally, co-cultures with different *Candida* strains induced higher TAM expression in both cancer and normal cell lines. Lesion biopsy samples from patients with OSCC also showed elevated expression of the TAM receptors relative to that of healthy tissues from the same patient as well as samples from healthy volunteers. These findings further support a link between OSCC and *Candida* as well as suggest that this association may be specifically linked to expression of TAM receptors along with other unexplored co-factors.

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ARCHAEAL AND BACTERIAL DIVERSITY OF THE FIRST LAKE OF A GEOTHERMAL WATER RESERVOIR SYSTEM LOCATED IN SOUTHERN HUNGARY

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In Hungary, the replacement of geothermal waters previously utilized for industrial purposes is still not resolved. As a workaround, they are usually released to natural surface waters after being stored in reservoirs. Our investigation focused on the Barex Reservoir System near Szarvas which consists of four separate, but interconnected artificial water bodies. According to the water chemical analysis, the utilized thermal water was anaerobic, alkaline, rich in phenol derivatives and was of high salinity. Since little is known about the microbiological properties of these types of

environments, the aim of this study was to gain insight to the structure and composition of microbial communities and the influence of changing parameters. The first step of this long-term investigation was to reveal the phylogenetic diversity of microbial communities inhabiting the first lake water, sediment and biofilm developed on the reed surface. The spatial and temporal changes were tracked by DGGE, using primers 27fGC and 519r. Exploration of the phylogenetic diversity of bacteria and archaea was carried out by the construction of three clone libraries respectively, and sequencing 16S rRNA coding regions of DNA. Archaeal diversity was examined by the cloning of ribosomal sequences from the community DNA using primers A109f and A958r. Analyses have shown that bacterial communities of the sediment formed separate groups which showed little similarity to the communities of the lake water during the whole year. Both clone library analyses and DGGE patterns have shown that the influent water differed from every other sample. In the case of water samples, representatives of the Phylum Cyanobacteria were present in large proportions. Arthrospira platensis proved to be a constant member of the lake water community. In both biofilm and sediment samples members of the Genus *Hydrogenophaga* (Proteobacteria), the Ordines Rhodocyclales and Rhodobacterales and purple non-sulfur bacteria were found to be dominant. Archaeal communities were less diverse than bacterial ones based on the processing of three clone libraries and the similarity was more pronounced between the sample types. The archaeal diversity of the sediment was the most diverse among the sample types. The most common archaeal genera were *Metanosaeta* and *Metanocalculus*. The majority of bacteria identified from the Reservoir-System was haloalkalophilic, and might be capable of utilizing a wide range of carbon compounds.

TETRACYCLINE RESISTANCE TET(A) PLASMIDS TRANSFERRING MULTIRESISTANCE IN ENTEROTOXIGENIC *E. COLI* STRAINS FROM PIGS

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Enterotoxigenic *E. coli* (ETEC) bacteria are frequent pathogens causing watery diarrhea in weaned pigs. Plasmids encoding different enterotoxins (estA, estB, elt) and fimbrial adhesins (f4, f18) are of prime importance in the pathogenesis of ETEC strains. Recently, the complete sequence of the ETEC pTC plasmid was described, as a first representative of hybrid ETEC plasmids carrying the tetracycline resistance gene tet(B) in combination with enterotoxin genes estA and estB. Similarly, unraveling the impact of tet(A)-plasmids in the transfer of resistance and/or virulence would also be important for the efficient therapy of ETEC infections. The main objective of the present study was to provide a comparative description of antimicrobial resistance and virulence profiles of porcine post-weaning ETEC strains representing three middle-European neighboring countries: Hungary, Austria and the Czech Republic. Furthermore, the characterization of genetic vectors for tetracycline resistance was also attempted, with special regard to plasmids responsible for the transfer of tetracycline resistance gene tet(A) and putative co-resistance/virulence genotypes. A total of 87 porcine ETEC strains isolated from post-weaning diarrhea were included in this study. Majority of them were isolated in Europe: Hungary (n=16), Austria (n=34) and the Czech Republic (n=17), and 20 ETEC strains were derived from the USA. Antimicrobial resistance phenotype of the strains was determined for 17 antimicrobial compounds with clinical relevance. Strains resistant to tetracycline were subjected to further studies, including the PCR subtyping of the tet genes, and the detection and characterization of plasmids mediating tetracycline resistance and ETEC-specific virulence in two tet(A)-positive monoplasmidic derivatives of F18+ ETEC strains from Hungary and from the

Czech Republic (2172/11 and 11732/71 respectively). Regardless of the geographical origin, majority of the ETEC strains shared a common MDR pattern of sulfamethoxazole (91%), tetracycline (84%) and streptomycin (80%) resistance. The tetracyclin resistant phenotype was most frequently covered by the presence of the *tet*(B) gene (38%), when *tet*(A) was identified in 26% of the isolates. The virulence gene profile included enterotoxin genes (*elt*, *est*A and/or *est*B), as well as adhesin genes (f4, f18) without geographic differences. Characterization of these tet(A) plasmids revealed, that the IncI1 plasmid of the Hungarian strain mediated the co-transfer of *tet*(A), *aad*A1 (streptomycin/spectinomycin) and *str*A (streptomycin) genes, when the IncF plasmid of the Czech strain carried *tet*(A) in association with *cat*A1 for chloramphenicol resistance. Furthermore, the tet(A) plasmid of the Hungarian strain carried a class 1 integron with an unusual variable region of estX-aadA1 responsible to streptothricin-spectinomycin/streptomycin resistance phenotype. In conclusion, the presence of IncI1 and IncF type plasmids, responsible for the co-transfer of the *tet*(A) gene and additional resistance determinants were demonstrated for the first time in F18+ ETEC strains. In addition to the pTC hybrid plasmid of tet(B)-type, the first description of tet(A) plasmids was provided as a vector of multidrug resistance in porcine ETEC strains.

ENHANCEMENT OF PERFORMANCE OF MICROBIAL FUEL CELLS USING A NEW GEL-TYPE ANODE AND SEMI-CONTINUOUS FERMENTATION

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Microbial fuel cell (MFC) is a device that contains live microorganisms and it is able to convert directly organic chemical bounds to electrical energy. MFC is considered to be a promising sustainable technology, in particular when using wastewaters as substrates, offering the advantage of simultaneously treating wastewater and generating electric power. Unfortunately, so far this technology still face numerous problems such as low converting efficiency comparing with other chemical fuel cells, low electrical potential, capacity of applied bacteria etc. Generally, in MFC bacterial cells attach directly to electrode forming biofilm in anode chamber and transfer extracellular electrons to anode, thus the performance of MFC should be affected by quality of biofilm layer(s). In this study, a new anode-type containing gel-entrapped bacteria was constructed and operated semi-continuously. *Shewanella* bacteria cells were entrapped in alginate gel with some major modification. Effects of some components such as polyaniline (PANI), titanium-dioxide and ammonium-persulphate as well as graphite powder to increase the conductivity and to enhance the electron transfer were investigated. In case of the conductivity, three levels of PANI (0 g ·ml-1; 0.01 g ·ml-1; 0.02 g ·ml-1) and three levels of graphite powder (0 g ·ml-1; 0.01 g ·ml-1; 0.05 g ·ml-1) were applied. The gel electrodes were investigated in three identical size dual-chamber MFCs. The increase in concentration of PANI resulted the rise of the conductivity in the modified gels. Addition of 0.01 g·ml-1 and 0.02 g·ml-1 PANI caused 6-fold (from 10.4 S to 64.5 S) and 10-fold (to 104.4 S) higher conductivity, respectively. Moreover, electrical potentials were 1.5 times in the case of 0.01 g·ml-1 and two times higher in the case of addition of 0.02 g·ml-1 of PANI meaning two and three times increase in power density (from 4.83 W·m-3 to 10.05 and 14.62 W·m-3). Graphite powder had also significant effects on conductivity, voltage and power density performance. Addition of 0.05 g·ml-1 graphite powder caused 10-fold higher conductivity (10 S to 100 S), double higher voltage output (0.17 V to 0.34 V) and 4-fold higher power density (4.83 W·m-3 to 19.24 W·m-3), respectively. It worth to note that combination of PANI and graphite powder resulted 105-fold higher conductivity (10.4 S to 1087 S), 3-fold higher voltage generation (0.17 V to 0.44 V) and 7-

fold higher power-density output (4.83 W·m-3to 32.86 W·m-3). In conclusion, new type gel electrode contained entrapped Fe(III)-respiring bacteria has significant effects on increase of both conductivity and power density, thus it is good idea for enhancement of efficiency of MFC.

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DETECTION OF FUNCTIONAL VARIABILITY OF TWO AM FUNGI STRAINS WITH ELECTRICAL IMPEDANCE AND CAPACITANCE METHOD

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Applicability of electrical impedance (EI) and electrical capacitance (EC) measurements for investigation of functional diversity of arbuscular mycorrhizal fungi (AMF) strains were studied with two mycorrhiza-dependent hosts. EI and EC measurements in plant–soil systems can provide a good assessment of in situ root status without damaging plant and the results directly correlate with various root properties, such as fresh or dry weight, root surface area and root volume. AMF root colonization, biomass production, transpiration activity, EI and EC of *Rhizophagus intraradices* (*Glomus intrardices*) or *Funelliformis mosseae* (*Glomus mosseae*) inoculated and non-inoculated cucumber (*Cucumis sativus* L.) and bean (*Phaseolus vulgaris* L.) hosts were monitored during their growth under controlled climatic conditions. Compared to non-mycorrhizal plants, AMF colonization increased the transpiration rate, the root and shoot biomass production and the fruit number each. The *Rhizophagus intraradices* strain colonized both hosts a week earlier and the colonization rate was higher than that in case of the *Funelliformis mosseae* strain. The effect of the AMF strains differed more significantly on host response in case of the more highly mycorrhiza-dependent bean. The effect of AMF colonization can be detected by EI and EC measurements as increasing EC and decreasing EI. A significant difference appeared in the measured values between the control and the AMF treatments from the beginning of the colonization. This non-destructive method can be a suitable technique to detect the infection phase and the early colonization by AMF.

CATALYSIS OF SYNTHETIC REACTIONS IN NON-AQUEOUS CONDITIONS BY LIPASE ENZYMES FROM MUCOROMYCOTINA FUNGI

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Lipase activity of several fungal strains belonging to the orders Mucorales and Mortierellales (Mucoromycotina) was previously tested using liquid and solid-state fermentation methods. Enzyme yield of some isolates was outstanding when wheat bran was used as supplement in both submerged and solid state fermentations. However, under certain conditions, lipase enzymes can synthesize ester linkages resulting in various aroma-, phenyl- and alkyl esters and different polymers. In addition, some enzymes also have the ability to catalyze these esterification-transesterification reactions in organic solvents which presents, among others, higher yields and easier recovery of the products. Since the resulted compounds can be utilized in many biotechnological processes and

industrial applications, investigation of the synthetic reactions in organic solvents has special importance to find novel lipase enzymes for industrial process development purposes. The aim of this study was to test and characterize in non-aqueous conditions the transesterification capacity of lipase enzymes produced by Mucoromycotina fungi. Although numerous lipases have been isolated and characterized by zygomycetes, only a few enzymes have been tested for their synthetic activity in organic solvents. Transesterification activities were analyzed by suspending the lyophilized crude extracts of selected *Rhizomucor, Rhizopus, Mucor, Umbelopsis* and *Mortierella* lipase enzymes in n-heptane containing p-nitrophenyl palmitate and ethanol. Reactions were carried out for 30 min, and the released and extracted p-nitrophenol was monitored colorimetrically at 405 nm. For preliminary characterization of the reactions, sampling was performed at different time intervals; furthermore, the effect of incubation temperature on synthetic activity was also tested. Lipase enzymes exhibiting high transesterification activity were detected in case of each genus, furthermore, results showed that prolonged incubation time and high temperature conditions were generally enhanced the product yield. After purification and biochemical characterization of the enzymes, detailed analysis of the synthetic reactions will be carried out. Attention will be paid to the synthesis of alkyl-esters using various acceptor alcohols (e.g. methanol, propanol and isopropanol).

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AEROBIC AND ANAEROBIC DEGRADATION PATHWAYS OF AROMATIC HYDROCARBONS ARE BOTH ACTIVE UNDER MICROAEROBIC AND ANAEROBIC CONDITIONS AS REVEALED BY MICROCOSM EXPERIMENTS

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Aromatic hydrocarbons including benzene, toluene, ethyl-benzene, and xylene (BTEX) are frequent contaminants of groundwater, the major resource of drinking water. Most of these compounds are readily degradable by groundwater microorganisms both under oxic and anoxic conditions. Consequently, aerobic and anaerobic degradation pathways of BTEX-compounds are widely studied. However, most studies deal with clear oxic or anoxic conditions, and the behavior of degradation pathways are often studied by using pure strains in highly artificial laboratory environments. In subsurface environments the availability of oxygen is commonly restricted and the hydrocarbon contamination causes microaerobic conditions even in shallow groundwaters. The major questions are that which degradation pathways are active under these conditions and how the decreasing availability of dissolved oxygen affects the composition of the microbial community and the expression of functional genes of aerobic and anaerobic degradation pathways of BTEX-compounds. To assess these questions groundwater microcosm experiments were conducted. Microbial community composition and expression of catechol 2,3-dioxygenase ($C_{23}O$) and benzylsuccinate synthase (*bss*A) genes were investigated at 0.5 and 0.1 mg/l dissolved oxygen concentrations and 72 hours after oxygen depletion. Microbial community composition of the investigated groundwater was revealed by using metagenomic approach, while alteration of the community during the microcosm experiments was assessed by sequence-aided terminal-restriction fragment length

polymorphism (T-RFLP). Monitoring of the expression of functional genes was performed by using the method of single-nucleotide primer extension (SNuPE). The results revealed that the microbial community was dominated by members of the genus *Azoarcus*, followed by members of the genera *Pseudomonas, Thauera, Acidovorax, Albidiferax, Polaromonas, Dechloromonas* and *Geobacter*. It was also found that this community composition was unalterable during the microcosm experiments, irrespectively of the decrease of dissolved oxygen concentration. Consequently the same types of functional genes were detected in the microaerobic and in the anaerobic microcosms. Moreover, the mRNA transcripts of $C_{23}O$ and *bss*A genes were constantly detectable regardless of the availability of oxygen. These results assume that oxygen does not necessarily modulate the expression of the genes in question under in situ conditions. Results obtained may shed light on the processes which take part in the degradation of BTEX-compounds under microaerobic conditions.

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PCR-SCREENING OF LOWER VERTEBRATE SAMPLES WIDENS THE KNOWN HOST RANGE OF CIRCOVIRUSES: FIRST DETECTION OF CIRCOVIRUSES IN FROGS

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The continuous improvement of the detection methods has facilitated the discovery of numerous novel circo- and circo-like viruses in the past couple of years. Members of the *Circovirus* genus (in the Circoviridae family) have been demonstrated in different animal species worldwide, but their pathological role is not well understood. To date, only the bird circoviruses and the porcine circovirus type 2 (PCV-2) could be correlated with pathogenic capabilities.

In fish and other evolutionarily more ancient animals, such as arthropods, the presence of different circo-like viruses have been described rather recently. The new metagenomic approaches led to the discovery numerous novel circovirus-like sequences in different environmental samples and sewage water, but no reports are available from amphibians and reptiles.

The present study reports the detection and partial characterization of new circoviral DNA in fishes of different species (from the families Cyprinidae and Gobiidae), and for the first time in frogs (Bufonidae and Hylidae). A collection of negative samples from lower vertebrates, screened previously for the detection of adeno- and herpesviruses, were examined for the presence of circoviral DNA. A total of 236 fish (34 species), 9 amphibian (2 species), and 49 reptilian (4 species) samples collected randomly from Hungarian freshwaters, fish farms and pet shops were examined. The DNA was purified from pooled organs and cloacal swabs.

A generally used, broad-spectrum nested PCR was applied for the amplification of an approximately 350-bp fragment from the replication-associated protein gene. Out of the 294 samples, 6 piscine and 3 amphibian ones were positive, whereas every reptilian sample was negative. Interestingly, the DNA sequence derived from 2 of the 6 positive fish samples was identical with PCV-2. Phylogenetic analysis of the deduced amino acid sequences showed that each putative virus, detected in a common bream (*Abramis brama*), two green tree frogs (*Litoria caerulea*) and a

common toad (*Bufo bufo*) clustered within the *Circovirus* genus, while three sequences obtained from a round goby (*Neogobius melanostomus*), an asp (*Aspius aspius*) and a roach (*Rutilus rutilus*) appeared in the cluster of the circo-like viruses. Although none of the gene fragments, studied here, contained stop codons, for the confirmation of the newly-detected viruses, the sequence of their whole genome should be determined. This is in progress now.Our results widened the spectrum of vertebrates known to harbor circoviruses, with the amphibians. As the pooled internal organs usually contained samples from the digestive tract, we cannot declare with certainty that the positive samples signify necessarily active infection.
It is possible, that the circo- or circo-like viruses were present in the water and passively "contaminated" the animals. This applies especially to PCV-2, detected for the very first time in fishes, namely in a bream and a monkey goby (*Neogobius fluviatilis*). Nonetheless, the potential epizootiological importance of this finding merits further studies.

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ELECTRON TRANSPORT PATHWAYS FROM/TO THE HYN HYDROGENASE IN A PURPLE SULFUR BACTERIUM

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The phototrophic purple sulfur bacterium, *Thiocapsa roseopersicina* BBS uses sulfur compounds as electron donors and carbonate as inorganic carbon source for photoautotrophic growth. Electrons, generated from the oxidation of thiosulfate and sulfur are donated to the photosynthetic reaction center via cytochromes. The oxidation of sulfite to sulfate produces ATP or promotes the reduction of the quinone pool. *T. roseopersicina* BBS has four active [NiFe] hydrogenases. Hox1, Hox2 are soluble NAD+ reducing hydrogenases, while the other two enzymes (Hyn, Hup) are associated/bound to the membrane. Although, Hyn can evolve hydrogen in the presence of elemental sulfur, thiosulfate related hydrogen evolution is more efficient. Sulfite alone cannot drive the hydrogen evolution of Hyn but can be electron source for the Hox1 hydrogenase. The Hyn dependent hydrogen evolution seems to be light driven. The connection between Hyn and sulfur oxido-reductases is bidirectional: hydrogenase linked sulfur reduction could be detected. This can be inhibited by a QB site competitive inhibitor: terbutryn suggesting that the electrons from/to the Hyn hydrogenase go through the photosynthetic reaction center. This electron flow requires the presence of Isp2 coded in the hyn operon and located at the cytoplasmic side. According to our data Hyn hydrogenase related membrane electron transport model is outlined.

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COMPARISON OF THE PREVALENCE OF THE TYPE 3 FIMBRIA AND BIOFILM PRODUCTION CAPABILITY AMONG *KLEBSIELLA PNEUMONIAE* STRAINS ORIGINATE FROM SEWAGE TREATMENT PLANTS AND DIFFERENT CLINICAL SAMPLES

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We have continued the comparisons of the virulence patterns of *K. pneumoniae* strains isolated from different clinical samples such as blood stream infections (BSI) urinary tract infections (UTI) wound infections (WI) and form environmental samples such as wastewater (WW).

The aim of the present study was to investigate such virulence factors which may be essential in the first step of infection i.e. adhesion. We focused on the type 3 fimbria {mannose-resistant and *Klebsiella*-like haemagglutinin (MR/K)} and the biofilm production. The clonality of all the strains was checked to exclude the multiple representations in the strain collection with different DNA based methods. 88 BSI and same number of UTI isolates, 110 strains of WI and 113 WW isolates were included into the study. The WW strains were collected from 47 geographically different sewage treatment plants. We used tannic acid pre-treated bovine red blood cells for haemagglutination to show the presence of *Klebsiella*-like haemagglutinin on the bacterial cell surface. The biofilm production was measured by the optical density of the bound crystal violet dye by biofilm matrix during the growth of the bacterial cells on the 96 wells tissue culture plate. Type tree fimbria prevalence were similar in case of UTI (69,3%), BSI (68,1%), and WI (62,7%) strains, however WW isolates showed statistically significant less frequency (49.5%) compared to the clinical isolates ($\chi 2$, p value ranging 0,003-0,048). The UTI isolates showed the highest prevalence of biofilm production (89,7%) followed by WI (89,09%), WW (78,7%) and BSI isolates (76,13%). Significantly higher biofilm productions were found among the UTI isolates compared to BSI ($\chi 2$, p=0,015) and also in relationship between WI and BSI strains ($\chi 2$, p=0,016). Weaker significances were observed between UTI and WW strains ($\chi 2$, p=0,037) and also between WI and WW isolates ($\chi 2$, p=0,036). Summarising our data we may draw two conclusions.

The well-known correlation between the expression of some of subunits of type 3 fimbria which enhance the biofilm production was limited to the UTI isolates only but not to the other strains. For example, the BSI isolates showed second highest prevalence to express type 3 fimbria but showed the lowest biofilm production. This data may suggest that other factors also may be involved to the biofilm formation.

Our data suggest that *K. pneumoniae* may adapt to the environment where the bacteria inhabit. For instance, in the natural environment fewer strains carry type 3 fimbria where may be not indispensable having of this fimbria for the adhesion while among the clinical isolates may be essential to have fimbrial adhesive capability inside the host organism to make them able to establish an infection. Further studies are needed to validate these hypotheses.

MOLECULAR CHARACTERIZATION OF *ESCHERICHIA COLI* O157 AND CYTOLETHAL DISTENDING TOXIN -PRODUCING *E. COLI*

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Escherichia coli is an important member of humans' and animals' normal gastrointestinal tract microbiota. *E. coli* is usually a harmless commensal, but some strains have evolved by gaining specific virulence genes to highly dangerous pathogens. Pathogenic bacteria deploy numerous virulence mechanisms including adhesion, invasion, overcoming of host defences, and subversion of host cellular functions by secreted toxins and effectors. Pathogenic *E. coli* strains are classified into pathotypes based on the production of virulence factors and on the clinical symptoms that they cause. Clinical syndromes resulting from infection with pathogenic E. coli strains include: enteric / diarrheal disease, urinary tract infection, and sepsis/meningitis. The urinary tract infection and the sepsis meningitis causing strains are termed extraintestinal pathogenic *E. coli* (ExPEC), while the strains inducing gastroenteric disease are known as diarrheagenic *E. coli* (DEC). Enterohemorrhagic *E. coli* (EHEC) is one of the most intensively studied group of DEC. EHEC O157:H7 is a significant food-borne zoonotic pathogen. E. *coli* O157:H7 causes diseases in humans ranging from uncomplicated diarrhea to hemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS). Typical EHEC O157 strains produce one or more Shiga toxin (Stx; also called verotoxins), encoded by lambda-like converting phages, carry the locus of enterocyte effacement (LEE) pathogenicity island encoding the adhesin intimin and a large virulence plasmid. Ruminants and, in particular, healthy cattle are the major reservoir of *E. coli* O157:H7 and other Shiga toxin-producing *E. coli* (STEC). Bacterial toxins that modulate the eukaryotic cell cycle establish a toxin family, termed cyclomodulins. Cyclomodulins are an emerging functional family of toxins that perturb the eukaryotic cell cycle machinery with various mechanisms and determine whether the infected cell will grow and divide or die. Cytolethal lethal distending toxins (CDT) are considered as inhibitory cyclomodulins' prototype. CDTs are widely distributed among a variety of pathogenic bacteria originated from intestinal and extraintestinal infections of human as well as different animal species. Our studies provided new data in the laboratory diagnosis, pathogenesis and evolution of *E. coli* O157, STEC and CDT-producing *E. coli*. Molecular analysis revealed that the Hungarian bovine *E. coli* O157 strains represent two main clones: additionally to EHEC/ enteropathogenic *E. coli* (EPEC) O157:H7/NM clone we identified and characterized a novel geno-group of *E. coli* O157 strains. These atypical (stx and eae negative) O157 strains produce CDT-V and long polar fimbrae and could be evolved independently from the typical EHEC O157 strains. We identified a new member of the CDT family (CDT-IV). Our results suggest that the cdt-IV and cdt-I operons might have been acquired from a common source by phage transduction and evolved in their bacterial hosts. We were the first to sequence and annotate a 31.2-kb long, non-inducible P2-like prophage carrying the cdt-V operon of an atypical *E. coli* O157:H43 strain of bovine origin and the draft genome sequence of this strain was also reported. We demonstrated that the Stx-encoding phages are able to spread in vivo among wild-type *E. coli* strains.

I render thanks to my masters in the field as well colleagues and co-authors for their contribution.

PREPARATION OF DELETION LIBRARY IN PATHOGENIC YEAST *CANDIDA PARAPSILOSIS*

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Since the 1980s, fungi have emerged as major causes of human disease, especially among immunocompromised patients and those having serious underlying conditions. Instead of being obligate pathogens, many fungal species are opportunistic, that are only able to cause disease in individuals with damaged physical barriers or/and altered immune status. Besides *C. albicans*, which is the most common species responsible for invasive candidiasis, the incidence of *C. parapsilosis* has dramatically increased over the past decade therefore the understanding of the pathomechanism caused by this species becomes urgent. In our previous work several fungal transcriptional factors have been identified using RNA-Seq data, that were overexpressed during host-pathogen interactions. Based on these data we are able to generate *C. parapsilosis* strains that either lack these genes or overexpress them. To investigate *C. parapsilosis* gene function the creation of an overexpression library is under progress. We have recently adopted a gene knock out strategy from the work of Susanne Noble et al. [1]. The preparation of knock out library including the genes mentioned above is now in progress. Fusion PCR method was applied to generate gene specific deletion constructions to disrupt genes from the genome of *C. parapsilosis* CLIB leu-/his- auxotrophic strain. First of all we generated the gene specific flanking PCR products for the upstream and downstream regions of each of these genes, and the HIS1 and LEU2 marker PCR products. We used HIS1 marker from plasmid vector pSN52, and LEU2 from plasmid vector pSN40. After the fusion of these regions chemical transformation was used to create knock out strains. For each of the identifications we used colony PCR to confirm the total deletion of the genes. All of the transformants were barcoded using a 20bp tag to be able to identify the mutants during later in vivo infections. The null mutant strains are continuously being tested in different conditions such as growth abilities on certain temperatures and on different medias observing pseudohyphae formation as well. We have found null mutants that show differences in appearance such as increased pseudohyphae formation or regressed growth on different temperatures.
The comparison of the virulence of these null mutant strains with the use of infection models is now in progress. In the future with the use of this method we are able to identify key regulatory factors that may play a role in the virulence of *C. parapsilosis* during host-pathogen interactions.

[1] Noble, S.M., Johnson, A.D. (2005) Eukaryot Cell 4: 298-309.
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INTERACTIONS BETWEEN HUMAN MACROPHAGES AND *CANDIDA PARAPSILOSIS* – MODULATION OF HOST IMMUNE RESPONSE BY SECRETED FUNGAL LIPASES

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Candida species are opportunistic pathogens that are able to cause disseminated infection when the immunity of the host is weakened. Although *Candida albicans* has always been the leading cause of

invasive candidiasis, the incidence of *C. parapsilosis* has dramatically increased over the past decade. There are several fungal virulence factors that are known to play importnat role in the pathogenesis of candidiasis. One of these factors is the secretion of hydrolytic enzymes such as aspartyl proteinases and lipases. We have previously shown that lipase mutant *C. parapsilosis* strains have significantly decreased virulence in various infection models, and are killed more efficiently by mouse macrophages. In the present study, we compared the response of human peripheral blood monocyte-derived macrophages to a wild type (wt) as well as a lipase deficient (lip-/-) C. parapsilosis strain that has been previously established in our lab. When co-cultured with macrophages, both strains induced a significant increase in the expression of tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and PTGS-2 (prostaglandin-endoperoxide synthase 2) genes in host cells after 12 hours, as determined by quantitative real-time PCR. Notably, macrophages stimulated with lipase mutant *C. parapsilosis* showed at least two-fold higher expression of these pro-inflammatory mediators compared to those infected with lipase-producing (wt) *C. parapsilosis*. Furthermore, the lip-/- *C. parapsilosis* strain induced significantly higher TNFα, IL-1β and IL-6 protein production in macrophages after 24 hours compared to the wt strain. Interestingly, macrophages stimulated with the wt *C. parapsilosis* strain in the presence of lipase inhibitor Orlistat produced higher amounts of cytokines compared to those stimulated without the inhibitor. Additionally, we examined the phagocytosis of wt and lip-/- *C. parapsilosis* strains by human macrophages using quantitative imaging flow cytometry. We found that although after 2 hours both strains were phagocytosed to the same extent by host cells, the rate of internalization and phagolysosome fusion was higher in case of lip-/- *C. parapsilosis*. These findings confirm the role of fungal lipases as important virulence factors during *C. parapsilosis* infection, and support the hypothesis that these microbial compounds have anti-inflammatory potential. Our results contribute to the better understanding of the immune response induced by *C. parapsilosis*, and highlight the role of fungal lipases during host-pathogen interactions.

COMPARATIVE STUDY OF HOMOLOGOUS ENDOMANNANASES FROM DIFFERENT *THERMOBIFIDA* SPECIES

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The members of the genus *Thermobifida* known as compost inhabiting, outstanding lignocellulose degrader microbes, although one of the four *Thermobifida* species (*T. halotolerans* YIM90462) has been isolated from a salt mine, which is a very different environment. *T. fusca* YX is far more the best characterized strain from this taxon: in the last decade six cellulose hydrolase coding genes, two xylanases and an endomannanse have been cloned and characterized. Despite the fact that strain *T. fusca* YX is the model organism of thermotolerant aerobic cellulose decomposition, our knowledge on the cellulases and hemicellulases of other thermobifidas is considerably poor. Based on the resequencing genome project of *T. fusca* TM51 strain and a de novo genome sequencing of *T. cellulolytica* TB100 and *T. halotolerans* YIM90462 we cloned, expressed and biochemically characterized three homologus mannanase enzymes (Man5A). The de novo genome projects of *T. cellulolytica* and *T. halotolerans* have been accomplished by ABI's SOLID and Roche 454 sequencing system together, because based on the resequencing result by SOLID system alone, we couldn't design useable primers to amplify the homologous endomannanase genes from these two species. By the use of de novo genome project data endomannanses from *T. cellulosilytica* and *T.*

halotolerans were successfully cloned and expressed as His-tagged fusion protein in *E. coli*. The domain structures and the biochemical features of the investigated enzymes (specific activity, pH and temperature optimums) show high similarities: all the three endomannanase enzymes contain a CBM2 domain, have no activity on xylene or cellulose and show high pH and temperature optimum. Despite more than 80 percentage homology between the three enzyme's amino acid sequences, there are considerable differences in one prominent characteristic: endomannanase of *T. fusca* has the highest and Man5A of *T. halotolerans* has the lowest thermal stability.

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SULFIDE OXIDASE ENZYMES IN PHOTOSYNTHETIC PURPLE SULFUR BACTERIA

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Phototrophic purple sulfur bacteria can utilize various reduced inorganic sulfur compounds (eg. sulfide, elemental sulfur and thiosulfate) as electron donor during anoxygenic photosynthetic growth. In these bacteria flavocytochrome c and sulfide quinone oxidoreductase play a role in the first step of the electron transport from sulfide into the photosynthetic electron transport chain. These enzymes are members of disulfide oxidoreductase protein family. Flavocytochrome c (Fcc) is a periplasmic enzyme consisting of a large sulfide-binding flavoprotein (FccB) and a smaller cytochrome c subunit (FccA). Sulfide quinone oxidoreductases are monomeric membrane-bound flavoproteins which present in all domains of life. Sqr can transfer electrons directly from sulfide into the membrane quinone pool while Fcc indirectly via small periplasmic c-type cytochrome proteins. *Thiocapsa roseopersicina* is a photosynthetic purple sulfur bacterium. Three genes presumably encoding sulfide oxidizing disulfide oxidoreductase proteins were identified in the genom sequence: fcc, sqr and sqn. The Sqr and Sqn belong to different groups of Sqr-type proteins: group IV and group VI, respectively. There have not been yet detailed biochemical and structural studies about the members of the group VI. proteins. For biochemical and functional analysis Fcc and Sqn proteins fused to Strep II affinity tag were expressed in T. roseopersicina. The soluble dimeric Fcc and the membrane bound Sqn were successfully purified by affinity chromatography from periplasm and solubilized membrane fractions, respectively. Based on the absorption spectra of oxidized and sodium-dithionite reduced forms of purified Fcc and Sqn redox active heme c and flavin prosthetic groups were identified in the proteins which seem to bind covalently to the enzymes. Recombinant Fcc catalyzes the reduction of bovine heart cytochrome c in the presence of sulfide. The purified Sqn protein has sulfide-dependent quinone reductase activity.

Detailed biochemical and kinetic analysis of the Sqn revealed that the enzyme affinity for sulfide is low, accordingly Sqn could play role in the sulfide oxidation at high sulfide concentration. Our model is that Fcc, Sqr and Sqn enzymes have role in the sulfide oxidation metabolic pathways at different sulfide concentration in *T. roseopersicina* cells. Based on our results the model of sulfur metabolism of purple sulfur bacteria have been supplemented.

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CHARACTERISING CELLULOSE DEGRADING, ALKALIPHILIC ANAEROBIC ENRICHMENT CULTURES

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The aim of the present study was to characterise the microbial communities of different, cellulose degrading, anaerobic enrichment cultures. Samples for enrichment originated from Hungarian shallow, moderately alkaline lakes (Lake Velencei, Lake Szarvas and Lake Hévíz). For enrichment sediment, *Typha*-bed and wood samples were used, enrichment procedure happened in alkaline, mesophilic conditions. After enrichment all cultures were studied by SEM. Sediment enrichment culture contained lots of degrading plant materials but coherent biofilm was not observed, even many crystal forms could be detected. Number of bacterial cells in *Typha* enrichment was higher. In wood enrichment appearance of filamentous bacteria (not found in other cultures) was the most characteristic. Later the sediment enrichment samples were characterised also by molecular (T-RFLP and molecular cloning) as well as cultivation methods. Results showed that cultures originated from the different lakes differed strongly also microbiologically. The enrichment resulted that the community structures have been significantly changed during the first enrichment step while further transfers have not influenced the composition of the microbial communities.
Cultivation resulted microorganisms previously isolated from anoxic, alkaline environments (eg. *Actinotalea fermentans, Alkaliflexus imshenetskii, Natronincola ferrireducens, Anaerovirgula multivorans, Alkalitalea saponilacus*) but revealed also the appearance of many new taxa (far related to genera *Clostridium, Alkaliphilus and Spirochaeta*).

CULTIVATION OF A SLOW-GROWING BACTERIUM STRAIN INTO HIGH CELL DENSITY

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Nitrosomonas europaea is an autotrophic Gram-negative bacterium that gains all of its energy for growth from the oxidation of ammonia into nitrite ions (nitrification), according to the following equation: $2H^+ + 2e^- + NH_3 + O_2 \rightarrow NH_2OH + H_2O \rightarrow NO^{2-} + 5H^+ + 4e^-$ *N. europaea* (ATCC 19718) cultures are relatively easy to grow, although several factors (e.g. temperature, pH, aeration, effect of light, presence of metal ions) may have a negative influence on biomass formation. However, like most nitrifying bacteria, *N. europaea* also has a long generation time (appr. 8 hours). To achieve high cell density under submerged (batch) conditions is therefore not trivial [1].
A major prerequisite to achieve high cell density in batch culture is to ensure that inhibitory metabolic by-products remain at minimal concentrations. In case of *N. europaea*, the principal inhibitory metabolite being continuously formed during fermentation is nitrite. As a consequence, this bacterium cannot grow into high cell density under conventional batch conditions. One suitable method to overcome this problem is the application of a single-vessel dialysis membrane bioreactor system [2]. Dialysis membrane fermentors are highly efficient for the continuous removal of inhibitory or toxic products during cultivations, and bacterial cells can also be supplied with substrate via the membrane. Fermentations were performed in a Bioengineering reactor, with 2 L

total/1.5 L working and 6 L total/5.5 L working volumes in the inner and outer chambers, respectively. The two chambers are separated with a cellulose-ester based dialysis membrane (thickness 20±2 μm, cut-off number 104 Daltons, trans membrane flow 1.5 *10^3 ml/m2*h*bar). Inoculum cultures were grown in shake-flasks and comprised 5 % of the working volume of the inner chamber. The growth medium contained inorganic salts and ammonium-sulphate. At pH 7.8, the level of free ammonia is only approximately 4 % of that of the ammonium ion. Therefore, to ensure that appropriate amount of ammonia is available to support growth, pH of the medium was kept above this value by the automatic addition of concentrated (2 M) sodium carbonate. Because oxidation of ammonia is light-sensitive, cultivation was performed in total darkness. Growth of *N. europaea* was monitored via cell density determinations and by the measurement of nitrite formation. Maximal cell density achieved was over 3 times of the value achieved under concentional batch conditions using the same fermentation technology.

[1] Sándor, E. et al. (1999) Biotechnol Techn 13: 443-445.
[2] Karaffa, L. (2000) In: Integration of Membrane Processes into Bioconversions (eds. Bélafi-Bakó, K. et al.) Kluywer Academic/Plenum Publishers, New York, U.S.A pp. 223-229.

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MICROBIOLOGICAL PROCESSES BEHIND OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) PRODUCTION – RESULTS OF THE LAST EIGHT YEARS RESEARCH

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Pleurotus ostreatus is one of the most popular cultivated edible mushrooms due to its high nutritional value and therapeutic properties. It is also a model organism for white-rot basidiomycete with an extensively studied ligninolytic system. Although it has been cultivated on the large scale for at least a half century, many parts of the cultivation are based on empirical knowledge, and microbial processes are not known in details. Its production has two main parts, (1) the preparation of substrate with partial composting, pasteurizing, conditioning and the (2) sensu stricto cultivation of oyster mushroom with spawn inoculation into the substrate, substrate colonization and fruiting body (FB) formation. Our first aim was to reveal the microbiological and chemical changes during the substrate preparation. We demonstrated the existence of a significant bacterial community succession from the raw materials to the mature substrate with the following dominant bacterial genera: *Pseudomonas* and *Sphingomonas* at startup, *Bacillus, Geobacillus, Ureibacillus, Pseudoxanthomonas* and *Thermobispora* at the end of partial composting and finally several genera of Actinobacteria, *Thermus, Bacillus, Geobacillus, Thermobacillus* and *Ureibacillus* in mature substrate. Fungal species played part in the initial mesophilic phase of partial composting, but they disappeared after pasteurization. Enzymatic digestibility of the substrate was improved by 77%, whereas the cellulose and hemicellulose to lignin ratios decreased by 9% and 19%, respectively. The next step was to monitor, how oyster mushroom colonized this mature substrate, and what kind of interactions were formed between *Pleurotus ostreatus* and the prevailing microbiological communities. During primary colonization oyster mushroom grew quickly in the substrate producing large amount of laccase. The activity of manganese peroxidase increased only in the next phase,

which was followed by an intensive cellulose breakdown with high level of endoglucanase, cellobiose hydrolase and β-glucosidase activity. This latter had their peak during fruiting body production. Activity of lignin degrading enzymes was monitored on mRNA level, as well. There was a marked shift in bacterial community composition, where the members of family Bacillaceae became the dominant bacteria after complete colonization. In a future step we want to investigate, whether this dominance of Bacilli is a consequence of active bacterial proliferation or other bacteria are consumed by oyster mushroom and the remaining Bacilli are just survivors. In order to test the effect of bacterial presence oyster mushroom was produced on partially sterilized and normal mature substrate in a model system. Hyphae were growing significantly faster (25%) in normal tubes than in partially sterilized tubes, and FBs appeared one week earlier on normal tubes. Moreover the tubes with partially sterilized samples were more sensitive for external fungal contamination. Our results provide us a deeper knowledge about the details of oyster mushroom production, which is useful for improving more effective mushroom production protocols.

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LACTOSE CATABOLISM IN *PENICILLIUM CHRYSOGENUM*: PHYLOGENETIC AND EXPRESSION ANALYSIS OF THE PUTATIVE PERMEASE AND HYDROLASE GENES

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Strains of *Penicillium chrysogenum* (sensu lato) are used as industrial producers of penicillin. We investigated its catabolism of lactose, an abundant component of the diary residue whey that has been used extensively in penicillin fermentation, comparing the type strain NRRL 1951 with the producer AS-P-78. Both strains grew similarly on lactose as the sole carbon source under batch conditions, exhibiting almost identical time-profiles of sugar depletion. In silico analysis of the genome sequences revealed that *P. chrysogenum* features at least five putative bGal-encoding genes at the annotated loci Pc22g14540, Pc12g11750, Pc16g12750, Pc14g01510 and Pc06g00600. The first two proteins appear to be orthologs of two *Aspergillus nidulans* intracellular family 2 glycosyl hydrolases expressed on lactose. The latter three *P. chrysogenum* proteins appear distinct paralogs of the extracellular bGal from *Aspergillus niger*, LacA, a family 35 glycosyl hydrolase. The *P. chrysogenum* genome also specifies two putative lactose transporter genes at the annotated loci Pc16g06850 and Pc13g08630. These are paralogs of the gene encoding the high-affinity lactose permease (*lac*pA) in *A. nidulans* for which *P. chrysogenum* appears to lack the ortholog. Transcript analysis of Pc22g14540 showed that it was expressed exclusively in response to lactose, while Pc12g11750 was weakly expressed on all carbon sources tested, including D-glucose. Pc16g12750 was co-expressed with the two putative intracellular bGal genes on lactose and also on L-arabinose, but not on any other carbon sources, while its two paralog genes were apparently not transcribed under any condition tested. Pc13g08630 transcript was also formed exclusively on lactose, suggesting that it may indeed function as a lactose permease.

The data strongly suggest that *P. chrysogenum* exhibits a dual assimilation strategy for lactose, simultaneously employing extracellular and intracellular hydrolysis, without any correlation to the penicillin-producing potential of the fungus.

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CARBON SOURCE PROFILING THE EXPRESSION OF THE LELOIR-PATHWAY GENES IN *PENICILLIUM CHRYSOGENUM*

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Strains of *Penicillium chrysogenum* are used as industrial producers of penicillin and other related antibiotics. As such, *P. chrysogenum* is amongst the most important fungi employed in biotech industry. However, apart from traits related to penicillin biosynthesis, general aspects of their carbon metabolism have received little attention. In particular, its metabolic relationship with lactose and its monomer D-galactose remained obscure, which is surprising in light of the fact that on technical scale, penicillin was produced on lactose as a carbon source for several decades. Two strategies have been described for the catabolism of lactose in fungi: extracellular hydrolysis by a beta-galactosidase and subsequent uptake of the resulting monomers, e.g. D-glucose and D-galactose, and uptake of the disaccharide followed by intracellular hydrolysis. The biological interconversion of galactose and glucose takes place only by way of the Leloir pathway and requires the five enzymes galactokinase, galactose-1-P uridylyltransferase, UDP-galactose 4-epimerase, UDP-glucose pyrophosphorylase and phosphoglucomutase [1]. Galactose mutarotase also participates by producing the galactokinase substrate alpha-D-galactose from its beta-anomer. In this study we analyzed the expression of genes comprising the Leloir-pathway on lactose, D-galactose, D-glucose, D-xylose and L-arabinose. In silico analysis revealed that *P. chrysogenum* features at least one putative galactokinase encoding gene at the annotated locus Pc13g10140, which shows strong similarity to the yeast galactokinase, and was expressed on lactose and D-galactose only. This is in contrast to results from other fungi such as *Aspergillus nidulans* and *Trichoderma reesei*, where galactokinase appeared constitutively expressed. The expression profile of the galactose-1-phosphate uridyl transferase at annotated locus Pc15g00140 was similar to that of galactokinase. As for UDP-glucose-epimerase encoding genes, five candidates at the annotated loci Pc16g12790, Pc21g12170, Pc20g06140, Pc21g1370, Pc18g01080 were identified. These five proteins appear to be orthologs of the respective (and characterized) *A. nidulans* and *A. fumigatus* enzymes. Pc16g12790, Pc21g12170 and Pc20g06140 were not expressed in any of the carbon sources tested, while from the other two loci (Pc21g1370, Pc18g01080) transcript was formed on each carbon source. The rest of the Leloir-pathway genes (UDP-glucose pyrophosphorylase at Pc21g12790) and phosphoglucomutase (Pc18g01390 and an ortholog of *A. nidulans* AN2867) also expressed constitutively. Notably, we identified a constitutive and apparently independent (though at present hypothetical) galactose mutarotase encoding locus (Pc20g08410). An independent mutarotase enzyme – if proven to exist – would be intriguing as this activity in most fungi exists as a domain within the UDP glucose epimerase protein, while in *T. reesei* it is missing [1].

[1] Fekete, E. et al. (2008) PNAS 105: 7141-7146.

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HETEROLOGOUS EXPRESSION OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN IN PICHIA PASTORIS AND ITS ANTIFUNGAL ACTIVITY AGAINST FILAMENTOUS FUNGAL ISOLATES FROM HUMAN INFECTIONS

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The *Neosartorya fischeri* antifungal protein (NFAP) is the member of the defensin-like antifungal protein family secreted by ascomyceteous filamentous fungi. It consists of 57 amino acids, and has a molecular mass of 6615.5 Da, and a calculated pI of 8.93. In our previous work we demonstrated that NFAP effectively inhibits the growth of numerous filamentous fungi including potentially human and plant pathogens. In silico structure modelling revealed that NFAP contains five β-strands connected by three loops, and showing a β-barrel topology in general which is stabilized by three intramolecular disulfide bridges. Despite of the available knowledge of the nature of its 5′-upstream transcriptional regulation elements, the practical application of NFAP is still limited because of its low yield production by the original producer. For the future investigation and practical application it would be important that NFAP could be producible in a non-sensitive, easily fermentable, "generally recognized as safe" (GRAS) fungus For this reason, in this study we carried out the heterologous expression of NFAP in the yeast *Pichia pastoris*, and investigated its antifungal activity on filamentous fungal isolates from human infections compared to that was exerted by the native NFAP from *N. fischeri*. *P. pastoris* KM71H transformant strain harbouring the pPICZαA plasmid with the mature NFAP encoding gene produced the protein. The final yield of the hNFAP was six-fold compared to the NFAP produced by N. fischeri NRRL 181. Based on the signal dispersion of the amide region, it was proven that the hNFAP exists in folded state. The purified hNFAP effectively inhibited the growth of fungal isolates belonging to the *Aspergillus* and to the *Fusarium* genus, but all investigated zygomyceteous strain proved to be insusceptible. There was no significant difference between the growth inhibition effect exerted by the native and heterologous NFAP. Our results provide a base for further research, e.g. investigation of the connection between the protein structure and the antifungal effect using site directed mutagenesis.

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ESTABLISHMENT OF A MULTIMICROBIAL ROOT CANAL INFECTION MODEL FOR DENTAL EXPERIMENTS

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The root canal infection is one of the most common infection types in periodontal practice. The eradication of the polymicrobial consortium is a difficult problem because of histologic structure of the tooth i.e. dentin tubulus. The insufficient antimicrobial treatment can be the source of further inflammatory complications of tooth, which can lead to the extraction of tooth. Lot of different ex vivo root canal microbiological models exist using them for endodontic researches. Relatively few

models use more than one microbe, however it is well known fact that the root canal infections are polymicrobial., Our aim was to work out an ex vivo polymicrobial root canal infection model, wherein we could artificially generate a microflora that is similar to chronic root canal infection and it is appropriate to compare the efficiency of different endodontic disinfection methods. Our other objective was to establish a triple microbial infection, wherein three different microbes cover the broad spectrum of microbes regarding of the surviving capability of them. A Gram positive, a Gram negative bacterium and a fungi species was used. We chose the three of the most frequently identified pathogen in chronic root canal infections i.e. *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans*. First we had to determine the most appropriate growth medium and the incubation time therefore we used 17G sized standardized injection needles as a model of the circumstance of root canals. The samples were taken by dental paper points and colony count was determined. We used mono and double infection as well to examine how the species interact with each other in different media and in different incubation time. After we achieved our aim, the three microbial cultures existing at the same time, we switched from the needles to extracted human teeth. The teeth had (n=40) one root canal which were prepared by engine-driven Mtwo files (VDW, Munich, Germany) up to size 30/0.06 in same working length (d=13 mm). The teeth were sterilized and inoculated with the three microbes and we took samples daily. We found that the three microbes could grow together till the third day of the incubation, but from the fourth day CFU of *Candida albicans* was decreased. As many time we repeated this method the mixed culture was present in the root canal. This method maybe useful tool for further dental experiments e.g. testing the efficacy of different disinfectant for eradicating microbes from the root canal.

ANTIBIOTIC-BILE SELENIT-F BROTH/AGAR

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The diagnosis of *Salmonella* spp. is based upon isolation of the etiologic agent in culture. Prolonged fecal excretion is well known because of intestinal *Salmonella* infection. Stool specimens contain numerous microbial strains, between are usually of the normal micro flora. However, many prohibit isolation small number of *Salmonella* spp., which multiply freely in Selenite F broth medium and suppress the growth of the *Salmonella* spp. (it is *E. coli* ssp., *Enterobacter* and *Klebsiella* spp., *Pseudomonas* spp., *Citrobacter freundii*, *Proteus* spp.). *Salmonella* infection of man and animal has increased in the world wide, and it is one of the world's most important zoonoses-diseases, with a high incidence, morbidity, but relatively low mortality. Conventional method for isolation and detection of *Salmonella* spp. is unreliable. Selenite F broth was not sufficiently toxic to inhibit fecal coliforms microbes completely. The concentration of bile in the gall bladder is several times higher than in the intestinal luminal. Antibiotic selective inhibited growth micro flora. The object of this study is to modification the original Selenite F broth formula, elaborate more selective Selenite F broth or agar media, which inhibited bacterial contaminants of specimens, whereas they did not obstruct the growth of *Salmonella* spp. Selenite F broth with agar, eliminates non-motile bacterial species from the start and provides growth motile *Salmonella* spp. If we used selective Selenite F broth, the author wants to suggest to adding combination the anti-metabolism selective supplement "Sigma": Novobiocin N-1628, Sulfanilamide S-9251, Sulfabenzamide S-9757, or Nitrofurantoin N-7878 and Bile Acid-oxidized B-8256. Combination: Sulfanilamide 0,5g, Nitrofurantoin 0,0025g and Bile Acid-oxidized 0,2g.; or Novobiocin 0,01g, Sulfanilamide 0,3g, Sulfabenzamide 0,05g and Bile Acid-oxidized 0,2g. The best result be realized with *Salmonella* motility medium, add 3,5/4g agar to one-liter modified Selenite F broth. In this chance is useful tube with small pipe inside. Selective

selenite motility medium gives more rapid and purity results, for the isolation of *Salmonella* spp. from stools, especially when incubated 18-24 hours in water bath at 370C or 430C. This ″antibiotic-bile-acid selenite broth or agar" gives a high specificity and sensitivity when compared with Selenite F broth. Not motility *Salmonella* spp. isolated from instead of inoculation. Very important that to have well-differentiated selective agar, so we can detect with case the presence of small number of *Salmonella* spp. and lactose positive *Salmonella* spp. (*S. Newport, S. Tennessee*) which cause confusion, among other Enterobacteriaceae spp. Bismuth Sulphite Agar is particularly useful for the isolation lactose-fermenting salmonellae. Salmonella spp. gave positive reactions for: arginine, lysine, ornithine, citrate, H_2S, glucose, mannitol, sorbitol, rhamnose, melibiose, arabinose, trehalose, xylose, galactose, levulose, maltose, dulcitol, KCN, methyl red and negative reactions for: urea, tryptophan, indole, Voges Proskauer, gelatin, inositol, sucrose, amygdaline, adonitol, esculin, dextrin, inulin, raffinose, salicin, malonate.