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MONITORING THE BIOGAS PRODUCING ARCHAEA COMMUNITY VIA MOLECULAR BIOLOGICAL METHODS

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Biogas is one of the most promising renewable energy carriers since it has got the potential to produce energy from numerous biomasses, combining the production of green energy with the elimination of hazardous waste streams. Biogas, the main product of the anaerobic digestion (AD) process can be utilized by burning in combined heat and power (CHP) units or after purification it can be treated equivalently to the natural gas. If we want to enhance the produced biogas yield, or digest unconventional substrates, a deeper understand of the microbial community involved in the fermentation is requisite. This can be achieved with the culture independent methods. These molecular biological tools (TGGE, DGGE, ARDRA, RISA, T-RFLP) are widely applied today. High protein content substrates are considered toxic for the biogas fermentation due to the high level of ammonia released upon protein degradation. In this study systematic experiments were conducted in 5 litre CSTR (continuous stirred) fermentors, fed wit protein-rich substrates (casein or pig blood). We selected T-RFLP technique to determine the abundant archaeal microbial groups from the assortment of genomic DNA in the fermentation sample. Fluorescent labeled primers targeting the large subunit of the methyl-coenzyme-M-reductase (*mcr*A) gene were used to generate the mixed PCR product from the purified gDNA. The restriction endonuclease, RsaI was applied in order to generate the RFLP patterns of the selected samples. We concluded that the unconventional substrate source affected not only the primary consumers (hydrolyzing bacteria) but the archaeal community as well. A significant shift could be observed, confirming the dominant role of the genus *Methanoculleus* in the conversion of protein-rich material into biogas.

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SURVEILLANCE OF HUMAN ROTAVIRUSES IN 2007-2011, HUNGARY

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Group A Rotaviruses are the major viral pathogens causing acute dehydrating gastroenteritis among children less than five years of age. The availability of rotavirus vaccines has resulted in an intensification of post vaccine strain surveillance efforts worldwide to gain information on the impact of vaccines on prevalence of circulating rotavirus strains. In this study, the distribution of human rotavirus G and P types in Hungary is reported. In addition, the VP4 and VP7 genes of G1P[8] strains were sequenced to monitor if vaccine-derived strains were introduced and/or some

strains/lineages were selected against. The study was conducted in 8 geographic areas of Hungary between 2007-2011. Rotavirus positive stool samples were collected from diarrheic patients mostly <5 years of age. Viral RNA was amplified by multiplex genotyping RT-PCR assay, targeting the medically most important G and P types. When needed, sequencing of the VP7 and VP4 genes was performed.In the study period, a total of 2380 rotavirus strains were successfully genotyped. Our results indicated the dominating prevalence of genotype G1P[8] (44.87%), followed by G4P[8] (23.4%), G2P[4] (14.75%) and G9P[8] (6.81%) strains. Uncommon strains were identified in a low percentage of samples (4.12%). Phylogenetic analysis of 318 G1P[8] strains identified 55 strains similar to the Rotarix strain (nt sequence identities; VP7, up to 97.9%; VP4, up to 98.5%) although their vaccine origin was unlikely.
Current vaccines would have protected against the majority of identified rotavirus genotypes. A better understanding of the potential long-term effect of vaccine use on epidemiology and evolutionary dynamics of co-circulating wild type strains requires continuous strain surveillance.

MOLECULAR PHYLOGENETIC RECONSTRUCTIONS WITH A DISCRETE MATHEMATICAL METHOD, THE BOOLEAN ANALYSIS

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Boolean analysis, method for sequence comparison uses the Iterative Canonical Form of Boolean functions [1, 2]. It considers sequence information in a way entirely different from standard phylogenetic methods (i.e. Maximum Parsimony, Maximum- Likelihood, Neighbor-Joining, and Bayesian analysis). The performance and reliability of Boolean analysis were tested and compared with the standard phylogenetic methods, using artificially evolved – simulated – nucleotide sequences and the 22 mitochondrial tRNA genes of the great apes. At the outset, we assumed that the phylogeny of Hominidae is generally well established, and the guide tree of artificial sequence evolution can also be used as a benchmark. These offer a possibility to compare and test the performance of different phylogenetic methods. Trees were reconstructed by each method from 2500 simulated sequences and 22 mitochondrial tRNA sequences. Considering the reliability values (branch support values of consensus trees and Robinson–Foulds distances) used for trees produced by different phylogenetic methods, BOOL-AN appeared as one of the the most reliable method. It can be concluded that Boolean analysis is a promising alternative to existing methods of sequence comparison for phylogenetic reconstruction and congruence analysis. Therefore this method can also be applied for inferring phylogenies of taxa with ambiguous evolution, like non-marine planktonic picocyanobacterial isolates (*Synechococcus* and *Cyanobium*). I investigated their phylogeny (based on the 16S ribosomal RNA genes) both with BOOL-AN and standard phylogenetic methods.

[1] Jakó, É. et al. (2009) Mol Phylogenet Evol 52: 887-97.
[2] Ari, E. et al. (2012) Mol Phylogenet Evol 63: 193-202.

FUROCOUMARINS: A NEW, POTENTIAL GROUP OF HIV-1 INHIBITORS?

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Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), is still one of the leading causes of death in low-income countries. In high-income countries, highly active antiretroviral therapy (HAART) improved the life expectancy of HIV infected individuals. Rapid replication, high mutation rate and frequent recombinations of HIV can contribute, however, to the development of drug resistant variants, which can replicate even in the presence of antiretroviral drugs, leading to the failure of therapy. Accordingly, development of new inhibitors is required. Usage of naturally occurring compounds probably could increase the applicability and availability of drugs also in low income countries, where the epidemic is the most considerable. The aim of this study was to examine the anti-HIV effects of three furocoumarin compounds from *Ruta graveolens* and *Evodia hupenhensis*. We studied the cytotoxicity and inhibitory effect of byakangelicin, 8-geranyloxy psoralen and izopimpinellin on MT4 cells by MTT cell proliferation assay in vitro. The antiviral activities were determined by p24 antigen assay. The results demonstrated that only 8-geranyloxy psoralen had an inhibitory effect on HIV-1 replication. The mechanism of action of 8-geranyloxy psoralen remains to be elucidated.

ANAEROBIC FERMENTATION OF DISTILLERY THIN STILLAGE

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Industrial processes create large amount of waste which are difficult and costly to decontaminate and properly dispose off. The distillery waste of starch or sugar based ethanol production is a light brown liquid with high organic content. Because of the high concentration of organics, distillery waste is a potential source of renewable energy and it can be fermented under anaerobic conditions. The main problem of the degradation of this material as a mono-substrate is the depletion of the trace elements during anaerobic degradation. The distillery waste usually does not contain enough trace elements and vitamins which are necessary for the proper microbiological activity. The anaerobic fermentation of the distillery stillage waste was carried out in explicitly designed biogas reactors working in continuous operation mode with a working volume of 5 liters plus 1 liter headspace. The devices could be fed continuously or intermittently through a piston-type delivery system, which controls the substrate volume introduced into the digester. As the feeding was performed, the same volume of fermented material was removed through an overflow via U-shaped tubing in order to maintain a gas-tight closure and constant working volume. The biogas fermentors were equipped with a spiral strip mixing device driven by an electronic engine. In this experimental set-up we have confirmed that it is possible to degrade distillery stillage as a mono-substrate in an anaerobic reactor efficiently if the supply of the trace elements and vitamins are adequate.

PATTERN OF LIGNOCELLULOSE DEGRADING ENZYME ACTIVITIES DURING COLONIZATION OF OYSTER MUSHROOM SUBSTRATE

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Oyster mushroom (*Pleurotus* spp.) is the second most popular cultivated edible mushroom in Hungary following button mushroom. Despite this fact, there is insufficient knowledge on the details of production, which has two main parts, the preparation of substrate and the sensu stricto cultivation of oyster mushroom. In the previous years our research group studied the bacterial succession during the substrate preparation from the raw materials to the mature substrate. In our new project, we would like to follow how oyster mushroom colonizes this mature substrate. The aim of the present work was to monitor this colonisation with determination of lignocellulose degrading enzyme activities. A model system was created. Autoclaved, gamma-sterilized and normal substrate was filled into tubes, inoculated with oyster mushroom spawn at one end. Control tubes, without inoculation were also prepared. The tubes were incubated at 26°C, 65% RH for one month. Sterilized tubes were prepared, to explore, whether microbes have a direct effect on the colonization pattern. Before complete colonization the tubes were cut into slices. Lignocellulose decomposing enzymes (endocellulase, exocellulase, beta-glucosidase, endoxylanase, xylosidase, laccase, manganese peroxidase and exochitinase) were extracted from the slices with phosphate buffer, enzyme activities and reducing sugar content was determined. There was no difference in colonization speed among normal and sterilized substrates. Activity of laccase was the highest in the region of the front hyphae. As sterilization partially destroyed the structure of the substrate, and toxic compounds for the mushroom might have been released the sterilized samples had a relatively higher laccase activity. Endocellulase showed highest activity in the formerly colonized regions of normal substrate. Since cellulose chains might have been fragmented during sterilization it had lower activity in the sterilized tubes. Activity of exocellulase and beta-glucosidase did not show considerable differences between regions of normal and sterilized substrate. In the uncolonized regions all enzyme activities were low, which suggests, that the bacteria did not have a direct substantial role in lignocellulose decomposition during substrate colonization. The observed differences among normal and sterilized tubes can be explained mostly with the alteration of the substrate structure not with the absence of bacteria. To get a deeper insight into the colonisation pattern of normal substrate, further parallel investigations are required.

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TOXOCARIASIS A RARE PARASITOSIS IN COUNTY MUREŞ, ROMANIA

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The larval forms of nematodes belonging to the genus *Toxocara* causing infection in humans are: *Toxocara canis* (specific to dogs), *Toxocara cati* (specific to cats). They lead to syndromes called *Larva migrans visceralis* (LMV) or *Larva migrans ocularis* (LMO), due to ingestion of viable,

embryonated eggs transmitted by soil. The LMV syndrome appears in children under 5 years and the most affected organs are the liver, lungs and Central Nervous System (CNS). The LMO is present in children aged between 5- 10 years, and is characterised by unilateral ocular impairment. We review the cases of children suspected of *Toxocara* infection. Each case came from rural area and they all were less than 8 years old. Two cases were associated with decreasing of the sight and three with cough and expectoration. One child was detected accidentally, without having any symptoms. No CNS implication was evidenced. The clinical findings were not specific, laboratory results showed increased white blood cell counts with hypereosinophilia and lower red blood cell counts. In one case severe anaemia was detected. The increased IgE titers were guiding us to the diagnosis of toxocariasis, wich was confirmed by specific IgG anti- *Toxocara* ELISA and by Western Blot. Using the ELISA test, titers over 1,1 UI/ml were considered as positive results. Appropriate treatment was based on Albendazol, with good compliance. The normalised eosinophil counts and disappearing of symptoms showed improving of their status. In conclusion, toxocariasis has to be considered in children from rural area with coughing and expectoration or visual acuity problems. This disease can be prevented by strictly respecting personal hygiene measures, by avoiding contamination of soil and playgrounds designated for children, by washing fruits and vegetables before use, by thorough hand wash before meals, by avoiding children's geophagy, applying regulate anthelminthic treatment of pets, especially for dogs under 6 month.

MOLECULAR IDENTIFICATION AND POLYPHASIC CHARACTERIZATION OF *ASPERGILLUS* ISOLATES CAME FROM KERATITIS CASES FROM INDIA

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Aspergillus strains are among the most common organisms causing fungal keratitis in tropical and subtropical areas. The main risk factor for the infection is trauma by vegetable matter during agricultural activities. Among *Aspergillus* species mainly *A. flavus, A. terreus, A. fumigatus* and *A. niger* have been isolated from fungal keratitis cases. During this study, 52 *Aspergillus* strains isolated from keratitis cases in South India were examined. Based on morphological studies, all isolates were classified to the *A. flavus* species. For the molecular identification, part of the calmodulin gene was amplified and sequenced. As a result, 46 isolates were identified as *A. flavus*, while four as *A. tamarii*, one as *A. terreus* and one was found to belong to the *A. pseudotamarii* species. This is the first report on the identification of *A. pseudotamarii* from a human infection. *A. pseudotamarii* is able to produce both B and G type aflatoxins. Aflatoxin producing abilities of the isolates were tested in YES culture media. The toxin producing abilities of the isolates were determined by HPLC analysis. Aflatoxin B1 was produced in the range of 50,2 ng/ml to 75,3 µg/ml by *A. flavus* isolates, while the concentration of aflatoxin B2 was between 34,4 ng/ml and 352 ng/ml. We examined the aflatoxin producing ability of *A. pseudotamarii* also in BHI and RPMI media, which imitate the human body fluids. *A. pseudotamarii* produced aflatoxins only in YES medium (2,6 ng/ml aflatoxin B2, and almost 1 µg/ml aflatoxin B1). Regarding the genetic variability of the isolates, most of the *A. flavus* isolates and the *A. pseudotamarii* strain belonged to the MAT1 mating type. Further investigation of the genetic variability of the isolates and the antifungal susceptibility testing with disc diffusion and E-test methods are in progress.

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NEXT-GENERATION SEQUENCING BASED FUNCTIONAL GENOMICS METHODS IN TRANSCRIPTOMICS - CHIP-SEQ, RNA-SEQ AND GRO-SEQ

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In September 2012, the ENCODE (Encyclopedia of DNA Elements) consortium scientists published 30 papers in high impact journals based mainly on systematic ChIP-seq and RNA-seq analysis of various human cell lines and tissues. The results prove that the latest advances in next-generation sequencing technology provide powerful tools for deciphering the transcriptional regulation mechanism. From the results we know better now where are the transcription factor binding sites (TFBSs) genome-wide, or how can we easily identify them from ChIP-seq data. We know how the histone modifications show the transcriptional activity, and where are the nucleosome-depleted regions in the chromosomes. From the RNA-seq data we know not only the expression level of the coding genes, but we can exactly see the alternative splicing products. We can also detect non-coding RNA transcription across the genome. By separating polyA+ and minus RNAs and RNAs from the nuclei and the cytoplasm, we can determine the life and history of the different kind of RNA products. From 2008 the GRO-seq, a new next-generation sequencing functional genomics technology is also provides interesting details about the transcriptional machinery of the cell. We can now see almost real time, where and in what extent the transcription occurs in the cell. This provided some new details, which cannot be found in the textbooks. We know now, that there are paused polymerases sitting on the DNA at the transcriptional start site (TSS) or that the RNA synthesizing polymerase II does not stop at the polyadenylation site but goes further, sometimes as long as 10-15 kbp. We have also learnt that there is a definitive transcriptional activity at the active enhancer regions, which were thought only binding TFBSs. This three technique is very powerful per se, but analyzing their results together will greatly help us to understand the very basic processes in gene regulation and transcription. To cope with this "data tsunami" however, we need not only to buy suitable hardware infrastructure, but also we have to develop powerful new databases and software tools. In this presentation I will try to show how these methods helps us in understanding the biological processes. I summarize the bioinformatics challenges in processing ChIP-seq, RNA-seq and GRO-seq data, and present some of our results based on this techniques.

MANIPULATING THE SOIL MICROBIOME TO INCREASE SOIL FERTILITY AND PLANT NUTRITION

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A microbiome is defined as the totality of microbial content, their genetic elements, and environmental interactions in a particular environment. The interaction between plant and its surrounding is a dynamic process in which plant monitors its environment and reacts to changes. Soil microbiomes play a key role in sustaining soil quality, and soil microbial properties have been proposed as sensitive indicators of changes in soil quality. The functions of microbiomes can influence plant health and crop productivity. Plant–microbe interactions research revealed that plant is able to shape its rhizosphere microbiome, as evidenced by the fact that different plant species host specific microbial communities when grown on the same soil. Our approaches in recent studies were to identify, characterize and select *Rhizobium* and other PGPR strains for use in agriculture as biofertilizer. This study was conducted in vitro for selection of faba *Rhizobium* and other related plant-growth promoting rhizomicrobiota (PGPR) tolerant strains to different ecological factors e.g., high temperature, dryness, acidity, heavy metals, pesticides, inorganic fertilizers, etc. using solid and liquid media, and then in a greenhouse pot experiments, a single or multiple co-inoculatants introduces to faba bean of 9 days old seedlings grown in sterile and unsterile clay loam brown forest soil samples of 45% moisture content at 28°C treated with different pesticides and sewage sludge and in another pots contaminated by some phytopathogens. Microbial and biochemical activities in the faba bean rhizosphere and plant analysis were investigated after 9 weeks of plant growth. Results showed significant differences between the soil properties and application of selected tolerant strains of *R. leguminosarum, Pseudomonas fluorescens, Bacillus subtilis, Saccharomyces cerevisiae* and *Trichoderma viride* to in vitro ecological parameters are able to control heavy metal mobilization, pesticide degradation and suppress pathogens in the rhizosphere of faba bean with adjusted mixing ratio. Results indicated that soil inoculation by effective *R. leguminosarum* Lóbab Z strain in combination with *P. fluorescens* B77, *B. subtilis* BS05, *S. cerevisiae* NASS7002 and *T. viride* TV99 as PGPR strains are required for maximizing *Vicia faba* yield and protect plant disease and improve soil quality in the term of soil enzymes and microbial activities and plant nutrient content. A key strategy to enhance soil inoculant performance to increase plant production is the selection of PGPR strains to improve N2-fixtion and to survive under stressful soil conditions and greater competitive ability in comparison with usage of agrochemicals. The study discusses some challenges for strain selection and introduces some novel uses for agrobiotechnology. There is an urgent requirement to develop rapid and easy technologies for formulation and mass production of multiple inoculants at a commercial scale for field application.

IN VITRO AND IN VIVO EFFICACY OF CASPOFUNGIN AGAINST FLUCONAZOLE RESISTANT *CANDIDA KRUSEI* AND *C. INCONSPICUA* CLINICAL ISOLATE

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Efficacy of caspofungin (CAS) was tested against two *Candida krusei* and two *C. inconspicua* isolates. CAS MIC values and killing activity was determined in RPMI-1640 plus 50% human serum. BALB/c mice immunosuppressed by cyclophosphamide were infected intravenously through the lateral tail vein. The infectious dose was 107 CFU/mouse for C. krusei and 2x107 CFU/mouse for *C. inconspicua*. Five-day intraperitoneal treatment with 1, 2, 3, 5 and 15 mg/kg CAS doses was

started 24 hours postinfection. On day six postinfection all mice were sacrificed, both kidneys were removed, weighed and homogenized aseptically. Aliquots of 0.1 ml of the undiluted and diluted (1:10) homogenates were plated onto Sabouraud agar plates and CFUs were determined after 48 h. Kidney burden was analyzed using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons. In killing studies CAS was fungicidal against *C. krusei* and *C. inconspicua* at >=16 mg/L and >=0.5 mg/L concentrations, respectively. In our neutropenic murine model all CAS doses were effective against *C. inconspicua* clinical isolates.

GLYCOGEN DRIVEN DARK HYDROGEN PRODUCTION IN A PHOTOSYNTHETIC BACTERIUM

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Several microorganisms are able to produce or oxidize molecular hydrogen. Our model organism, *Thiocapsa roseopersicina* is a Gram-negative anaerobe, purple sulphur photosynthetic bacterium. It mainly utilizes sulphur compounds as electron donors but can grow under photomixotrophic conditions, as well. The strain can fix molecular nitrogen, store several compounds and produce molecular hydrogen during photochemolitoautotrophic growth. It contains four active NiFe hydrogenase enzymes with different properties. Basically two of them are connected to the photosynthetic membrane and the another two are localized in the cytoplasm. The aim of this work is to disclose the linkage between the Hox1 soluble hydrogenase, the storage compounds and the redox processes of the cell. The Hox1 soluble hydrogenase consists of five subunits: Hox1Y and H are the small and large hydrogenase subunits, the Hox1F and U are diaphorase subunits with NAD^+-reducing activity while the Hox1E subunit has an electron transport role. The Hox1 enzyme is able to reduce electrons and oxidize protons in response to the environmental redox conditions. Hydrogen can be produced by means of the Hox1 hydrogenase under continuous illumination and in the dark. Under photochemolitoautotrophic conditions, excess thiosulfate can drive hydrogen production likely via central membrane redox and bioenergetic processes. The similarity between the Hox1EFU subunits and the dissociable diaphorase subunits of the membrane-located NADH-ubiquinone oxidoreductase complex may refer to a functional resemblance. The hydrogen production in the dark is also observed using cells previously grown photosynthetically. In the dark, the electrons for hydrogen production can be derived from the degradation of glycogen. In order to prove this hypothesis, the glycogen phosphorylase genes were disrupted in the genome and the hydrogen production as well as the stored glycogen content were monitored. When the glycogen breakdown was blocked, the hydrogen production was dramatically relapsed as compared to the control strain. However, the glycogen content remained on the original level. These might be a very important processes in photobiohydrogen producing technologies since these routes allow the cells to evolve hydrogen during both in the daytime and the night.

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SCREENING AND CHARACTERISATION OF NEW LIPOLYTIC YEAST STRAINS

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Extracellular lipolytic enzymes are produced by several yeast and fungal species which belong to carboxylesterases (EC 3.1.1.1.), true lipases (EC 3.1.1.3.) and different types of phospholipases. True lipases and carboxylesterases are responsible mainly for degradation of lipids of plant and animal origin. Yeasts are important sources of lipases produced for industrial and biotechnological purposes and several yeast species were reported as sources of commercial lipases. There is, however, an increasing demand for the production of new lipases which could be used either for hydrolysis of lipids and also for the synthesis of novel compounds. Our aim was the isolation of lipolytic yeasts from a sunflower seed processing and oil producing plant and investigation of the lipase production and biodiversity of the selected yeast strains. Samples (liquid, semi-solid and solid) were collected from 17 different places of the oil manufacturing plant. Screening of the lipolytic yeasts was carried out on agar plates containing tributyrin as a carbon source and positive colonies generating halos around the colonies were isolated. Altogether 99 lipolytic strains showing yeast and yeast-like morphology were isolated from the samples. Chromogenic medium WL nutirent agar was used in the first step of morphological differentiation of the isolates. According to the colony and microscopic morphological studies 79 isolates could be separated into 15 groups while 20 isolates remained solo. Basidiomycetes yeast strains were recognised by using the urease reaction. Lipase and esterase activities of the isolates were determined on tributyrin and Tween-80 agar plates, respectively, at 25 and 30 °C by using the agar diffusion semi-quantitative technique. Isolates with high lipolytic activites were identified by rDNA sequencing. There were twenty yeasts strains identified which had the highest lipolytic activity and all belonged to the Basidiomycetes.

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PHYLOGENETIC ASSIGNMENT OF PYROSEQUENCING AMPLICON SEQUENCES

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Placement of environmental sequences upon a reference phylogeny has been the "gold standard" method for taxonomic assignment of Sanger sequences. More recently, pyrosequencing technology has largely replaced Sanger methods in environmental DNA sequencing studies. Phylogenetic placement methods, as practiced earlier, became impractical with the data set sizes produced by pyrosequencing. Accordingly, variations of a workflow consisting of sequence clustering and taxonomic assignment based on k-mer statistics or pairwise alignment found widespread application. There are still reasons why read-by-read phylogenetic placement is expected to be superior to these methods though. In order to make this practicable for a large 16S rDNA V1 pyrosequencing data set consisting of about 2 million reads, we developed a software pipeline for the alignment and phylogenetic placement of large numbers of marker sequences.
I will introduce this tool and illustrate it with results of our analyses of temporal patterns of SAR11 ecotype distributions at the Bermuda Atlantic Time Series site.

FULLY BAYESIAN COMPUTATION FOR COMPARATIVE METAGENOMICS

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Statistical comparison of abundances of gene categories pooled across all taxa is among the standard approaches to analysing metagenomic data sets. We recently demonstrated that relative abundances of gene categories as used in such analyses are biased by the average genome sizes of the communities compared. Correcting for this and gene length bias enables estimation of gene abundances on an ecologically meaningful scale, as community averaged gene copy numbers. We experimented with fully Bayesian methods addressing this issue which can propagate error in estimation of nuisance parameters (here average genome sizes) in a statistically sound manner. Sampling from posterior distributions using Markov Chain Monte Carlo (MCMC) is a versatile tool for parameter estimation in the Bayesian framework, however, data set sizes typical for a metagenomic analysis are far beyond those that can be handled using general purpose sampling software. We developed an adaptive MCMC implementation which can scale to real life metagenomic data sets with tens of thousands of parameters and allows a flexible exploitation of posterior distributions for addressing a wider range of questions than allowed when using standard statistical techniques. Our results suggest that, computationally less demanding approaches (marginal likelihood, empirical or approximate Bayes) might be preferable, the full flexibility of Bayesian computation is not out of reach for the rather parameter rich problems of metagenomics.

MICROBIOLOGICAL RESPONSES AND MN-STRESS ALLEVIATION IN THE MYCORRHIZOSPHERE OF *ELYMUS ELONGATUS*

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The microbiological responses of manganese (Mn) metal stress was studied in the mycorrhizosphere of *Elymus elongatus* (L.) energy grass, grown in a slightly humous and calcareous sandy soil in a lysimeter experiment, Őrbottyán, Hungary. The increasing doses of Mn-mud, as a waste of Úrkut Mn-ore was used and utilized. Doses of 0, 500, 1000, 2500 and 12500 mg/kg Mn was incorporated into the 20 cm of soil-layer before the sowing parallel with or without the inoculation of arbuscular mycorrhiza fungi (AMF, INOQ, Germany) in 2 % amount (V/V) at half of the lysimeters. Beside the growth and biomass production of energy grass, the AMF colonization properties, the fluorescent diacetate analysis (FDA) and the colony forming units (CFU) of heterotrophs and micromycetes were estimated. The increasing doses of manganese could be successfully tolerated at the highest doses as a positive respond of the AMF inoculation. In those AMF-treated lysimeters a reduced Mn-content was found in the shoot biomass and largely enhanced amounts (about 30.000 mg/kg manganese) in the root system. At lower (500 mg/kg) Mn rate the shoot production of AMF treated plants was found to be doubled with a significant increase also of the root systems. FDA activity and of soil-microbes was reduced concomitant with the increasing doses of manganese, except the AMF

inoculated soils, where it could be remained rather constant and independent from the Mn-addition. CFU counts of bacteria and fungi showed case-dependent values, with a reduced abundance of spore-formers at the higher Mn doses of AMF treated soil. Microbiological impacts are one of the important parts of the surviving mechanisms of Elymus elongatus plants at Mn-stressed conditions.

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FERMENTATIVE HYDROGEN PRODUCTION FROM SYNTHETIC WASTEWATER USING COMPLEX MICROBIAL CONSORTIUM AS INOCULUM

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Biohydrogen (H_2) production from synthetic wastewater as substrate was studied in anaerobic small scale batch reactors. Selectively enriched anaerobic mixed consortia sampled from different environments were used as parent inoculums to start the bioreactors. Prior to the inoculation the samples were sequentially pretreated with heat, acid, ultrasonication and a combination of all three methods. The small scale reactors were operated at mesophilic temperature (30 ± 2°C) in batch mode. Experimental data showed the feasibility of hydrogen production using wastewater as substrate. Acidophilic microenvironment favored H_2 production over neutral microenvironment. In addition to this, the pretreatment methods influenced visibly the hydrogen generation of the different mixed bacterial consortiums. The described process opens the way towards simultaneous renewable energy generation in the form of H_2 and wastewater treatment.

PORCINE ENTEROVIRUSES: RECENT FINDINGS OF A LONG TIME IGNORED PICORNAVIRUS GROUP

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The historical ‘‘porcine enterovirus’’ (PEV) group of family Picornaviridae was originally consists of 13 serotypes (PEV-1 to -13) isolated from domestic pigs. These serotypes were recently reclassified into three genera: *Teschovirus, Sapelovirus* (formerly called PEV-A group) and *Enterovirus* (PEV-B group). Until recently, only two serotypes of PEV-B group (PEV-9 and -10) were identified, both are isolated in 1979. Little information was available about the epidemiology, host range, clinical impact and genetic diversity of these viruses. Using reverse transcription-polymerase chain reaction (RT-PCR) and next generation sequencing methods, our research group has identified and characterized two additional PEV-B enteroviruses from fecal samples of healthy domestic pigs and wild boars in Hungary. Based on phylogenetic analysis of the complete genomes, these viruses represents two novel PEV genotypes and circulating separately among the domestic pigs (PEV-14, [1]) and wild boars (PEV-15, [2]). Furthermore, a natural interspecies recombinant bovine/porcine enterovirus (ovine enterovirus type 1, OEV-1) was also detected and characterized from fecal samples collected in two consecutive years from domestic sheep (*Ovis aries*). Sequence and phylogenetic analysis of the complete genome revealed that OEV-1 is a novel member of the

PEV-B group, implying the endemic presence of PEV-B viruses in sheep. On the other hand, the 5'-untranslated region of OEV-1 showed a high degree of sequence identity to bovine enteroviruses (BEV) which explains the interspecies recombinant origin of OEV-1 [3]. OEV-1 is the first interspecies recombinant enterovirus in animals, detected in sheep. Furthermore, PEV-B was identified in domestic pig, wild boar and domestic sheep indicating that PEV-B viruses have wider host range than it was thought previously. Detection and characterization of further PEV strains from different geographic areas and different host species are important to understand the worldwide distribution and heterogeneity (genotype/serotype) of animal enteroviruses including PEV-B and their association with symptomatic infection of economically important farm animals.

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INVESTIGATION OF THE PEPTIDE ANTIBIOTICS PRODUCTION OF *TRICHODERMA* STRAINS USING A MICROBIAL SCREENING APPROACH

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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 enzymes, antibiotics, as plant growth promoters and decomposers of xenobiotics. Furthermore, according to their multiple biological properties they are exploited as sources of industrial enzymes and applied in the agriculture as commercial biofungicides. On the other hand, certain Trichoderma species may be harmful as opportunistic human pathogens and as the causative agents of green mould disease in mushroom farms. Peptaibols are secondary metabolites from the group of fungal peptide antibiotics. These compounds are linear, amphipathic polypeptides composed of 7-20 amino acids. They usually contain several nonproteinogenic amino acid residues such as isovaline and α-aminoisobutyric acid, which are important to form their characteristic structures. The number of known peptaibols is constantly growing due to their widespread biological application potentials and the intensive research focused on this area. A complex screening programme aimed at the identification as well as structural and conformational elucidation of new peptaibols along with testing their biological activities towards different target organisms could provide new antimicrobial, immunosuppressive and neuroleptic drug candidates for human medicine, as well as potential components of biocontrol preparations intended for agricultural applications. The first step on this way is the development of a suitable biological screening system which enables the large-scale examination of *Trichoderma* strains for peptide antibiotic production. During this study, *Trichoderma* strains were isolated from different locations in Hungary and identified at the species level based on morphological and molecular characters. Peptaibols were extracted from the mycelia of agar cultures and their antibacterial activities were tested against bacterial strains of *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* and *Escherichia coli*, while the antifungal effects were recorded against fungal strains of *Fusarium oxysporum, F. culmorum* and *Rhizoctonia solani*. Most of the isolates showed remarkable antimicrobial activities, with high variability among examined strains.

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COMPARISON AND HETEROLOGOUS EXPRESSION OF DIFFERENT *XANTHOPHYLLOMYCES DENDRORHOUS* CRTS GENES IN *MUCOR CIRCINELLOIDES*

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Keto-derivatives of the β-carotene, e.g. canthaxanthin and astaxanthin have excellent antioxidant activities. These pigments are used by the food and feed industries and their market is growing. Previously, we used different methods to integrate the *crt*W gene encoding a bacterial β-carotene-ketolase from *Paracoccus* sp. N81106 into the M. cricinelloides genome, the resulting transformants produced canthaxanthin as the main carotenoid compound. *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*) is a carotenoid producing basidiomycetes yeast, which accumulates astaxanthin (3,3'-dihydroxy-β,β-carotene-4-4'-dione) as the main carotenoid compound. The *crt*S gene of *X. dendrorhous* encodes a cythochrome-P450 hydroxylase, which is thought to be responsible for the β-carotene – astaxanthin conversion. Previously, it has been shown, that the enzyme has β-carotene 3,3'-hydroxylase activity [1] and also has a hypothethical β-carotene 4,4'-ketolase activity [2], but the latter suggestion has not been verified yet. In the present study, we cloned and compared the nucleotide and the encoded amino acid sequences of the crtS genes of wild type and astaxanthin over-producing mutant *X. dendrorhous* strains. Differences in the nucleotid and thus in the amino acid sequences were identified. Two *crt*S genes with different sequences were amplified from one of the wild type strains indicating that it is heterozygous for this gene. Plasmids were constructed carrying the isolated *crt*S genes under the control of the promoter and terminal regions of the *Mucor* glyceraldehyde-3-phosphate dehydrogenase 1 (*gpd*1). The plasmids were introduced by PEG/CaCl2-mediated protoplast transformation into β-carotene producing wild-type and canthaxathin producing (*crt*W-harbouring) mutant *M. circinelloides* strains. Carotenoid production of the transformants and the fate of the introduced DNA were analysed.

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EXAMINATION OF GENETICALLY INFORMATIVE MOLECULAR MARKERS IN *ERYSIPHE NECATOR*

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Erysiphe necator Schw. [*Uncinula necator* (Schw.) Burr.] (Ascomycotina, Erysiphales) is an obligate biotroph growing only on grapevine tissue. All green tissue (stems, leaves, flowers and fruit) of grapevine may become infected by powdery mildew showing whitish-gray mycelium with a powdery appearance caused by the fungus. Powdery mildew epidemics emerge year after year in most important crops, including several cereal, fruit, vegetable and ornamental crops. Although in most cases powdery mildew infections do not kill the infected crops, these might cause serious yield and quality losses. Chemical control remains the main way to reduce the incidence. Recently several highly active fungicides have been introduced but with some of them failures of disease control have been observed in vineyards. Information about the plant pathogen fungi is essential for the effective

and economic protection. A plant pathogenic fungal population with high level of genetic variation is likely to adapt more rapidly to fungicides or resistant host plants than populations with little or no genetic variations, and information on the level of migration between populations and on the presence or absence of sexual reproduction within a population may indicate how rapidly will novel (fungicide resistant or more pathogenic) genotypes spread between populations. *E. necator* is already resistant against most fungicides, thus making alternative strategies for its control essential. A clue to these strategies lies in the understanding of the genetic structure and dynamics of its populations. In the course of our work numerous isolates of grapevine green tissue infected *E. necator* samples were collected from various locations of Hungarian wine regions: Eger, Tokaj, Ászár-Neszmély, Kunság, Pécs and Villány. 20 of these isolations were randomly chosen to check different genetically informative markers. Four nuclear loci, ITS/IGS, β-tubulin, translation elongation factor EF1-α and 11 microsatellite markers which have become the first choice for the genetic examines. In the course of our experiments all genetic markers were amplified successfully. The microsatellite markers showed a high variability in fragment length and number of observed alleles. The experiments revealed that these genetic markers could accommodate exceedingly in examination of *E. necator* populations from phylogenetic and population structure point of view.

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PREVALENCE OF NOVEL HUMAN POLYOMAVIRUS 9, WU AND KI IN HEALTHY AND IMMUNOCOMPROMISED INDIVIDUALS

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Until 2007, only two human polyomaviruses (HPyV), BK (BKPyV) and JC (JCPyV) viruses were known. KIPyV and WUPyV polyomaviruses were identified in respiratory samples of children with acute respiratory symptoms in 2007. Seroepidemiological studies revealed that similarly to BKPyV and JCPyV, in adult population the seropositivity reaches 70-80 %. Although the pathogenic roles of these viruses have not been clarified, PCR based detection revealed 0.4-9 % prevalence in various samples from immunocompetent and immunocompromised patients and higher frequency in children and immunocompromised individuals. HPyV9 was described from blood and urine samples of kidney transplanted patients in 2011. Our aims were to study the prevalence of HPyV9, WUPyV, KIPyV and to determine whether natural immunosuppression due to pregnancy and the immunosuppression required for renal transplantation result in higher prevalence of these viruses. Plasma, urine and throat swab samples were collected from healthy, pregnant and renal transplant individuals. Up to now, WU and KI viruses were not detected in the collected and analyzed urine, respiratory and plasma samples from healthy and pregnant individuals. At the same time, both viruses were found in all samples types from renal transplant patients. The prevalence of WU and KI was similar in the different sample types. HPyV9 DNA was determined in all sample types of healthy individuals, pregnant women and renal transplant patients. There were no significant differences between pregnant and non pregnant women in HPyV9 DNA frequencies for plasma, urine and respiratory samples. Among renal transplant patients higher PCR positivity was revealed. These results suggest that strong immunosuppression required for renal transplantation may result in higher susceptibility for KI and WU viruses or may result in reactivation of latent infections.

DIFFERENTIATION BETWEEN HUNGARIAN AND BRAZIL HUMAN PAPILLOMAVIRUS TYPES AMONG FEMALE ANOGENITAL HPV INFECTIONS

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Within some decades after the discovery of human papillomaviruses (HPVs), adequate laboratory diagnostic methods and vaccination for primary prevention have been introduced. We have experienced divergences in HPV prevalence between the continents, and our aim was therefore to compare data from a European and a South-American country. The Brazilian study was carried out at Hospital Maternidade Leonor Menedes de Barros, one of the largest public maternity hospitals in the city of São Paulo, Brazil. The Hungarian study was carried out at Department of Obstetrics and Gynaecology, University of Szeged in Szeged. HPV PCR and HPV types were determined by known diagnostic PCR methods (Roche). A very effective automated nucleic acid isolation procedure was performed. 333 samples were examined in Szeged and 301 in Sao Paolo, among 15-24-year old women. >80% of the participants in Sao Paolo were married, or lived with a partner, while 90% of the Hungarian women were single. The proportions of those who had their first sexual intercourse at 15 years old were 43.9% (BR) and 20.9% (HUN). The prevalences of low-risk HPV types were 14.3% (BR) and 2.9% (HUN). Examinations of three age groups (15-18, 19-21 and 22-24 y) revealed that among the Brazilian women, the high-risk positivity decreased in the higher age groups, but increased among the Hungarian women. The distributions of HPV types differed in the two countries. Analysis of the results from different aspects demonstrated many correlations and opposite results. The high prevalence of cervical carcinoma in Brazil with relatively high HPV prevalence may be explained by the rarity of cervical cytological examinations. The frequency of HPV vaccinations was very low, which may be due to the high expense of the vaccines [1-3].

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DISTRIBUTION OF THERMOPHILIC FUNGAL COMMUNITIES IN THE AIR OF COMPOSTING PLANTS

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Incidence of different fungal spores in air is affected by definite physical, chemical and biological factors. Thermophilic fungi as dominant agents of composting processes are defined with a minimal growth limit of 20°C yet their dormant elements may survive in air even in big quantity. The amount of fungal elements in the air of different composting plants (using passive or static aerated technology) was determined by the use of a three-stage Andersen air sampler. Potato dextrose agar was used for primary culturing and the exposed plates were incubated at 50°C for a week. Regularity was observed in the distribution of the colony forming units of different thermophilic fungi in relation to the different stages of the composting process. The amount of the thermophilic fungi proved to be 4.5-9.5 × 10^4 CFU m^{-3}, while that of the mesophilic fungi was 3.5-7.0 ×10^5 CFU

m^{-3} in the lowest air layer above the compost piles. The aerating turn-over of the piles increased the number of the thermophilic fungal elements by two magnitudes (2.5-6.5 × 10^6 CFU m^{-3}) and resettlement of the number of fungi to the original state occurred only after 6-8 hour. The amount (4.5-7.5 × 10^2 CFU m-3) of these fungal elements at spots 100 m away from the compost piles significantly exceeded the amount (<10^1 CFU m^{-3}) that could be measured in ordinary air. The technology applying semipermeable membrane to cover the aerated static piles having numerous advantages even proved to emit two magnitudes less hygienic microbial air contaminants than the passive technology. Having a knowledge of the relative frequency of thermophilic fungal genera developed in the composting materials, we determined the order of aeropersistency based on their incidence rate in the air: *Talaromyces* and *Paecilomyces* species proved to be the most frequent thermophilic fungi, *Myceliophthora, Thermomyces* and *Rhizomucor* species took place in the middle field, while *Thermoascus, Malbranchea* species found to be appeared rare in the air of composting sites. Phialoconidial ontogeny and melanoid pigments in the cell wall holding the destructive effect of the radiation were considered as the major biological and physical factors increasing the incidence of different thermophilic fungi in the air.

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INTERRELATIONSHIP BETWEEN SULFUR OXIDATION AND HYDROGEN EVOLUTION OF HYN HYDROGENASE IN *THIOCAPSA ROSEOPERSICINA* BBS

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Our model organism, *T. roseopersicina* is a representative member of the Chromatiaceae family of purple sulfur bacteria. These bacteria are capable to utilize various sulfur compounds such as thiosulfate, sulfur and sulfide as electron donor and carbonate as sole carbon source for anoxygenic phototrophic growth. The strain contains four active [NiFe] hydrogenases. Hox1 and Hox2 are soluble and Hox1 is likely connected to the central quinone pool while Hox2 is related to glucose metabolism. Hyn and Hup are periplasmic membrane-bound hydrogenases. Hup is mainly an uptake hydrogenase. Hyn has remarkable stability under extreme conditions and its gene organization is rare. Between the coding regions of the hydrogenase large and the small subunits, 2 ORFs could be found, namely isp1 and isp2. Isp2 is similar to heterodisulfide reductases of Archaea and DsrK of the dissimilatory sulfite reductase, Dsr heteromultimer complex involved in the sulfur oxidation. These findings point out the possible connection between sulfur metabolism and Hyn hydrogenase. In this work, the connection between membrane bound Hyn hydrogenase and sulfur oxidation of *T. roseopersicina* has been investigated at physiological level. We found that increased amount of elemental sulfur as sole electron donor promotes hydrogen evolution by Hyn and hydrogen sulfide formation. Under these circumstances, sulfite stimulated hydrogen production and decreased hydrogen sulfide formation. Moreover, sulfite alone could't drive hydrogen evolution, only in the presence of elemental sulfur. It has been also shown that the strain which has higher Hyn expression level produces more hydrogen but less hydrogen sulfide in cells grown on elemental sulfur as compared to the wild type cells. This indicates a competition between hydrogen sulfide formation and hydrogen evolution. In the presence of elemental sulfur, additional sulfide and sodium

thiosulfate had no significant effect on hydrogen formation. Therefore, it was concluded that sulfur oxidation is the main electron source for the Hyn hydrogenase mediated hydrogen production.

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SULFIDE OXIDATION IN A PURPLE SULFUR PHOTOSYNTHETIC BACTERIUM, *THIOCAPSA ROSEOPERSICINA* BBS

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Phototrophic purple sulfur bacteria can utilize various reduced inorganic sulfur compounds such as sulfide, elemental sulfur and thiosulfate as electron donors during anoxygenic photosynthetic growth. Members of disulfide oxidoreductase protein family: flavocytochrome c (Fcc) and sulfide quinone oxidoreductase (Sqr) were identified in these photosynthetic bacteria. These enzymes play a role in the first step of the electron transport from sulfide into the photosynthetic electron transport chain. Flavocytochrome c is a periplasmic enzyme consisting of a large sulfide-binding flavoprotein (FccB) and a smaller cytochrome c subunit (FccA) which binds a single or two heme c in different species. In vitro FccAB can catalyze electron transfer from sulfide to small periplasmic c-type cytochromes that then may donate electrons to the photosynthetic electron flow. The membrane-bound sulfide quinone oxidoreductases transfer electrons from sulfide directly to membrane quinone-pool. *Thiocapsa roseopersicina* is a purple sulfur photosynthetic bacterium. Three genes presumably encoding sulfide oxidizing disulfide oxidoreductase proteins were identified in the genom sequence of *T. roseopersicina*: *fcc, sqr* and *sqn*. For biochemical and functional analysis of products of these genes, the proteins fused with affinity tags were expressed in *T. roseopersicina*. The Sqn and dimeric Fcc were successfully purified by affinity chromatography via Strep-tag. In the UV-visible absorption spectra of oxidized and sodium-dithionite reduced forms of purified FccAB and Sqn characteristic peaks of a redox active flavin prosthetic group was identified. The proteins bind the flavin group covalently. The pure recombinant flavocytochrome c catalyzes the reduction of bovine heart cytochrome c in the presence of sulfide. In in vitro experiments the membrane-associated recombinant Sqn and strongly membrane-bound recombinant Sqr can catalyze sulfur-dependent quinone reduction. Our results clearly demonstrate that these flavoproteins (FccAB, Sqr, Sqn) play role in the sulfide oxidation in *T. roseopersicina*. Deeper understanding of the biochemical differences and the role of the enzymes requires further analyses.

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DETECTION OF A WIDE RANGE OF ANTIBIOTIC RESISTANCE GENES OF *BACTEROIDES* SPP.

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The species of the genus *Bacteroides* are important constituents in the intestinal tract, are significant human anaerobic pathogens and among these harbour the highest number of described antibiotic resistance mechanisms and the highest resistance rates to the applicable antibiotics. We aimed to study to detect a series of described antibiotic resistance genes (*cep*A, *cfx*A, *cfi*A, *nim*,

*erm*B, *erm*F, *erm*G, *lin*A, *mef*A, *msr*SA, *tet*M, *tet*X, *tet*X1, *tet*Q, *tet*36 and *bex*A) of clinical isolates of these medically important group of bacteria (128 B. fragilis and 34 non-fragilis *Bacteroides* - NFB) in Real-Time PCR experiments. All these strains were selected from a collection of isolates obtained from a European antibiotic resistance study. We found the following prevalences for the examined genes: *cep*A (BF 79,7%, NFB 35,3%), *cfx*A (BF 14,8%, NFB 29,4%), *cfi*A (BF 9,48%, NFB 0,0%), *nim* (BF 1,6%, NFB 0,0%), *erm*B (BF 1,6%, NFB 0,0%), *erm*F (BF 22,7%, NFB 29,4%), *erm*G (BF 6,3%, NFB 0,0%), *lin*A (BF 21,9%, NFB 20,6%), *mef*A (BF 13,3%, NFB 8,8%), *msr*SA (BF 7,0%, NFB 0,0%), *tet*36 (BF 0,0%, NFB 0,0%), *tet*M (BF 0,8%, NFB 0,04%), *tet*X (BF 10,2%, NFB 8,9%), *tet*X1 (BF 4,7%, NFB 2,9%), *tet*Q (BF 78,1%, NFB 85,3%) and *bex*A (BF 4,7%, NFB 17,6%). The most prevalent genes were the *cep*A and *tet*Q genes that code cephalosporin and tetracycline resistances, respectively. The *cep*A, the *cfx*A (cefoxitin resistance) and the *bex*A (flouroquinolone efflux pump) genes distributed differentially among *B. fragilis* and NFB species. Having previous data on the antimicrobial resistance of these strains we were able to correlate the presence of the resistance genes and the corresponding antimicrobial resistance phenotype. The *cfx*A gene did not influence much the cefoxitin resistance rates, while in the case of the carbapenemase coding *cfi*A gene the number of the *cfi*A-positive isolates overcame the resistance rate. Most of the clindamycin-resistant strains harboured the *erm*G, *erm*F, *mef*A and *msr*SA genes. However, *erm*F and *mef*A genes could be fond in clindamycin-susceptible isolates as well. The *erm*B, *mef*A and *msr*SA genes were coincident in 10 isolates which by our view is caused by the common presence of the CTnGERM1 conjugative transposon in these strains. No single gene could be found that had been causing tigecycline resistance. Our studies demonstrated the importance of the detection of resistance genes among antibiotic-resistant *Bacteroides* strains. We could gain information of the spread of antibiotic resistance genes of *Bacteroides* species which is important in that regard that *Bacteroides* are a reservoir of such genes in the intestinal microbiota.

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LONG-TERM MONITORING OF MICROBIAL COMMUNITY DYNAMICS AND CATECHOL 2,3-DIOXYGENASE GENE EXPRESSION IN A HYPOXIC, PETROLEUM HYDROCARBON CONTAMINATED GROUNDWATER

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Petroleum hydrocarbons are frequent contaminants of soil, groundwater or marine environments. Among these pollutants aromatic compounds including benzene, toluene, ethyl-benzene and xylene (BTEX) are the most prevalent. In terrestrial subsurface ecosystems the contamination can threaten subsurface aquifers, a major resource of drinking water. Therefore, remediation of polluted subsurface environments is usually required. Microbial degradation of aromatic compounds occurs rapidly under aerobic conditions, however the availability of dissolved oxygen in subsurface environments is commonly restricted. Recent studies have shown that bacterial communities of oxygen-limited, BTEX contaminated groundwater ecosystems are usually dominated by Betaproteobacteria and catechol 2,3-dioxygenase (C23O) genes encoding subfamily I.2.C extradiol dioxygenase enzymes are ubiquitous and active in these environments. Since these enzymes are

considered to be adapted to hypoxic conditions, they may play primary role in the microaerobic degradation of BTEX compounds. However, the majority of those subfamily I.2.C extradiol dioxygenases which appear to have environmental relevance cannot be linked to any cultured bacteria. In order to address this question the dynamics of in situ bacterial community and changes in the expression pattern of subfamily I.2.C C23O genes in a hypoxic BTEX contaminated groundwater were assessed over a 13-month period using sequence-aided terminal-restriction fragment length polymorphism (T-RFLP) and single-nucleotide primer extension (SNuPE) respectively. The bacterial 16S rRNA fingerprinting revealed a considerably stable community composition with the predominance of Beta- and Gammaproteobacteria. Members of the genera *Rhodoferax, Azoarcus, Pseudomonas* and unknown bacteria related to Rhodocyclaceae were mostly major components of the community. Subfamily I.2.C C23O mRNA transcripts were constantly detectable, showing that they played a significant role in the degradation of aromatic hydrocarbons. Clone libraries revealed the presence of six different clusters of subfamily I.2.C C23O genes. In order to reveal expression pattern changes that may occurred during the monitoring period we developed a SNuPE assay. This quasi fingerprinting of gene expression provided opportunity to link the investigated function to specific microbial populations. Consequently a yet uncultured member of the genus *Rhodoferax* and an unclassified Rhodocyclaceae bacterium were identified as possible key microaerobic BTEX degraders. The results can improve our understanding of BTEX degradation under oxygen limitation and may benefit bioremediation research by demonstrating the usefulness of SNuPE for the monitoring of microbial populations involved in degradative process.

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UNIQUE FEATURES OF PHOTOAUTOTROPHIC PICOPLANKTON IN LAKES AND PONDS OF THE CARPATHIAN BASIN - RESULTS OF TEN YEARS RESEARCH

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In the last few decades, the discovery and recognition the importance of photoautotrophic picoplankton (PPP, planktonic algae smaller than 2 μm) have changed our view on the ecological processes in aquatic habitats. In many cases, this functional group of microorganisms is significant contributor to primary production and therefore plays an important role in the nutrient cycle and constitutes a relevant part of the food-web. PPP investigations are traditional in Hungary, but the taxonomic composition and the real diversity of these aquatic communities was unknown previously. Therefore besides epifluorescence microscopic studies, in our research, we focused on the identification of PPP members using PCR-based methods in the hypertrophic turbid soda ponds, in Lake Balaton and in the saline lakes of Transylvania. The temporal changes of individual genotypes and their relation to physicochemical parameters were also studied. Results have revealed several unique features of this group in the lakes and ponds of the Carpathian Basin: the highest PPP cell abundance values ever were observed in the soda ponds of the Kiskunság Region, marine PPP genera were detected in the saline lakes of Transylvania (also contributed to a double deep chlorophyll maxima in Lake Ursu), a new genus of pico-sized green algae has been isolated and

described as *Chloroparva pannonica* from a soda pond. Some additional findings were: seasonal changes were reflected by the changes in the PPP community structure, unambiguous relationships were retrieved between the abundance of some PPP genotypes and environmental parameters.

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EXAMINATION OF THE CELLULAR INVASIVITY AND THE OUTER MEMBRANE PROTEIN (OMP) PROFILE OF *PSEUDOMONAS* STRAINS CULTIVATED UNDER DIFFERENT TEMPERATURE

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The *Pseudomonas aeruginosa* is a very important human pathogen, for example, it is a major cause of airway inflammation in cystic fibrosis. Special conditions of this illness like viscous mucus are favorable for the bacteria to colonize in the airways and promote the bacterial invasion of the surrounding cells. The *Pseudomonas* strains are Gram-negative. So their pili and outer membrane proteins (OMP) play an important role in the cellular invasive process. These OMP-s are on the surface of bacterial cells and therefore they come first into contact with the human cells, and play an important role in the host's reaction. Sometimes these OMP-s are constitutive, but some of them are induced by environmental stress. Such inducing environmental condition may be the temperature. Sometimes their genetic information is extrachromosomal. Our aim was to find connection between the invasive capability and OMP profile of *Pseudomonas* strains cultivated under different temperature. We cultivated our *Pseudomonas aeruginosa* strains at 25°C, 37°C and 42°C. We used an A549 lung carcinoma semiconfluens cell invasion system to measure invasive capability and electrophoretic (PAGE and Lab-on-a-chip) methods to characterise the OMP profile. We realised that the environmental temperature of cultivation could influence the invasive capability and OMP profile of *Pseudomonas* strains. We suspect that these changing OMP-s are stress-proteins, for their accurate identification mass spectrometric studies are planned [1].

[1] Felső, P., et al. (2011) AMIH 58, S 143.

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

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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 supressor protein p53 and the cell cycle regulatory protein Rb, respectively. These interactions generate disorder in the cell cycle control and failures in the DNA repair, so these can lead to genomic instability and contribute to malignant transformation. The aims of this study were to determine the sequence and amino acid variations of E6 and E7 oncogenes of HPV type 31, and 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 (nt 94-554) and E7 (nt 525-868) 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 phylogenetic 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 HaCaT 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 HaCaT cells.

ISOLATION, CHARACTERIZATION AND MOLECULAR IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM DIFFERENT *CAPSICUM ANNUUM* CULTIVARS

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Endophytes are harmless plant-associated microorganisms, which reside inside the plant tissues. Endophytic bacteria can play important role in plant vitality and may confer resistance to abiotic or biotic stress effects. On the other hand, researches have recently shown that these bacteria may prevent or - in some cases - even promote the penetration of human pathogenic bacteria into the plant tissues, which can cause serious food safety problems. Our goal is to examine the endophytic bacteria of different organs of *Capsicum annuum* cultivars and analyze the potential effect of the endophytes from agricultural practice and food safety aspects. Therefore, in order to reveal the role of endophytic bacteria in plant defense, and the hazard of helping pathogenic bacteria in penetration into plant tissues, interactions between the endophytic and human pathogenic bacteria are to be analyzed. Our recent study deals with the isolation, morphological characterization and molecular identification of endophytic bacteria from hydrocult and soil grown plants of three *Capsicum annuum* varieties. Endophytic bacteria were isolated from seeds and internal tissues of different organs (primary roots, root hairs, stems and leafs) after surface disinfection by sodium hypochlorite and ethanol. Samples of the disinfected seeds and organs were placed into TGY (tripton-glucose-yeast extract) broth and incubated at 25^{o}C by gentle shaking. Aliquots of positive samples were subcultured in TGY, differential chromogenic (WL) and coliform selective (VRBL) agar plates for the isolation of the endophytic bacteria and selection of coliforms among them. Pure cultures of colonies grown on different media were tested for Gram property, colony and cell morphology, and spore forming ability. After grouping the isolates, selected strains were analyzed by molecular techniques such as RAPD-PCR and genus specific PCR. Species identity of the isolates has been determined by sequencing the PCR amplicons of the rDNA and *rpo*B genes.

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FUSARIUM KERATITIS IN SOUTH INDIA: IDENTIFICATION, PHYLOGENY, ANTIFUNGAL SUSCEPTIBILITY AND EVALUATION OF A RAPID IDENTIFICATION METHOD FOR THE *FUSARIUM SOLANI* SPECIES COMPLEX

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The number of cases of keratomycosis caused by *Fusarium* spp. increased significantly over the past several years in South India. In this study we investigated Fusarium isolates derived from human keratomycosis isolated during the years of 2004-2005 and 2010-2011 at the Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, Tamilnadu, India. A total of 125 *Fusarium* isolates were subjected to molecular identification. Most of the isolates were confirmed as members of the *F. solani* species complex (n=106, 85%), followed by isolates belonging to the *Gibberella fujikuroi* species complex (n=7, 6%), the *F. dimerum* species complex (n=6, 5%), the *F. oxysporum* species complex (n=3, 2%) and the *F. incarnatum-equiseti* species complex (n=3, 2%). This was the first case when *F. andiyazi* was isolated from human keratomycosis. Within the *F. solani* species complex, a remarkable and significantly supported phylogenetic structure was recovered by the phylogenetic analyses, and the South Indian isolates from keratomycosis separated in one clade. The in vitro antifungal susceptibilities of the isolates to the clinically used antifungal agents (amphotericin B, clotrimazole, econazole, fluconazole, ketoconazole, itraconazole, natamycin and terbinafine) were determined by CLSI M38-A2 broth microdilution method. Based on the susceptibility data, terbinafine, natamycin and amphotericin B proved to be the most effective antifungal drugs. Synergistic interactions were revealed between the two most effective antifungal agents (natamycin and terbinafine) by checkerboard microdilution method. Based on a unique recognition site of EcoRI in the partial sequence of the translation elongation factor 1α gene, a new method was developed for the rapid identification of the members of *F. solani* species complex.

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MICROSCALE COMPARISON OF AMF DIVERSITY OF NATIVE AND INVASIVE WOODY PLANTS

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The ca. 230 species of arbuscular mycorrhizal fungi (AMF) are associated with about 80% of land plant species in a mutualistic symbiosis. They are ubiquitous in terrestrial ecosystems and play an important role in nutrient cycling as well as reducing biotic and abiotic stress for plants and modifying interspecies interactions through differentially benefitting distinct plant species. Their importance in arid and semiarid environments - such as the Great Hungarian Plain, our study site - is high as they alleviate the adverse effects of these areas. Considering their omnipresence and the low taxonomic diversity compared to their host partners it is widely accepted that the AMF species

are generalists i.e. associate with a broad spectrum of hosts. The question whether there exist specialists among the fungal species, thus seems relevant. This might be answered by comparing the AMF communities found in the roots of native and invasive plant species. In Tatárszentgyörgy, we collected tightly intertwined roots of the native *Juniperus communis* and the introduced *Ailanthus altissima*, both woody species. Partial LSU region of fungal nrDNA was amplified by PCR, using AMF-specific primers. The primers were chosen according to the results of a previous experiment where we tested the efficiency of 4 primer sets on an artificial AMF community. After cloning and sequencing we obtained 622 sequences which formed 69 groups at a 3% similarity threshold. Most of the sequences belonged to groups known only from environmental studies but some sequences could be assigned to described species such as *Glomus macrocarpum*, the type species for Glomeromycota and *Paraglomus majewskii* a recently described basal taxon of the phylum. Some groups were detected either in *Juniperus* or *Ailanthus* while others in both plant species.

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BIOINFORMATICS IN VIROLOGY

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Bioinformatics (nucleic acid and amino acid sequence analyses) dramatically speeded up the understanding the relations among viruses and the finding of novel viruses. Pure biological properties usually can not give enough quantitative data to measure and correctly interpret the exact difference of viruses, and consequently to understand their evolution and establish appropriate taxonomy. PCR can quickly supply sequences of potential viruses in samples, but without homology searches, it would not be possible to identify a virus or a possible contaminant. Excellent and quickly growing databases are available to make homology searches immediately. It is also advisable to build your own confidential database for homology search. Freely available programs can be used to visualize, interpret and compare the electrophoretograms of DNA sequencing, to assemble full viral genomes, to identify possible sequencing mistakes, to predict protein coding regions, to annotate the genomes, to identify genome variants and submit the sequences and their annotation (interpretation) to GenBank. Special programs can be applied to discover and locate homologous recombinations. Without identifying recombinations, phylogenetic calculations may yield incorrect conclusions for newly detected viruses. The results of bootstrap analyses should also be handled cautiously as they highly depend on the number of sequences and can exaggerate similarities. PCR primers can be designed with broad or narrow species specificity as needed for different applications. With available programs, even three dimensional structures can be predicted if homologous proteins had been structurally studied earlier. Besides these promising advantages of using bioinformatics, it is also easy to make big mistakes and draw bad conclusions if not proceeding correctly. An example is the simple pair wise comparison of full genomes of larger viruses instead of comparing the corresponding genes. The correct selection of the right phylogenetic calculations is highly debated, too. Applying "too sophisticated" phylogenetic calculations for very short sequences may cause surprising mistakes. Finally, correct predictions need bioinformatics, but it is not possible to perform trustable calculations without sufficient amount of data. Higher number of sequences yields more solid conclusions. Preferably, sequencing should be performed simultaneously with the bioinformatic analysis to fine tune both of them constantly.

METABOLIC AND PROTEIN-PROTEIN INTERACTIONS INVOLVED IN THE SULFANILIC ACID DEGRADATION IN *NOVOSPHINGOBIUM SUBARCTICUM* SA1

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Novosphingobium subarcticum SA1 (*Sphingomonas subarctica* SA1, formerly identified as *P. paucimobilis*) is an aerobic Gram-negative bacterium capable to grow on sulfanilic acid as sole carbon and nitrogen source. Moreover, because of its diverse metabolic pathways it can utilize a wide range of natural and anthropogenic aromatic compound such as protochatechuate, p-aminobenzoic acid, 4-hydroxybenzoate etc. The genome of the strain sequenced by new generation genome sequencing technologies revealed numerous genes of enzymes potentially involved in the oxidation of aromatic compounds. Among these, genomic regions harbouring genes responsible for sulfanilic acid degradation were identified. One of these operons contains the genes of the whole metabolic pathway from 4-sulfocathecol to TCA cycle. The genes of enzymes which are responsible for the first key step are in another loci. Investigation of the proteins appearing upon sulfanilic acid induction disclosed proteins likely involved in the sulfanilic acid transport, conversion as well as the iron transport. These results might indicate that the elevated levels of iron containing enzymes taking part in ring hydroxylation and cleavage require additional iron transporting pathway. The recently performed whole cell transcript analysis confirmed these observations. A localization study disclosed that the proteins likely involved in the deamination and ring hydroxylation were membrane associated. This might have a self-protecting role since the cellular uptake and the conversion of toxic compound should be tightly linked processes. Recombinant enzymes were produced in homologous host and proteins interacting with either of these proteins are being analyzed. Our results suggest that a sensitive membrane-associated complex is responsible for coupled uptake and conversion of sulfanilic acid.

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MICROBIAL ABUNDANCE DURING PROPYLENE-GLYCOL AND FORMATE DEGRADATION IN A COLUMN EXPERIMENT WITH FOREST SOILS

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In northern airports there is an intensive use of propylene-glycol (PG) and formate (FO) based aircraft de-icing fluids. Previous experiments revealed that the degradation of PG is faster, compared to FO, which is less limited by the freezing temperatures when the snow melts, biochemical changes needed for its acceleration [1]. The main objective of the experiments was to study the role of the microbial population in the degradation process of de-icing chemicals. Column experiments (column volume 170 ml, length 10 cm) were conducted with homogenised soil obtained from Gardermoen Airport, Oslo, Norway, from 30 to 70 cm depth in 6 soil columns. The soil columns were saturated from bottom to top using a flow rate of 0.5 pore volumes (PV) per day (0.7

ml/h) to prevent air entrapment. The experiment was conducted at 4°C. Effluent fractions were collected 3-5 times/day by a fraction collector. The background solution contained 6.9 mg/L $NaNO_3$, 27.0 mg/L $CaSO_4x2H_2O$, and 1.6 mg/L $KHCO_3$. Bromide (1 mmol/L LiBr) was used as a tracer to determine column flow characteristics. The columns were treated by: S1 and S2: 50 mg/L PG + 500 mg/L FO, S4 and S5: 50 mg/L PG, S6: 1000 mg/L PG. S3 were used as non-contaminated control. Flow-through cells were installed for online monitoring the redox potential. To monitor the abundance of total heterotrophic microbes in the effluents Most Probable Number (MPN) method have been used, beside the analysis of pH, electric conductivity, total organic carbon, bromide, formate, other PG metabolites, main anions, cations, iron and manganese. MPN analysis were also conducted to study the tolerance level of microbes against various PG and FO levels. Microorganisms found in these soils can tolerate 10 times more PG and FO concentration that can be available during the snowmelt (at about 2500 mg/L in the effluents). An increased microbial abundance was realised with the enhanced pollution levels, followed by a decreased concentration of used contaminants. Significant drop of the redox potential showed the presence of anaerobic microbial degradation in the columns, and the possible fast adaptation to the changing environmental conditions. In S1, S2 columns the PG reached faster its initial concentration of 50 mg/L than FO, showing the differences in microbial degradation between the 2 chemicals. An enhanced degradation was realised in S4, S5 columns. The addition of 1000 mg/L PG to column S6 on the other hand showed that there is a limit to microbial degradation, the initial doses could be hardly degraded at 4 oC. Isolating, identifying and testing the abundant bacterial strains are needed in order to move forward to biodegradation experiments.

[1] Libisch, B., et al. (2012) Environ Technol 33: 717-724.
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HIGH THROUGHPUT NGS SEQUENCING OF *PROPIONIBACTERIUM ACNES*: IDENTIFICATION OF NOVEL VIRULENCE FACTORS

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Propionibacterium acnes is an opportunistic Gram-positive anaerobic bacterium that forms part of the human skin microbiota. It is an opportunistic pathogen associated with a wide range of infections and conditions including acne vulgaris, medical device and ophthalmic infections, sarcoidiosis, SAPHO syndrome and prostate cancer. Acne is a multifactorial disease of the pilosebaceous follicle and affects >70% of adolescents and young adults. To date, the exact role of *P. acnes* in the pathophysiology of the disease is unclear. This is all the more challenging as we have recently shown that the organism can be classified into at least six phylogroups, known as IA1, IA2, IB, IC, II and III that also display differences in their serology, biotype, cellular morphology and infection profile. In order to better understand the pathogenic potential of these different phylogenetic groupings, and identify novel virulence factors, we have determined the genomes of >20 strains by Next Generation Sequencing. Our analysis has revealed numerous SNP/MMP variations between the different groupings, and we have also identified several gene clusters unique to particular lineages that may be important in pathogenicity. In particular, our genome analysis revealed different level of lipase activity between distinct phylogroups, which may be important in the context of acne as the fatty acids released due to the action of lipase are inflammatory. To investigate this, we tested lipase activity from different strains of *P. acnes* by thin-layer-

chromatography. Isolates, including non-acne derived strains, were selected to represent different eMLST sequence types from the different phylogenetic clusters. We found that non-acne isolates show limited fatty acid reduction activity, while acne isolates efficiently degrade triacylglyceride. Overall, our data demonstrates that *P. acnes* exhibits high levels of genetic plasticity which accounts for successful adaptation to different environmental niches.

WATER AS A CRUCIAL FACTOR OF DETERMINING MICROBIAL ABUNDANCE IN THE RHIZOSPHERE OF GRAPEVINE (*VITIS VINIFERA* L.) AMONG DIFFERENT GROWING CONDITIONS

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The microbial community in red-clay rendzina soil was examined in the rhizosphere of grapevine (*Vitis vinifera* L.). Experimental area was located at Szentmiklóshegy hills of Mecsek area, near Pécs city. Grapevine variety of Jázmin was inoculated previously with mycorrhiza (AMF) inoculums in 2008. After 4 years of AMF inoculation soil samples were collected from 15 sampling points between the rows of plantation from two different layers (0-30, 30-60cm) as it is described by the TIM protocol [1]. Beside the soil moisture content, total number of bacteria were estimated on Nutrient Agar medium from the soil samples concomitant with the estimation of dehydrogenase enzyme activity (data shown in another study). Samples from lower depths has higher amount regarding the total countable number of bacteria than upper layers. There was S40-30: 6,5 10^4 in comparison with the S430-60, as 7,15 10^4 per 1 g of soil on a dry weight basis. At grapevine plantations the main root mass can reach deeper parts of soil – in some cases it is more than 2 meters -, therefore we can find higher microbial activity in the deeper layers. The same difference can be observed between northern and southern parts of the experimental area. Samples from the northern section has higher total number of bacteria values (S130-60: 7,75 10^4 > S530-60: 6,4-10^4 - total number of bacteria per 1g). Those differences were found to be in correlation with the greater water content in soils of the investigated northern areas and/or in the AMF inoculated plants.

[1] Czakó-Vér, K., et al. (2011) Növénytermelés Suppl. 60: 211-214.

COMPARISON OF THE EFFECT OF DIFFERENT BACTERIAL FERTILIZERS ON A CALCAREOUS CHERNOZEM SOIL

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Nowadays, the application of bacterial fertilizers has been spreading in agriculture. Different bacterial strains, enzymes and vitamins are allocated into the soil, which can influence the properties of the soil. In a pot experiment the impact of different bacterial fertilizers were studied. The soil applied was a calcareous chernozem from the area of Debrecen-Látókép. It was a mildly acidic loamy soil, with medium nitrogen and good phosphorus and potassium content. Our test plant was perennial ryegrass (*Lolium perenne*, L.). The bacterial fertilizers applied possessed with different properties in compound, physical condition, the amount and species of microbes, etc. The treatments applied were the following: 1-Control, 2-NPK fertilizer, 3-Wheat straw, 4-Bactofil A

bacterial fertilizer, 5-NPK+Bactofil A, 6-Straw+Bactofil A, 7-EM-1 bacterial fertilizer, 8-NPK+EM-1, 9-Straw+EM-1, 10-Microbion UNC bacterial fertilizer, 11-NPK+Microbion UNC, 12-Straw+Microbion UNC. The physical, chemical and microbiological parameters of the soil and the test plants biomass were measured in laboratory. In the treatments were established the positive effect of self-applied and combined bacterial fertilizers treatments on soil. Our results were supported the difference of bacterial fertilizers. On the basis of our results the following can be concluded: - The bacterial fertilizers itself and with NPK and straw treatments amended the soluble nutrient content of soil. The most effective was the Microbion UNC (NO_3^-N and AL-K_2O). - The bacterial fertilization increased the total number of bacteria (included cellulose decomposing bacteria) while the straw+biofertilizer combinations stimulated the number of microscopic fungi. - The bacterial fertilization itself and with combinations generally stimulated the enzyme's activity, except the saccharase. - The CO_2 production of soil increased, the CO_2-C content decreased by the use of the bacterial fertilizers. - The green mass of the ryegrass grew by the effect of the NPK and combinations of NPK+Bacterial fertilizer. The bacterial fertilization amended the soil parameters examined in most cases. Medium and close relations were proved with Pearson-correlation analysis between the results of treatments. The 12 treatments in the experiment were arranged in a random block design with three replications. For evaluation the results, one-factor analysis of variance on statistical data was applied. The use of bacterial fertilizers with straw could recommend in this soil type as an effective amendments/fertilization method.

COUNTRYWIDE DISSEMINATION OF EXTENDED-SPECTRUM ß-LACTAMASE- AND PLASMID-ENCODED AMPC-TYPE ß-LACTAMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* IN HUNGARY

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Although the most common mechanism to develop 3rd generation cephalosporin resistance among *K. pneumoniae* strains causing nosocomial infections is the production of extended spectrum β-lactamase (ESBL) enzymes all over the world, the role of plasmid-encoded AmpC-type β-lactamases is continually growing, as well. In many members of the Enterobacteriaceae family, AmpC-type β-lactamases are chromosomally encoded and inducible. Since these genes appeared on transferable plasmids, AmpC-type β-lactamases emerged in species lacking the chromosomal AmpC-type gene (e.g. *K. pneumoniae*), as well. Acquisition of such plasmids enables *K. pneumoniae* isolates to develop resistance to 3rd generation cephalosporins and cephamycins. Currently, based on the aminoacid sequence, eight enzyme families of the plasmid-encoded AmpC-type β-lactamases were described (CMY, FOX, ACC, LAT, MIR, ACT, MOX and DHA). Plasmid-encoded AmpC-producing Enterobacteriaceae strains represent a new threat since they usually show multidrug-resistant phenotype and antibiotic therapy of infections caused by such strains is strongly limited. In Hungary, the first plasmid-encoded AmpC-producing *K. pneumoniae* isolate was detected at the end of 2009. In the following years, expeditious, countrywide dissemination of AmpC-producing *K. pneumoniae* isolates was observed. The aim of our work was to characterize these isolates. The phenotypic confirmation of the putative AmpC-producing isolates was performed by ESBL combined disk test and ESBL&AmpC ID test. Antibiotic susceptibility was determined by disk diffusion tests and antibiotic gradient strips. The presence of AmpC- and ESBL-encoding genes was verified using PCR. Typing was performed by pulsed field gel electrophoresis and MLST. Between

January 2010 and June 2012, 1129 *K. pneumoniae* isolates resistant to 3rd generation cephalosporins were submitted to the National Center for Epidemiology to confirm the resistance mechanism. Based on the results of phenotypic and molecular tests 318 *K. pneumoniae* strains proved to be AmpC-producers. Of these, 290 strains produced CTX-M-type ESBL, as well. By PCR screening, all isolates were positive for carrying the *bla*DHA gene. Sequence analysis of β-lactamase genes detected *bla*DHA-1 and *bla*CTX-M-15 in selected isolates. PFGE typing revealed ten pulsotypes, the vast majority of the strains belonged to KP053 (sequence type ST11). The DHA-1 and CTX-M-15-producing KP053 clone spread to 28 centers across Hungary and affected 161 patients. It is important to emphasize that 32% of the AmpC-producing *K. pneumoniae* isolates were resistant to at least one carbapenem. Presence of these strains in clinical infections can result in the loss of one of the most important, last-line class of antibiotics, the carbapenems. Therefore, the rapid, countrywide spread of AmpC-producing *K. pneumoniae* strains may create serious problems in antimicrobial therapy, and endangers Hungarian health care facilities.

POLYMYXIN-RESISTANCE IN ENTEROBACTERIACEAE

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Polymyxins are „last hope" antibiotics against multidrug-resistant Gram-negative bacteria. They are amphipathic cationic polypeptides that cause disruption of the outer membrane by binding to the lipopolysaccharide. Emergence of polymyxin resistant strains was observed worldwide in different species of Enterobacteriaceae, notably, *Salmonella* spp., *Klebsiella* spp., *Enterobacter* spp. and *Escherichia coli*. The possible mechanisms of resistance are the following: substitution of lipid A with 4-amino-4-deoxy-L-arabinose or phosphoethanolamine, efflux pump/potassium system and increased production of capsule polysaccharide. The substitutions depend on two operons, arn and pmrCAB. Both of them are regulated by the PmrAB two-component system. The PmrB sensor kinase can be activated by different stimuli, e.g. low pH or elevated Fe^{3+} concentration. It phosphorylates the PmrA regulatory protein, which activates the genes of arn operon and the *pmr*C gene. The genes of arn operon are responsible for the synthesis of 4-amino-4-deoxy-L-arabinose, and *pmr*C codes a phosphoethanolamine-transferase. The phosphoethanolamine and arabinose are attached to free phosphate groups of lipid A. They increase the net positive charge of the outer membrane, making it difficult for polymyxins to attach to it. The arn operon, the PmrAB system or their homologues were found in *Salmonella enterica* serovar *Typhimurium, Escherichia coli* and *Klebsiella pneumoniae*. As a means of polymyxin-resistance, phosphoethanolamine substitution is more characteristic of non-fermentatives than Enterobacteriaceae, although several species of this family have a gene coding a phosphoethanolamine-transferase, but their role in resistance to polymyxins is not explored, yet. The PmrAB system has a regulator, too, the PhoPQ. The PhoQ sensor kinase can be actived by low Mg^{2+} and Ca^{2+} concentrations. It phosphorylates the PhoP response regulator, which activates the *pmr*D gene. The PmrD protein connects to the phosphorylated PmrA and prolongs its phosphorylation by preventing phosphatases to reach it. As a consequence, PmrA remains active and sustains the activation of the arn operon longer. Most of the species carrying the genes of PmrAB carry the PhoPQ, too, but there are differences in the regulatory relations and pathways. The capsule polysaccharide over-production, which was observed in *Klebsiella* spp., promotes resistance to polymyxins by preventing the molecules to reach their

target. Resistance to polymyxins can occur after exposure to the drugs during therapy, but there are known cases where resistance developed without any previous contact with these antibiotics. There were reports where treatment with aminoglycosides and fluoroquinolones selected polymyxin-resistant *Klebsiella pneumoniae* and *Enterobacter cloacae* strains.

GENETIC CHARACTERIZATION OF NOBLE ROTTED *BOTRYTIS CINEREA* ISOLATES FROM EGER WINE-GROWING REGION

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Botrytis cinerea Pers.:Fr., the anamorph of *Botryotinia fuckeliana* causes grey mould on a high number of crop plants in the temperate zones worldwide including grapes. In vineyards, the frequent occurrence of *B. cinerea* prior to harvest results in serious losses of fruits and deterioration of wine quality. *Botrytis* infection requires moist conditions and if the weather stays wet, the malevolent form, "grey rot" can destroy crops of grapes. Grapes typically become infected with botrytis when they are matured, but when then exposed to drier conditions become partially raisined and the form of infection brought about by the partial drying process is known as "noble rot". Grapes when picked at a certain point during infection can produce particularly fine and concentrated sweet wine. The realisation of grape protection for these different wine making technologies is difficult. A clue to these strategies lies in the understanding of the genetic structure and dynamics of its populations. In the course of our work we have investigated the genetic properties of grape pathogenic populations of *B. cinerea* in two small vineyard of Szomolya village which is located in the wine-growing region of Eger, Hungary. Eger, the region of Bull's Blood is well known from its dry wines and not for their botrytised wines which can made here as well in a very high quality. About 200 noble roted isolates were collected from these two vineyards during the year 2011 and 96 were examined between them. Individual strains were obtained by single-spore isolation. Characterization of their genotype was done by analysing of the β-tubulin and MSB1 minisatellite sequence, which is located in the intron of the ATP synthase. Data and sequences was analysed by phylogenetic methods. A moderate variability was found among the investigated sequences. The minisatellite allel map contrast strikingly, the variability of allels are great. We could also detect some differentiation between the isolations form the different territories and grape varieties. In summary, our data underline a significant specialization of populations from this territory what difference could arise by using a dissimilar grape protection technology.

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ANALYZING POTENTIAL ENTRY INHIBITORS OF HIV ON CELLLAR LEVEL

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The aim was the analysis and evaluating the anti-HIV effect in vitro of synthetic thyolated pyrimidine nucleotides termed UDs and chemically modified derivates. For the successful HIV infection, the initial molecular events determined by gp120 and gp41 surface glycoproteins, as well

as cellular receptors especially CD4 are with primary significance. MT-2 human T-cell lines were treated with the compound UDs in various concentration 30 min prior to the infection of cells with laboratory prototype replication competent HIV-1IIIB, and clinical isolates from HIV infected individuals respectively in a 96-well plate. M.o.i. of HIV-1 were between 0.1 – 2.0. Syntitia formation were monitored for an additional 72 hrs, and inhibition concentration (IC50) was determined in cultures , where syntitia formation was 50% or less compared to the ones without compounds. Antiviral effect of the various UD derivates were also determined quantitatively in MAGI (multinuclear activation of galactosidase indicator) assay. This in vitro infectivity assay is based on the activation of HIV-tat regulated galactosidase gene of transfected HeLaCD4-LTR/β-gal monolayer cells. Cells were treated by the compounds 30 min prior to HIV-1 infection (compounds were not removed). Forty hours after infection infected cells were counted in situ with a light microscope by virue of their blue color after incubation with 5-bromo-4-chloro-3-indolyl- β-D-galactopyranoside (X-Gal). IC50 and IC90 were determined by Hill analysis. Cytotoxicity of UD compounds were determined with XTT in vitro toxicology assay kit (Sigma). This was based on the activity in living cells, where mitochondrial dehydrogenases of viable cells reduce the tetrazolium-carboxyanilide ring of XTT yielding an orange color detected photometrically. Results with UD derivates showed a prominent antiviral effect in the concentration of ~5 ug/ml. As UDs inhibits the glyceraldehydes-3-phosphate dehydrogenase (GAPDH), results suggest that these thiolated nucleotides may interfere with function of the essential –SH groups of CD4 molecule (the primary receptor of HIV), and also of HIV gp120(env) and may function as an entry inhibitor for HIV.

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ON THE WAY OF UNRAVELLING OF THE FIRST EUKARYOTE NICOTINATE UTILIZATION PATHWAY IN THE MODEL ORGANISM *ASPERGILLUS NIDULANS*

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Despite the fact, that many microorganisms harbour the capacity of the utilization of nicotinic acid as N- or C-source, the degradation pathway of nicotinic acid was studied in only a limited number of prokaryotes [1, 2]. In eukaryotes the pathway is obscured and the only known degradation step was unraveled when enzyme activity of PHII from *Aspergillus nidulans* (purine hydroxylase II, coded by hxnS [3, 4]) was found to be involved in convertion of nicotinic acid into 6-hydroxy-nicotinic acid. Our effort to reveal the degradation pathway in *A. nidulans* led us to the identification of three genomic clusters NDC1 (Nicotinic acid Degradation Cluster 1 - *hxn*S, *hxn*R, *hxn*P, *hxn*T, *hxn*Y, *hxnZ*) and NDC2 (*hxn*X, *hxn*V) separated by 40 kb from each other on chromosome IV and NDC3 (*hxn*N, *hxn*M) on chromosome I. Genes of NDC2, NDC3 and two genes of NDC1 (*hxn*S and *hxn*P) were found to be regulated by HxnR transcription factor, a member of the NDC1 cluster and also by 6-hydroxy-nicotinic acid. Deletion mutants for hxnR-P-T-Y-Z NDC-1 related genes, the NDC2 members *hxn*X-V and NDC3 genes *hxn*N-M were developed to identify genes of nicotinate degradation pathway and support further studies to explore their biological function.
We showed that besides HxnS, three gene products, HxnX and V from NDC2 and HxnM from NDC3 were implicated in the degradation pathway.

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VIROCLIME – THE EFFECT OF CLIMATE CHANGE ON WATERBORNE VIRUSES. HUNGARIAN CASE STUDY

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Climate change may affect the spread and the survival of pathogenic microbes, including viruses. VIROCLIME, an EU FP7 Project, was designed to study the effect of climate change on the fate, transport and risk management of waterborne viruses in five different countries. In an 18-months sampling programme, environmentally sensitive 'hot-spots' were investigated for viral pathogens and indicator bacteria. Virus surveillance data will be combined with meteorological and hydrological data to create a model to estimate the risk of water-related diseases under a range of climate change scenarios. In the Hungarian case study, river water samples (10L) from four sampling points along River Tisza were analyzed bi-weekly between January 2011 and May 2012. Raw and secondary treated sewage (50mL and 50mL) were also sampled in a waste water treatment plant which is a potential source of viruses. Water samples were concentrated by direct flocculation. QIAamp Viral RNA Mini kit was used for nucleic acid purification. Human adenovirus, norovirus GI and GII, JC polyomavirus were detected with real-time quantitative polymerase chain reaction (qPCR) methods. Faecal indicators were enumerated using the microplate method. The virus titers and bacterial counts of 129 surface river water samples, 65 secondary effluent and 66 raw sewage water samples were determined. The most prevalent viruses in all matrices were the human adenoviruses. Human virus titers of the river water samples varied across the range 10^3-10^5 genome copies/L. Around 60% of the samples contained JC virus, and noroviruses were detected in only 30-40% of the river waters (possibly due to their less robust RNA genome). The sewage samples were quite stable, the average virus titre of the raw sewage samples was 10^7GC/L. The concentrations decreased 1log10 or sometimes 2log10 by secondary sewage treatment. The treatment process was most effective in attenuating noroviruses and least effective for the elimination of the human adenoviruses. The highest human virus concentrations were measured at the first sampling point which was impacted by urban drainage and effluent flux. According to the seasonal distribution, the highest human viral loads were in spring and summer 2011, and the lowest were in winter 2011 and spring 2012. The bacterial counts varied in the range 10^2-10^3CFU/100mL in the surface waters and the highest values were observed in the winter months. Wastewater treatment was most effective for the elimination of the indicator bacteria, in which 3log10 reduction was detected. It was revealed in the 18-months sampling programme, that human pathogen viruses persist constantly in the water environments. These viruses can not be eliminated properly with secondary waste water treatment process. The expected outcome of the VIROCLIME project is a model of the environmental fate of viruses, based on the accumulated viral, meteorological and hydrological data.

PHYLOGENETIC ANALYSIS OF *BORDETELLA BRONCHISEPTICA* STRAINS ISOLATED FROM DIFFERENT HOST SPECIES

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Bordetella bronchiseptica causes respiratory diseases in a variety of animals, including pigs (atrophic rhinitis), dogs (kennel cough), cats, rabbits, and guinea pigs (bronchopneumonia). It has been implicated as an infrequent cause of infection in humans, primarily in immunocompromised patients. This Gram-negative bacterium produces several virulence factors (toxins and adhesins) that are responsible for the broad host spectrum. The flagella, a possible virulence factor, are negatively regulated by a two-component system (BvgAS). In previous studies we described eight major *fla*A (flagellin) types with the PCR-RFLP analysis of 95 strains from our collection. The aim of our present work was to sequence and phylogenetically analyse the representative strains (30) of major *fla*A PCR-RFLP types. Our goal was to reveal whether a connection was between *fla*A types and hosts at the nucleic acid level, furthermore if signs of host-adaptation were manifested at the amino acid level, too. All strains tested were prepared for PCR by a simple boiling procedure of the bacterial colonies, and an 1165 base pairs length of *fla*A was amplified with specific primers. The nucleotide sequences were determined from amplicons in both directions using commercial sequencing facilities. The data were analysed by the BioEdit Sequence Alignment Editor and multiple sequence analysis was performed using CLUSTALW version 1.8. The phylogenetic trees were established by using the neighbour- joining method from Jukes–Cantor corrected distance estimation. The percent identity among the studied *B. bronchiseptica* strains varied from 86.6 to 100% in nucleotide acid level, whereas it was from 82.2 to 100% at the amino acid level. The phylogenetic analysis revealed four distinct groups of strains. Group I contained strains isolated from rabbits, dogs, horses and humans. In group II were located human strains and two uncommon strains originated from a pig and a turkey. Interestingly, the *fla*A of *B. pertussis* (wherein the flagellar operon has been considered to be inactivated due to multiple pseudogenes and IS insertions) belonged to this group too. Group III was very uniform with strains originated mostly from pigs. Group IV formed a separate branch with strains originated from cats, guinea pigs, humans and a turkey. The distinction of the four groups was validated at the amino acid level, each group has unique amino acid patterns in the hypervariable region of *fla*A. Our results highlighted the zoonotic risk of *B. bronchiseptica*. The signs of the host- adaptation were noticed in the case of strains originated from pigs, cats and guinea pigs.
The presented established results suggest that the flagella has a role not only in motility but also in other microbial processes, such as adherence to host cells and the invasion of the host cells.

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EFFECT OF IMMOBILIZATION OF CELLS AND/OR PRESENCE OF CYCLODEXTRIN ON BIODEGRADATION OF HYDROPHOBIC COMPOUNDS

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Nowadays, the appearance of unctuous contamination in the environment is still a serious problem. The insolubility of these compounds in water is a limiting factor for their degradability. Several microbes can produce surfactants providing an easier way for solubilizing hydrophobic organic substrates. One of the most famous group of microorganisms synthesizing these surfactants is the Rhodococcus genus. These surfactant might be extracellular or bound to the membrane. If continuous bioreactor is used for bioremediation of the pollutants, the cells should be immobilized to avoid of their washing out and/or to protect cells from external effects. However, the immobilization of cells might have a negative effect during hydrocarbon degradation, when cells have cell wall-bound surfactants. If cells are immobilized, the cell wall-bound surfactants are unable to interact with the hydrophobic components floating on water surface, therefore, there was a significant decrease in the efficiency and speed of hydrocarbon degradation as compared to cells producing extracellular surfactants. The aim of this work was to compare the hydrocarbon degrading efficiency of free and immobilized cells. Two *Rhodococcus erythropolis* strains (one having wall-bound and another one producing free surfactant) were used. The biodegradation efficiacy was greatly affected by the properties of the surfactants. The cell immobilization did not effect the hydrocarbon degrading capacity of the *R. erythropolis* PR4 strain having extracellular surfactants, however a significant decrease could be observed in the activity of the *R. erythropolis* MK1 having cell wall-bound surfactant. In case of hydrophilic substrate, a quicker adaptation was shown at *R. erythropolis* MK1 using either immobilized and free-floating cells. Cyclodextrin was applied for the enhancing the bioavailability of cells to the hydrophobic molecules.
The addition of cyclodextrin to the *R. erythropolis* MK1 culture stimulated the bioconversion rate, which effect could not be observed for the *R. erythropolis* PR4.

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FOLLOWING THE TRIPLE COMBINATION TREATMENT OF HEPATITIS C VIRUS INFECTION IN ROUTINE DIAGNOSTICS

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Hepatitis C virus (HCV) is the leading cause of chronic liver diseases. Approximately 170 million people are affected by chronic HCV infection worldwide. Former standard of care for treatment of hepatitis caused by HCV was the combination of pegylated interferon and ribavirin, however sustained virological response evolved only in 50% of cases. Hence, the new standard of care for hepatitis caused by HCV accepted in 2012 has a very important role in increasing this 50% rate significantly. According to the new protocol a triple combination of drugs is used instead of the double combination. In addition to pegylated interferon and ribavirin (nucleoside analogue) a third direct acting antiviral, a protease inhibitor is added to the treatment. Although the new method has been used since the beginning of this year, our team has managed to follow the progress of therapy of two patients who were being treated with the triple combination. Both of the patients had been treated with pegylated IFN+ribavirin before but failure of treatment provided a good reason for restarting therapy with the triple combination. Our laboratory performed quantitative determination of viral nucleic acid during the previous therapy and current treatment. We determined the initial

nucleic acid concentration and monitored values after 3 and 6 months. Test results show that triple combination seems effective in the cases of both patients.

INTER SAMPLE SEQUENCE REPEAT ANALYSIS OF MAJOR GROUPS OF DARK SEPTATE ENDOPHYTES OF SEMIARID GRASSLANDS

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The majority of the terrestrial vascular plants live together with different non-pathogenic fungi. These fungal partners can be endophytes, that colonize plant tissues during some period of their life cycle yet cause no symptoms of tissue damage to their hosts. Dark septate endophytes (DSE) represent a form-group of root colonizing endophytic fungi. DSE fungi are relatively frequent in (semi)arid and harsh environments and they probably play an important role in ecosystems with low water availability, studies of their diversity in those regions are sporadic. In a previous study, we found that the majority of the plants of semiarid areas of the Great Hungarian Plain were colonized by a few dominant DSE groups showing neither host and area specificity nor seasonality. However, the isolates were grouped using the sequences of the internal transcribed spacer (ITS) region of nrRNA gene. Using more variable DNA regions strains may indicate intra-ITS-group heterogeneities and probable subclades might correlate with different hosts or microhabitats. Our aims were (i) collect more strains of three dominant DSE groups from different hosts and microhabitats (ii) to study the variability of the strains of these groups using Inter Sample Sequence Repeat (ISSR). A finer-scale analysis using ISSR markers can point out specificity masked by the ITS sequence similarities and those might help the better understanding of DSE fungi in semiarid environments. Group-specific primer pairs targeting the ITS region were designed for three dominant DSE groups (*Cadophora* sp., *Rhizopycnis vagum*, *Pleosporales* sp.) to make accurate and fast diagnosis of the isolates possible. Root samples were collected from woody and herbaceous plants of sandy areas close to Fülöpháza. Nine of the ISSR primers generally used for fungi were selected for analysis. The pattern of each nine primers was informative and could be used to screen the genetic variability of the three DSE groups. In this presentation we show the results and the correlation of the groups resulted by the ISSR patterns with biotic (e.g. hosts) and abiotic (e.g. microhabitats) factor.

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THE INVESTIGATION OF QNRD, PLASMID-MEDIATED QUINOLONE RESISTANCE DETERMINANT IN ENTEROBACTERIACEAE

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Plasmid-mediated quinolone resistance determinants maintain low-level fluoroquinolone resistance and facilitate the selection to higher level. To date, they belong to three groups: Qnr determinants, aminoglycoside acetyltransferase(6')-Ib-cr modifying enzyme and efflux pumps (QepA and OqxAB). Five different linages of Qnr determinants are known: QnrA, QnrB, QnrC, QnrD and QnrS. In our

study we were investigating the prevalence of *qnr*D by PCR and sequencing in a collection of 756 non-replicate Enterobacteriaceae clinical isolates identified in Italy. All the isolates were resistant or reduced susceptible to fluoroquinolones according to the latest EUCAST breakpoints. Five *qnr*D positive isolates, namely four *Proteus mirabilis* and one *Morganella morganii* were detected. Plasmids harbouring the qnrD gene were further analyzed by Southern blot, conjugation and plasmid sequencing. A nonconjugable low molecular weight plasmid was common to all the positive isolates and Southern blot verified the plasmidic localisation of *qnr*D gene. All the five *qnr*D-plasmids were entirely sequenced and two of them accounting for 2687 bp and 2684 bp, were deposited at Genbank with accession numbers as follows: JN183060 and JN183061. The nucleic acid sequences were analyzed and no other known coding sequence was found besides *qnr*D. The four *P. mirabilis* strains were clonally unrelated by pulsed-field gelelectrophoresis and by random amplified polymorphic DNA. This feature suggests a frequent association of *qnr*D gene with this species. The qnr determinants can be used as resistance markers.

VARYING FITNESS COST ASSOCIATED WITH RESISTANCE TO FLUOROQUINOLONES GOVERNS CLONAL DYNAMIC OF EXTENDED SPECTRUM ß-LACTAMASE (ESBL)-PRODUCING *KLEBSIELLA PNEUMONIAE*

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Since varying fitness cost associated with resistance to fluoroquinolone type antibiotics proved a crucial determinant in the clonal dynamic of methicillin-resistant *Staphylococcus aureus* the impact of fluoroquinolone resistance on the clonality of ESBL-producing *Klebsiella pneumoniae* was investigated. Strains from major international clones of ESBL-producing *Klebsiella pneumoniae* (ST11, ST15 and ST147) maintained considerable vitality even when showing high MIC values to ciprofloxacin as was demonstrated in propagation assays. In contrast, isolates from various minor clones proved either incapable of assuming high level resistance or suffered significant fitness cost when attaining high MIC values. Thus, strains from minor clones of ESBL-producing *Klebsiella pneumoniae* seem to be unable to disseminate in wards where fluoroquinolone type antibiotics are used extensively. These findings and the extensive dissemination in Hungary of a relatively recent clone (ST525) that suffered just slight fluoroquinolone-associated fitness cost clearly show that the testing of fluoroquinolone-associated fitness cost can predict the capacity of newly-emerged clones of ESBL-producing *Klebsiella* to disseminate in adult ICUs. Mutations in the fluoroquinolone determining regions of the *gyr*A and *par*C genes of all isolates were determined and their potential role in the diverse fitness cost of the strains analysed. High level resistance induced to ciprofloxacin (512 mg/L) in a VIM-4-producing ST11 strain resulted in a dramatic loss of fitness. The practical implications of this observation are under investigation.

INVESTIGATIONS ON VIRULENCE PHENOTYPES IN ASYMPTOMATIC BACTERIURIA *ESCHERICHIA COLI* ISOLATES

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Asymptomatic bacteriuria (ABU) is defined as a condition when a significant number ($\geq 10^5$ cells pro ml) of the same bacterium is present in two independent urine samples without producing clinical symptoms. This setting is facilitated by female gender, pregnancy, indwelling catheters, elderly and diabetes mellitus. Though ABU strains frequently possess with a battery of virulence genes only a small portion of them appears in the phenotype. It has also been observed that ABU strains may outcompete the incoming pathogens by bacterial interference. The goal of this work was to assess the appearance and patterns of virulence phenotypes in ABU *E. coli* isolates from diabetic children. These investigations may foster further studies in order to identify effective and safe candidates for bacterial interference applicable with emphasis to diabetics. Phenotypic assays for virulence markers included: haemolysis, haemagglutination (mannose sensitive and resistant), siderophore production (aerobactin and enterobactin), serum resistance, biofilm production, binding to matrix components (collagen types I and IV, fibronectin, laminin), adhesive and penetrating capacity to urinary epithelial cells, and cytotoxicity. The genetic background of the haemolytic character was specified by PCR for *hly*A, *hly*F and *she*A. Out of 14 virulence properties none of the strains produced more than five ones. None of the isolates possessed with all the five most important virulence traits (adherence to and invasion of epithelial cells, serum resistance, siderophore production and haemolytic capacity). One strain presented with no virulence factors tested. This latter strain seems worth studying for its interference capacity against uropathogenic bacteria. Animal experiments to assess the in vivo interference capacity and safety are in progress.

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CHANGES IN MICROBIAL COMMUNITY METAGENOME UPON ADAPTATION TO PROTEIN SUBSTRATE

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Facing energy crisis, the world is in need of green, efficient, carbon-neutral energy sources to replace fossil fuels. Biogas, formed by anaerobic digestion of organic materials, provides sustainable, renewable energy. There is a significant potential in biogas production from industrial waste, partly because waste treatment reduces the environmental impact of these materials and partly because biogas can replace fossil natural gas in all of its applications. In this work biomethane production from protein-rich waste was studied in 5-litre continuously stirred tank reactor (CSTR) experiments at 37°C. The new generation SOLiD DNA metagenomic sequencing technique was employed to determine the taxonomic distribution and relative abundance of the members of the microbial community. During the anaerobic degradation (AD) fermentations, fed

with protein-rich substrates and the sole source of biomass, the composition of microbial community was tested at three time points: at the start, halftime and at the end of the adaptation process. A shift in the population balance was clearly observed as part of the adaptation process to protein-rich substrates. Considering the entire period to adapt the microbial community to this unconventional substrate, the Bacteria domain, and within Bacteria the Firmicutes and Proteobacteria phyla showed the greatest alterations. The classes Clostridia and Bacilli and Gammaproteobacteria, belonging to these phyla, constitute the majority of the Bacteria in the biogas fermentor. The biogas yield from the substrate (decomposition rate of the protein substrates) increased and both the activities of hydrolyzers and syntrophic acetate oxidizing bacteria (SAOB) were remarkably amplified. The results substantiated that the adaptation of the microbial community to the high protein content substrate is achievable and this modification leads to the intensification of biogas production.
The findings extend the range of organic "waste" substrates, which are suitable for renewable energy production and predict routes for rational design of more effective microbial communities for industrial scale biogas production technologies.

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DIFFERENT PCR PRIMER SYSTEMS REVEAL STRIKINGLY DIFFERENT COMPOSITION OF ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITIES

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Arbuscular mycorrhiza (AM) formed by the majority of terrestrial plants and the fungi belonging to phylum Glomeromycota is the most widespread symbiosis on the Earth. The number of described species of AM fungi (AMF) is relatively low: ~230 species have been described, however molecular diversity studies revealed a much higher species richness. Because of pitfalls of spore based methods, PCR-based molecular diversity screening techniques are commonly used to study AMF communities. For this, several primers and primer systems for general and specific amplification of AMF have been designed and used. In this study we tested whether four widely used primer-systems resulted in a similar community structure when AMF communities were studied. Both NS31-AM1 primers and the AML primers target the SSU gene of nrDNA, da Silva's primers amplify a segment of the LSU nrDNA while Krüger's nested primer-system amplifies the ITS and partial LSU of nrDNA. An artificial AMF community was established using DNA extracted from spores originating from a reference AMF collection. Three different mixtures of AMF species representing different orders of Glomeromycota were made and screened by the primers listed above. 96 clones from each primer-test of each mixture were sequenced. The four systems gave strikingly different results regarding both AMF species richness and the frequency based diversity estimators calculated. Our results suggest that the species number of AMF can be much higher than thought before based on molecular diversity studies and stress the importance of simultaneous use of different primer systems for screening AMF diversity.

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POSTANTIFUNGAL EFFECT OF CASPOFUNGIN IN 50% SERUM AND EFFICACY OF SINGLE HIGH DOSE OF CASPOFUNGIN AGAINST THE „*PSILOSIS*" GROUP

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Caspofungin (CAS) activity was determined in vitro and in vivo against two-two *Candida orthopsilosis, C. metapsilosis*, and *C. parapsilosis sensu stricto* isolates. Postantifungal effect (PAFE) was determined in RPMI-1640 with and without 50% human serum. Duration of PAFE was defined as the difference in the time required for control and test isolates to grow 1 log10 following drug removal. Neutropenic mice were intravenously challenged with $5x10^6$ CFU/mouse. Mice were assigned to seven treatment groups for *C. orthopsilosis* and *C. metapsilosis* (no treatment, 1, 2 and 3 mg/kg CAS daily for five days, a single 5, 10 and 15 mg/kg CAS doses).
For *C. parapsilosis* there were five treatment groups (no treatment, 3 and 4 mg/kg CAS daily for five days, a single 15 and 20 mg/kg CAS doses). All regimens were started 24 hours postinoculation, the drug was administered intraperitoneally. At the end of treatment all mice were sacrificed and fungal tissue burden in kidney pairs was determined by quantitative culturing. Kidney burden was analyzed using the Kruskal-Wallis test with Dunn's post-test. PAFE of CAS at 16-32 mg/L concentrations for *C. parapsilosis, C. orthopsilosis* and *C. metapsilosis* were 8.7->36.7, 21.1->37.5 and 4.6->37.3 hours, respectively. In case of mice inoculated with *C. parapsilosis*, the kidney tissue fungal burdens were significantly decreased by a daily 4 mg/kg CAS dose and 15 and 20 mg/kg single CAS doses when compared to the untreated group.

EXAMINATION OF PLANT GROWTH-PROMOTING HORMONE PRODUCTION OF BACTERIA ISOLATED FROM STRESS SOILS

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In Hungary, as worldwide, economically important food and forage plants are grown mostly on low fertility soil. In our country, more than 40% of the cropland is of low fertility and - in terms of soil's microbial life - degraded, "stress"-soils (strongly acidic, alkaline-saline, structureless, sandy soils, etc). Economical and environmentally-sustainable farming in these areas is difficult due to the deteriorated quality of soil. The new generation of soil vaccines will include naturally present (not genetically modified), stresstolerant and adaptive soil bacteria strains, which are able to efficiently stimulate crop growth, increase resistance capacity and crop yield in „stress"-soils. The Biofil Ltd.'s researchers – in collaboration with strategic partners – select and examine the bacteria with state-of-the-art microbiological technology and molecular biological and biotechnological tools. The outcome shall provide a complex environmentally safe and biological-based tool in order to limit the use of chemical fertilizers and synthetic soil conditioners, and thereby reduce environmental load. We isolated bacteria from soils which impose an extreme environment for the bacteria. We collected soil samples from natural habitats and ploughed lands of Hungary. We isolated hundreds of strains from the samples and among these strains there are around 100 that produce indole-acetic acid (IAA). Thereafter we selected the ones with the highest capacity for further examination. We verified IAA production by the TLC method, after that we selected the ten most effective strains and examined the IAA production in extreme conditions. During the research, we performed

examinations in a wide range of salt concentrations and pH-levels. Our goal was to select bacterial strains which can produce growth-promoting hormones in "stress"-soils, helping plants.

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DEVELOPMENT AND APPLICATION OF PHAGE THERAPY BASED PESTICIDES: PRACTICAL EXPERIENCES

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Bacterial plant pathogens are able to cause severe economic losses, especially in cases when no effective control methods are available. An alternative approach of treatment of plant pathogenic bacteria is the utilization of bacteriophages. Bacteriophages (phages) are viruses, which are obligate intracellular parasites of bacteria. Our group demonstrated the effectiveness of a phage-based pesticide in case of fire blight. *Erwinia amylovora*, member of Enterobacteriaceae, is the causative agent of this disease of some Rosaceae plants including apple, pear, cotoneaster, pyracantha and hawthorn. Streptomycin was the conventional drug used to control fire blight, however, its extensive use has resulted in the emergence and spread of streptomycin-resistant *E. amylovora* strains, making the treatments ineffective. We investigated the applicability of phage therapy for treatment of fire blight. 39 bacteriophage strains were isolated against E. amylovora, and tested their effectiveness and host range under laboratory conditions. Two phage strains (PhiEaH1 and PhiEaH2) were chosen for field experiments. A phage cocktail containing PhiEaH1, PhiEaH2, UV-protectant and alginate was applied for spraying 66 apple trees, whereas 70 trees remained untreated control. A significant difference could be detected in the appearance of new fire blight cases among the treated and untreated trees. Penetration experiments of bacteriophages into host plants revealed that phages are able to get into the sprayed plant. Analysis the complete genome of *E. amylovora* phage PhiEaH2 will be also presented. Preliminary results of phage therapy against other plant pathogenic bacteria will be demonstrated. Our results support that phage therapy may provide an effective solution for controlling fire blight and other bacterial plant diseases.

ESTABLISHMENT OF PHAGE THERAPY CENTRE: BACTERIOPHAGE THERAPY AGAINST *STAPHYLOCOCCUS AUREUS*

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Spreading of multidrug resistant pathogenic bacterial strains is one of the biggest challenges of clinical microbiology. Although huge money is invested into the development of newer and newer antibiotics, we are always behind bacteria. The breakout from this vicious circle can be presented by using new alternative methods, like phage therapy. Bacteriophages (phages) are viruses that are obligate intracellular parasites of bacteria, which could be applied to control also multidrug resistant bacteria. The consortium of Enviroinvest Co., University of Pécs and University of Szeged decided to establish a virtual scientific centre in order to develop phage therapeutic product against human-, animal- and plant pathogenic bacteria. One of our first aims was to isolate and characterize (both

morphologically and genetically) bacteriophages against methicillin-resistant and vancomycin-resistant *Staphylococcus aureus* (MRSA and VRSA). *S. aureus* is a Gram positive bacterium that is a pathogen of pyogenic inflammatory diseases, systemic infections, food-poisoning and toxic-shock syndrome. 14 phage strains were isolated against MRSA, from which 2 are able to infect also VRSA bacterial strains. All the isolated phages belong to Caudovirales. Laboratory application trials demonstrated the efficiency of these viruses against MRSA. All tested bacteriophage strains showed narrow host range with the exception of two strains, which were able to infect also VRSA bacteria. Optimization of fermentation of PhiSAH1 was carried out resulting a high titer phage suspension. Full genomic sequences of 4 phage strains were determined. Preliminary nasal colonization results will be also presented. Summarizing our results it could be concluded that bacteriophage therapy may be effective against both MRSA and VRSA.

THE EFFECT OF ORGANIC AND CONVENTIONAL AGRICULTURE ON THE PHYSICAL, CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF THE SOIL

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The role of soil microbiota is very important, since it can largely influence the fertility of soils. Soil management can either support or limit the activity of soil microorganisms. Our aim was to compare the effects of organic and conventional agriculture on the physical, chemical and microbial properties of the test soil. The long-term field experiment has been carried out by Géza Kovács in Martonvásár for 15 years. The samples were collected from the organic and conventional plots in October 2011. The soil samples were analysed in detail. The pH values, humus content, electric conductivity, mechanical composition and nutient status were determined. The number of soil microorganisms were determined by the traditional plate count method for bacteria (nutrient agar) and fungi (Rose Bengal agar). The soil microbial activity was characterised by FDA test for total catabolic enzyme activity. Zymo Research Soil Microbe DNA Mini Prep TM was used for soil DNA extraction. The total extractable soil DNA content was measured spectrophotometrically. Significant differences of soil physical, chemical and microbial properties (CFU, FDA activity, total extractable soil DNA content) were found between the organic and conventional plots. In the organic plots the electric conductivity, the pH values, the humus content, the NO3-N content and total N content were significantly higher. Moreover, the CFU values of heterotroph bacteria and fungi, as well as the FDA enzyme activity were also significantly higher. Comparing to the conventional plots the extractable soil DNA contents were significantly higher in the organic plots. It can be concluded that the good practice in organic agriculture supports the microbial diversity as well as activity of soils.

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APPLICATION OF THE IMAGE ANALYSIS-BASED BIOCONTROL INDEX CALCULATION METHOD FOR THE ASSESSMENT OF IN VITRO ANTAGONISTIC ABILITIES OF *TRICHODERMA* ISOLATES FROM VEGETABLE RHIZOSPHERE SAMPLES

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In 2006, a novel accurate method has been proposed for the evaluation of in vitro antagonistic abilities of biocontrol candidate *Trichoderma* strains [1]. Based on the measurement of areas of confronted fungal colonies, biocontrol index (BCI) values can be calculated that are characteristic to *Trichoderma* strains, thus representing a useful measure to describe their in vitro antagonistic abilities. Since its description, the method has been adopted by other working groups [2]. The original protocol has been optimized for the measurement of *Trichoderma* antagonism against the plant pathogenic fungus *Fusarium culmorum*. In this study, the image analysis-based BCI calculation method was extended to five further plant pathogenic fungal species, *Alternaria alternata, A. solani, Phoma cucurbitacearum, F. solani* and *F. oxysporum*. Twenty *Trichoderma* isolates belonging to nine different species of the genus (*T. asperellum, T. atroviride, T. citrinoviride, T. gamsii, T. hamatum, T. harzianum, T. longibrachiatum, T. pleuroticola, T. virens*) and deriving from the rhizosphere of different vegetables (carrot, celery, kohlrabi, parsley, pumpkin, salad, spice paprika, tomato) grown at different locations in Hungary (Balástya, Hatvan, Ózd, Szeged-Szikssós, Szentes and Veszprém) were confronted on agar plates with 8 strains of the above mentioned 5 plant pathogenic fungal species. BCI values were calculated based on the image analysis of the plates and compared to the respective BCI values obtained for a commercially available biocontrol strain of *T. atroviride*. Certain examined strains of *T. asperellum, T. atroviride, T. hamatum, T. harzianum* and *T. pleuroticola* proved to have higher BCI values than the commercial biocontrol strain of *T. atroviride*, suggesting, that – except from *T. pleuroticola*, which is known as a green mould pathogen of cultivated mushrooms – these strains may become promising candidates for biocontrol applications. Furthermore, the results of this study underline that the image analysis-based BCI calculation method provides accurate quantitative values for the evaluation of in vitro antagonism, which helps the characterization of biocontrol abilities of fungal antagonists.

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APPLICATION OF THE FISH METHOD IN MEDICAL MICROBIOLOGICAL DIAGNOSTICS: NOTES FROM A TEST DEVELOPER

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Fluorescent in situ hybridization (FISH) is generally used for the sensitive detection of single bacterial cells in complex environments. The method uses fluorescent dye-labeled oligonucleotides or synthetic analogs targeted to the conservative specific sequences of the ribosomal RNA. The rapidly growing rRNA databases, as well as the continuously expanding amplification and detection technologies provide rich possibilities to explore new territories in the microbial ecology and evolution. In contrast to the rapid development in these fields, medical microbiology could not profit

too much from this technology, so far. Based on an international patent and the connected know-how, a couple of years ago we started to develop a rapid FISH based diagnostics intended to for the identification of various, medically significant microorganisms from biological fluids. This lecture will provide a candid, first-hand insight into the initial phases of the process of diagnostic's development. Although we have not reached the end of the full product development cycle as yet, our interim results may provide useful application tips for the prospective users of FISH-based microorganism detection techniques. At present it is the one-step, culture-free clarithromycin resistance determination of Helicobacter pylori using the FISH method that provides silver lining for the presence and future of our FISH based diagnostics. The initial, unique results of a 4,400 patient based study of the *H. pylori* clarithromycin resistance pattern in Hungary will also be presented.

PHYLOGENETIC DIVERSITY OF PLANKTONIC BACTERIAL COMMUNITIES INHABITING LAKE HÉVÍZ

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Lake Hévíz is the largest warm water natural spa lake of Europe. The curative effect of the water can be originated in the mineral salts derived from crater sprigs and the special sediment that forms a transition between moorish and volcanic types. Containing a combination of components with different origin, the water of Lake Hévíz harbors peculiar bacterial communities, which may play important roles in the preservation of the natural state and the curative effect of the lake. Therefore, the aim of the present study was to gain comprehensive information about the spatial and temporal distribution of planktonic microbiota. The phylogenetic diversity was studied by cultivation based and cultivation independent molecular biological Denaturing Gradient Gel Electrophoresis (DGGE) and cloning methods. Depth distribution of bacteria in the lake was studied following a sampling in November 2009, while horizontal and seasonal distribution in April, July and October 2010 and 2011 by DGGE based on the 16S rRNA gene of Bacteria and Cyanobacteria. From a composite sample taken in April and October 2011, strain collections by using different culture media and 16S rRNA gene based clone libraries were established.
Comparing the bacterial communities, seasonal alternations in microbial community structures were observed according to the band numbers and intensities. Dominant DGGE bands were identified as representatives of class α- and β- Proteobacteria and phylum Cyanobacteria and Chloroflexi. Clone library constructed was also dominated by members of class α- and β-Proteobacteria while the strains mainly belonged to class α-Proteobacteria but representatives of phylum Bacteroidetes, Actinobacteria and class γ-Proteobacteria were also abundant.

A NEW ZEARALENONE BIODEGRADATION STRATEGY USING NON-PATHOGENIC *RHODOCOCCUS PYRIDINIVORA*NS K408 STRAIN

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Zearalenone (ZEA) is a nonsteroidal estrogenic mycotoxin produced by several *Fusarium* spp. on cereal grains. ZEA is one of the most hazardous natural endocrine disrupting chemicals (EDC) which induces hyper estrogenic responses in mammals. Consequently, detoxification strategies for contaminated crops are crucial for food safety. In this study we have developed a bacterial based detoxification system using a non-pathogen *Rhodococcus pyridinivorans* K408 strain. Following 5 days treatment of ZEA with *R. pyridinivorans* K408 strain HPLC analyses showed an 87.21% ZEA-degradation efficiency of the bacterial enzyme systems. In another approach, the strain biotransformation ability has also been confirmed by a bioluminescent version of the yeast estrogen screening system (BLYES), which detected an 81.75% of biodegradability of ZEA, in a good agreement with the chemical analyses. Furthermore, the capacity of *R. pyridinivorans* to eliminate the estrogenic effects of ZEA was tested by using an immature uterotrophic assay. Prepubertal female rats were treated with vehicle (olive oil), 17β-estradiol, ZEA (0.1-1-5-10 mg/kg body weight) and LB broth containing 500 mg/l ZEA that has already been incubated with or without *Rhodococcus pyridinivorans* K408 strain. Uterine weights were measured and the mRNA level changes relating to apelin, aquaporin 5, complement component 2, and calbindin-3 genes were measured by qRT-PCR. These genes represent the major pathways that are affected by estromimetic compounds. Zearalenone feeding significantly increased the uterus weight in a dose dependent manner and at the same time upregulated complement component 2 and calbindin-3 expression as well as decreased apelin and aquaporin 5 mRNA levels comparable to that seen in 17β-estradiol exposed rats. In contrast, LB broth in which ZEA was incubated with *Rhodococcus pyridinivorans* K408 prior to the feeding, did not display any estrogenic effect neither on uterine weight nor on the expression of estrogen-regulated genes. Consequently, the identification of *Rhodococcus pyridinivorans* K408 strain in ZEA biodegradation proved to be a very efficient biological tool that is able to eliminate the complete estrogenic effects of ZEA. It is also remarkable that this biotransformation pathway of ZEA did not result in any residual estrogenic effects.

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MALARIA SITUATION NOWADAYS IN HUNGARY

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Malaria was an endemic disease in Hungary for many centuries. The highest number of reported cases was several thousands per year (1933-1943), but the actual number of malaria cases was estimated as high as 10-100 000. The major breakthrough came in 1949 by the organized antimalarial campaign applying DDT for mosquito killing. The drastic reduction of the vectors resulted in the rapid decline of malaria cases. Since 1956, there have not been reported any indigenous case in Hungary. In 1963, Hungary entered on the Official Register of the WHO to the areas where malaria eradication has been achieved. Aim of this work is to Present the malaria situation nowadays in Hungary. During the period of 1963-2011, there were 215 Hungarians and 293 foreigners who imported malaria to Hungary. Majority of cases (266) were caused by *Plasmodium falciparum*. Further 242 cases were caused by *P. vivax* and other *Plasmodium* species. During that period, 7 fatal cases were reported (*P. falciparum*). Diagnostic tools: microscopic examination of Giemsa stained thin and thick blood film, antigen detection and DNA detection by

LC and multiplex semi nested PCR. The expansion of migration (both the increase of the number of foreigners travelling into Hungary and of Hungarians travelling to abroad) favours to the appearence of imported cases. In order to avoid the importation of malaria to Hungary, attention is called of all the persons travelling to malaria endemic countries to the importance of malaria prevention by the International Vaccination Stations located in the National Center for Epidemiology and in the Public Health Institutes of 19 counties and of Budapest.

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A UNIQUE BETA-XYLOSIDASE COMBINING CHRYSTALLOGRAPHY, ELECTON MICROSCOPY AND BIOCHEMISTRY DATA

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Earlier we cloned a gene encoding a β-D-xylosidase (TfBXyl43, EC 3.2.1.37) from *Thermobifida fusca* TM51 which is the terminal part of the xylan degrading enzyme-system of this organism. The recombinant TfBXyl43 was expressed in *E. coli* BL-21(DE3) and was purified to apparent homogeneity. The SDS-PAGE investigations showed that the molecular weight of the inactive monomer unit of TfBXyl43 is 62.5 kDa but the active form of the enzyme seems to has a dimeric, trimeric or tetrameric quaternary structure according the recent publications and our native PAGE examinations. The substrate specificity investigations indicated that the TfBXyl43 is an exo-glycosidase, hydrolyzing only xylobiose and xylotriose from the nonreducing end. Interestingly we could detect transxylosidase activity of TfBXyl43 which is unique among the inverting glycoside hydrolases. For elucidating the transglycosidase activity and oligomeric composition of TfBXyl43 chrystalizaton and paralell electron microscopic 3D reconstruction and image modeling were done. Crystals of xylosidase were grown by the hanging drop vapor diffusion method at 20°C, mixing equal volumes of xylosidase with a precipitant (12% PEG 3350, 0.2 M $MgCl_2$, 50 mM TRIS, pH=8.0). Diffraction data were collected at the Swiss Synchrotron Light Source (SLS) in Villigen, Switzerland, at beamline X10SA. The structure was solved by molecular replacement using Molrep with Xylan β-1,4-Xylosidase from Bacillus halodurans C-125. The final model contains two chains of β-Xylosidase. 5-bladed beta-propeller 3D structure status of TfBXyl43 was identified, this is the common structural feature of GH43 glycosidases. Interestingly, a metal ion (Mg^{2+}) bound at the surface between His341, Asp343, Gly370 and Asp540 was found. The possible role of the metal ion might be stabilization of the protein. For electron microscopy and reconstruction study images of negatively stained TfBXyl43 with a nominal defocus of ~1 μm were recorded on a Tietz 4kx4k CCD camera using a CM200 microscope at 160 kV (final magnification, 60,000×, object pixel size, 1.6 Å). Particles were hand-selected for 3D reconstruction. The initial 3D model for refinement was a sphere with a radius of 7nm, no symmetry was applied. The β-Xylosidase tetramer was rigid-body fitted using Chimera. According our observations TfBXyl43 is a tetrameric enzyme stabilized by Mg ions and belongs to the recently described beta5-propeller GH43 family. Further experiments focused on the active site and substrate-enzyme co-crystallization studies are needed for elucidating the unique transglycosidase feature of this inverting enzyme.

BIODEGRADATION OF FOOD INDUSTRIAL WASTES BY *RHODOCOCCUS* SP.

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Industrial pollution represents one of the major problems for the world. Although, recent technologies tend to reduce the emission of hazardous substances, nevertheless environmental pollution still reaches high levels. There are many toxic compounds of industrial wastes which must be neutralized. Biological approaches using microorganisms to convert polluting materials are environmentally and economically sound tools for cleaning our environment. Certain bacteria, such as rhodococci, are able to degrade a wide range of hazardous chemicals, e.g. aliphatic and aromatic hydrocarbons. In our laboratory, a *Rhodococcus* sp. strain was isolated from hydrocarbon polluted soil. It was successfully proven that the bacterium could efficiently decompose industrial hydrocarbons such as diesel oil and dead oil. Moreover, the strain could tolerate low temperature and certain salt concentrations therefore it might be applied in oil mineralization after marine catastrophes. In addition to oil industrial emissions, unctuous materials of food industry could cause dramatic harm by blocking pipe systems and treatment of wastewaters. In spite of the importance of the problem, its handling is still not solved. There are several approaches for removal of unctuous wastes from wastewaters, but these methods must be still improved. According to the beneficial properties of our strain in diesel oil degradation, it might be successfully used in the cleanup of food industrial wastewaters. In this study, our aim was to demonstrate the applicability of this strain in bioremediation of food industrial and municipal wastes. Lard, pig and poultry fat and cooking oil were used as sole carbon sources in minimal medium. The substrate utilization was demonstrated by measuring substrate level in the medium, the respiration activity and CO_2 production of the *Rhodococcus* sp. The strain consumed the available oxygen and released remarkable amount of carbon dioxide within a week, which means the bacterium can oxidize these materials. In addition, measurements of substrate concentration coincide with these data. Consequently, this strain is a promising waste cleaner in both oil and food industrial as well as housekeeping applications.

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SWITCHING ON RNA SILENCING SUPPRESSOR ACTIVITY BY RESTORING ARGONAUTE BINDING TO A VIRAL PROTEIN

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RNA silencing is a sequence specific cellular process that leads to RNA degradation, inhibition of translation of mRNAs or heterochromatin formation. RNA silencing has several functions one of the most important is to counteract viruses and transposons. Viruses evolved silencing suppressors to inhibit RNA silencing. Cellular proteins possessing WG/GW domains are known to bind Argonaute (AGO) via their tryptophan (W) residues. The P1 protein of the Sweet potato mild mottle virus (SPMMV) is a silencing suppressor that is able to counteract with active RNA induced silencing complex (RISC) by binding AGO . We found that Sweet potato feathery mottle virus (SPFMV) P1, the close homologue of the SPMMV P1, did not have any silencing suppressor activity. Remodeling

the Argonaute (AGO) binding domain of SPFMV P1 by introducing two additional WG/GW motifs converted it to a silencing suppressor with AGO binding capacity. To our knowledge, this is the first instance of transforming a viral protein of unknown function to a functional silencing suppressor.

COMPARATIVE STUDY OF VARIOUS XYLANASE ENZYME PREPARATIONS ON THE DIGESTIBILITY OF DDGS

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Dried distillers grains and solubles(DDGS), a byproduct of bioethanol production, has a relatively high fibre content, which limits its usability in animal feeding, usually it is fed only to ruminants. Increasing DDGS production has entailed a rising demand for a technology that makes it digestible for monogastric animals, primarily poultry. DDGS is a potential substrate for microorganisms, microbial conversion is thus an opportunity to enhance digestibility. *Thermomyces lanuginosus* is a thermophilic filamentous fungus that produces high levels of ß-xylanase in submerged and solid state fermentation. Our aim was to produce xylanase on DDGS substrate with *T. lanuginosus* in solid state fermentation and to compare the efficiency of commercially available and *T. lanuginosus*-produced xylanase on the digestibility of DDGS. *T. lanuginosus* was cultivated on solid media containing DDGS in different ratios (20m/m%, 30m/m% and 40m/m%) for a week. Xylanase activity of the samples was measured on Day7. The highest xylanase activity was measured at 20% DDGS content (3300 FXU/g). The efficiency of various enzymes was investigated in digestion model experiments. Commercial xylanase produced by *T. lanuginosus* (Sigma), an industrial xylanase, and the xylanase produced at different DDGS ratios were investigated. Tests were carried out in shaking flasks in which 30g DDGS was mixed in 200 ml distilled water and the pH was adjusted to 7. The enzymes were added to the suspensions in different ratio (0,5%, 1% and 1,5%, respectively). Test flasks were incubated at 37˚C for 24h. After the incubation period samples were centrifuged and the dry matter content of the supernatants were measured. Increase in dry matter content correlates with the effectiveness of the enzyme. Our results show that xylanase produced in the medium containing 20% DDGS could enhance the digestibility to the highest extent. Depending on the mixing ratio the dry matter content of the supernatant increased by 13-23% compared to the control. These values exceed the effectiveness of the commercially available and industrial enzymes (11-13%). Results indicate that highest xylanase activity was measured at 20% DDGS content of the medium. This enzyme preparation effectively increases the digestibility of DDGS.

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THE USE OF NEW MEDIA TO CULTIVATE OLIGOTROPHIC BACTERIA FROM AN ULTRA PURE WATER

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The highly oligotrophic waters provide an extreme environment for most of the microorganisms. Therefore cultivation of bacteria from an ultra pure water needs special media and conditions. Recently the R2A is the most common medium for cultivation of the oligotrophic bacteria from these habitats. In the present study the bacterial communities of the soft desalinated water tank of a

Hungarian power plant was investigated using special media, containing different vitamins, salts and also the extracts/lysates of previously isolated bacteria. 139 bacterial strains were isolated from 5 different new media on a random manner. Bacterial strains were grouped and the group representative strains were subjected to 16S rRNA gene sequencing. As we compared the taxonomical list of the newly isolated bacteria with those which were isolated previously from R2A medium (from the same environment) some overlappings (especially in case of Proteobacteria) could be observed though their diversity was higher from the new media. The cultivation resulted higher ratio of Gram positive strains than detected before (genera *Mycobacterium, Microbacterium, Leifsonia, Paenibacillus*). Among our isolates many taxonomically new bacteria could be found.

PREVALENCE OF SHREW-BORNE HANTAVIRUSES AMONG INSECTIVORES CAPTURED IN THE TRANSDANUBIAN REGION OF HUNGARY

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Hantaviruses belong to the family Bunyaviridae, that are tri-segmented negative-sense single stranded RNA viruses. They are carried by mice and voles, and cause a human disease called hemorrhagic fever with renal syndrome in Asia and Europe but several studies have proven that hantaviruses may also be carried by shrews. Shrew-borne hantaviruses were detected in several european countries such as Switzerland, Germany and Hungary as well. To get a clearer picture on potential new hosts for hantvairuses, we decided to detect and characterizate hantaviruses among all Hungarian shrew species. Animals were collected from four different locations, near to Gyékényes, Sármellék, Matty villages and Pécs. Total viral RNA was extracted by TRIzol method from lung tissues, then viral nucleic acid detection was carried out by OneStep RT- PCR using type-common L segment specific primers. A total of 313 individuals among the 6 following shrew species were investigated: *Sorex araneus, Sorex minutus, Neomys anomalus, Neomys fodiens, Crocidura leucodon, Crocidura suaveolens*. Out of the 313 shrews tested, 38 *Sorex araneus*, 1 *Sorex minutus*, 2 *Neomys fodiens*, 2 *Neomys anomalus* were positive for hantavirus infection.
Results indicate that Seewis virus carried by *Sorex* species is the most abundant, however, we were able to detect hantavirus also in *Neomys* species. Future genetic studies are necessary to characterize shrew-borne hantaviruses circulating in the country.

CONSERVED TRANSCRIPTIONAL REGULATORS IN THE *SCHIZOSACCHAROMYCES* GROUP

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The *Schizosaccharomyces* group consists of four species: *Sch. pombe, Sch. japonicus, Sch. octosporus*, and *Sch.cryophilus*. Their cells are eukaryotic and haploid, which divide by medial fission. Their genomes have been sequenced (http://www.broadinstitute.org) and the data enable us to compare their genes, proteins and cellular processes. As the fission yeast cells grow fast, it makes

these microorganisms very suitable for breeding. These features give attractive possibilities of combining the bioinformatic methods with experimental research. Our research group is interested in studying of cell division, with special emphasis on cytokinesis and its transcriptional regulation. Therefore, our aim was to gain information about the sequence- and functional homology of transcriptional regulators of the cell separation. After bioinformatic analysis of the proteins, we have found two proteins, which showed high degree of homology. Rsv1: Identities=99/167 (59%), Positives=120/167 (71%). Med31: Score = 214.927 (546), Expect=0.0 Identities=98/108 (90%), Positives=104/108 (96%). In the med31 protein we have found 37 of 139a.acids, which are constant from yeasts to human. The interspecific complementation of these genes has been carried out. The cloned genes of *Sch. octosporus* have been transformed into the mutant cells of *Sch. pombe*. The phenotypic features of the transformants revealed functional homology between the genes.

GENETIC DIVERSITY OF *MONILINIA LAXA* POPULATIONS FROM THREE GEOGRAPHIC AREAS OF HUNGARY

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The objectives of this study were firstly, to determine the genetic diversity of *Monilinia laxa* isolates from three distinct areas of Hungary, using the PCR-based ISSR technique, secondly, to prepare genetic diversity groups based on the ISSR dendrograms, and finally, to study the fungicide sensitiviy of isolates in relation to ISSR groups. 55 random amplified polymorphic ISSR markers, of which 22 were polymorphic and 32 monomorphic, were used to assess the genetic diversity and to study the structure of *M. laxa* populations in Hungary. 27 isolates collected from several orchards (subpopulations) in three georgraphical regions, in various inoculum sources and in various hosts, were used. 10 fungicides and 12 isolates selected from genetic diversity groups based on the ISSR dendrograms were used to determine the fungicide sensitiviy of isolates in relation to ISSR groups. The analysis of population structure revealed that genetic diversity within locations, inoculum sources, host and fungicides sensitivity (HS) accounted for 99% of the total genetic diversity (HT), while genetic diversity among locations, inoculum sources, host and fungicides sensitivity represented only 1%. The relative magnitude of gene differentiation between subpopulations (GST) and the estimate of the number of migrants per generation (Nm) averaged 0.005-0.009 and 53.9-99.2 respectively. The results obtained in dendrograms were in accordance with the gene diversity analysis. Grouping of isolates in the dendrogram was irrespective of whether they came from the same or different geographical locations. There was no relationship between clustering among isolates from inoculum sources, hosts and fungicide sensitivity. Obtained results in genetic diversity of *M. laxa* populations are discussed together with implications for the management of brown rot.

NEXT GENERATION SEQUENCING-BASED METAGENOMIC ASSESSMENT OF COMPLEX MICROBIAL COMMUNITIES

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The world around us is largely inhabited and maintained by a huge crowd of unseen microorganisms. Until the advent of genomics tools we were unaware of just how prevalent and important these organisms were to life. Though much work has been done in trying to understand this microbial world, the surface has only been scratched. Appearance of Next-Generation Sequencing technologies speeded up this process. The potential applications are as numerous as the samples to be analyzed. Life Tech's SOLiD/Ion Torrent platform is an excellent choice for resequencing projects and for digital gene expression analyses (whole transcriptome analyses (RNA-Seq), SAGE applications). The platform is suitable for metagenomics projects as well (analyses of complex environmental, food and special samples), dynamic changes, metabolic activities of microbial populations can be monitored. Our experiences in metagenomic analyses of various complex microbial communities (biogas plants, symbiotic relationships, special environments) will be discussed. The aims of these high-throughput studies were the assessment of taxonomic distribution and relevant metabolic pathways of the microbial communities. The resulting information might be exploited for efficient and safe biogas production, for the discovery of novel, so far unknown enzymes with potentials for future applications in energy generation, biodegradation, bioremediation processes.

MICROBIOLOGICAL SURVEY OF A TRICHLOROETHENE CONTAMINATED SITE AND ANALYSIS OF ITS IN-SITU REMEDIATION

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Chlorinated ethenes, such as trichloroethylene (TCE), are highly dangerous environmental pollutants and are among the most common groundwater contaminants worldwide. Careless handling resulted in large accumulation of halogenated compounds in the environment. Stimulation of dechlorinating microorganisms is potentially the most promising and cost-effective technology for remediating contaminated sites. The aim of this study was to evaluate the efficiency of the in-situ remediation process taking place in a TCE contaminated site, located in Hungary and if it was required, to determine a new and more effective biological remediation method. Microcosms were set up to assess the efficacy of the electron donor injected into the wells during in-situ biostimulation. The dynamics of biodegradation was monitored by using gas chromatography. In the course of the survey, groundwater samples were collected from monitoring wells with high and low TCE concentrations. A complex chemical and molecular biological approach was applied, including catabolic gene tests, to assess the existing dechlorination potential, and to determine the presence and activity of halorespiring bacteria. It was stated from the results of the microcosm experiments that the substrate applied in-situ was not susceptible to enhance the degradation of TCE, since no daughter products could be observed in the headspace samples. Dehalococcoides spp., a dehalogenating bacteria capable to perform the complete degradation of TCE to ethene, and its reductive dehalogenase genes (*bvc*A and *vcr*A genes encoding VC reductase enzymes) were detected in some of the groundwater samples with molecular biological techniques. However, PCR assays targeting genus *Dehalobacter* spp. and *Desulfitobacterium* spp. resulted in no detectable amplicons. These bacteria are capable of partial degradation of perchloroethene (PCE) and TCE to cis-dichloroethene (c-DCE). The chemical analyses indicated that microbial processes (e.g.

methanogenesis, sulphate reduction) could take place on the field. These processes occur in a redox potential range which is suitable for dehalogenation. The complex monitoring showed that the geochemical conditions were sufficient for a complete dehalogenation and that the key dehalogenating bacteria were present in the contaminated field. However a new, more efficient substrate amendment is suggested to enhance the biodegradation of TCE. To determine the adequate electron donor further microcosm experiments are needed.

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INVESTIGATION OF THE GENETIC BACKGROUND OF AMINOGLYCOSIDE RESISTANCE AND THE DISTRIBUTION OF VIRULENCE GENES AMONG *PSEUDOMONAS* ISOLATES

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Aim of this study was to investigate the genetic background of aminoglycoside resistance as well as distribution of virulence genes in non-outbreak Pseudomonas aeruginosa isolates collected in the Pulmonology Intensive Care Unit, University of Debrecen between 2008-2010. Altogether 125 isolates (111 from the ICU, 14 from other wards of the clinic) were tested for aminoglycoside modifying enzyme genes *aac*(6')-Ib, *aac*(3')-IIa, *ant*(2")-Ia, as well as 16S rRNA methylase encoding genes *arm*A, *rmt*A and *rmt*B by PCR. Occurrence of resistance integrons were studied by integrase-specific PCR, variable regions of integrons were sequenced and the gene cassettes were identified. Pulsed-field gel electrophoresis was used to assess genetic relatedness. In addition, presence of virulence-associated genomic regions pyoverdin receptor genes (FpvA-I to -III), phenazine production operones (PhzI, PhzII), the gene for alkaline protease (*apr*) and for the type IV fimbrial precursor protein (*pil*A) were also examined. The genes *aac*(3')-IIa, *arm*A, *rmt*B and *rmt*C were never detected, 52.1% (65/125) and 17.9 % (22/125) of isolates carried the *aac*(6')-Ib and *ant*(2")-Ia gene, respectively. Based on PFGE three major clusters of genetically related isolates were distinguished (A-C). Two of the nine isolates of Cluster A carried type I integrons, but these integrons contained no gene cassettes (In0). Virulence associated regions were *Fpv*A-I, *phz*I, *phz*II, *pil*A gene was found in some but not all isolates. All 25 isolates in cluster B carried a type I integron with a single ant(2")-Ia gene cassette (In159), all virulence related regions sought for were found except *pil*A. In cluster C all 60 isolates carried a type I integron with *aac*(6')-Ib, *bla*P1 and *ant*(3")-Ib cassettes identified in the variable region (In99), the virulence genes found were *Fpv*A-III, *phz*I, *phz*II and *apr*, while all isolates lacked *pil*A. A small cluster (D) with four isolates of two patients carried no integrons and a virulence phenotype similar to that of cluster B. Out of the 27 genetically independent isolates, one carried an empty (In0) and three isolates of one patient an *E. coli*-derived (In54) typed I integron, presence of virulence-associated regions was variable, in four of the isolates none of the studied virulence associated regions were found. Our results suggest that the presence of aminoglycoside resistance genes and/or integrons may have played a role in the successful endemic spread of *P. aeruginosa* strains.

MALDI-TOF MS FINGERPRINTING ALLOWS RAPID DISCRIMINATION OF PHYLOTYPES I, II AND III OF *PROPIONIBACTERIUM ACNES*

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P. acnes has been considered traditionally as a non-pathogenic member of the skin flora, however today it is accepted as an opportunistic pathogen associated with several diseases such as sever acne vulgaris, corneal ulceration, endophtalmitis, endocarditis, late-stage prostatic joint infection, and shunt-associated central nervous system infection. Beside investigating the different virulence factors, which may be responsible for the pathogenic processes involving *P. acnes*, it has also been shown that different clinical and normal flora isolates can form genetically different types (type I, II and III). The aim of the present study was to evaluate the possibility to use MS-based typing for this important anaerobic species after routine identification by MALDI-TOF MS Bruker Biotyper (version 3.0). *P. acnes* strains obtained from patients with sever acne, blood, joint infection or skin, were identified by conventional methods and by MALDI-TOF MS. Our approach was the same applied earlier for differentiation of *B. fragilis* isolates belonging to division I and division II. After obtained the mass spectra of *P. acnes* strains, the type of which were determined earlier as type I, II and III, mass spectrum sets were imported in the ClinProTools 2.2 software. Spectra were normalized and recalibrated using the respective functionalities of the software. Type characteristic peaks and peak shifts were searched for by careful visual investigation. In addition, peak variations between the different types of *P. acnes* were investigated by using our FlexAnalysis 3.3 software. Out of the main spectra (MSP) of strains with known types, differentiating library was created for *P. acnes* type I (IA and IB), II and III and this library was challenged with spectra of further 48 *P. acnes* isolates with different origins. Enhanced MLST (eMLST) analysis of the strains was also carried out independently. By careful analyses of the mass spectra of 11 strains with known types of *P. acnes*, specific peaks and peak shifts were found in the range of 6800 and 7400 Da, which could be selected as characteristic peaks for type I, II and III. Even differentiation of types IA and IB was possible with a characteristic peak with 7034 Da present in type 1A P. acnes and missing in type IB strain. Using this approach all 48 clinical P. acnes isolates could be typed after identification by MALDI-TOF MS. eMLST results were compared with the MS-based typing results. Since the introduction of MALDI-TOF MS for microbiological application, it has evolved from an experimental tool to a technology with significant benefit for routine microbiological laboratories first of all for the identification of bacteria and fungi. However careful evaluation of the MSPs of well known bacteria may give a possibility to use this technique for typing Propionibacteria.

WHAT IS THE ROLE OF HMG-COA REDUCTASE GENES IN THE CAROTENE PRODUCER *MUCOR CIRCINELLOIDES*?

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3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is an endoplasmatic reticulum associated protein. It catalyses the central step of the biosynthesis pathway of isporenoids, such sterols and carotenoids. This step is the conversion of HMG-CoA to mevalonate. Based on earlier studies, it is suggested that HMG-CoA reductase may also play role in the adaptation to the changes

of the environmental conditions, such as oxygen concentration [1] and osmotic stress [2]. The genome of the carotenoid producing fungus *Mucor circinelloides* contains three *hmg*R genes (*hmg*R1, *hmg*R2 and *hmg*R3). These genes were cloned and used in PEG-mediated protoplast transformation experiments. All types of transformants harbouring the different hmgR genes in extra copies showed enhanced carotene production and decreased sensitivity to statins, but in different extent. In these studies, increased copy number of *hmg*R3 affected the carotenoid production and the sensitivity to statins in the highest degree. Transcription of the three genes was also studied under different cultivation conditions (such as different carbon sources and aerob/anaerob growth) using the quantitative real-time PCR method. In these studies, *hmg*R1 showed a constitutively low, while *hmg*R2 showed constitutively high transcription level during the cultivation period. However, under anaerob condition, transcription level of *hmg*R2 decreased significantly, while that of *hmg*R3 increased. These studies suggested that *hmg*R2 may play role in the general metabolism, while *hmg*R3 may have a role in the sensing of the oxygen concentration. Silencing constructs were also built using the antisense mRNAs of the three genes (pAS1 from *hmg*R1, pAS2 from *hmg*R2 and pAS3 from *hmg*R3). We transferred these vectors into *M. circinelloides* by the PEG-mediated protoplast transformation method. All transformants, which contained the pAS3, showed a reduced growing rate and had an altered morphology with swollen, frequently branching hyphae. We suggest that hmgR3 is needed to the morphogenesis. Analysis of ergosterol content in the wild type and the transformants strains have also been started under different cultivation conditions by HPLC.

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HETEROLOGOUS EXPRESSION AND ANALYSIS OF *HOX*I FUNCTION IN *THIOCAPSA ROSEOPERSICINA*

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The photosynthetic purple sulphur bacterium, *Thiocapsa roseopersicina* harbours four functional [NiFe] hydrogenases. Two of them are attached to the periplasmic membrane (Hyn, Hup) and the other two are apparently localized in the cytoplasm (Hox1, Hox2). It prefers to utilize reduced sulphur compounds for anaerobic photochemolithoautotrophic growth, but simple organic substrates such as glucose and acetate can also be used as carbon, energy and electron sources. There is a facultative lithoautrophic proteobacterium, *Ralstonia eutropha* which has a soluble hydrogenase gene, *hox*I. In *R. eutropha* the *hox*I gene has an established role in the photosynthetic electron transport. Various constructs harbouring the *hox*I gene were created and transferred into different *T. roseoperscina* hydrogenase mutant strains. The expression of hoxI gene was verified in a mutant strain defective in both Hyn and Hup hydrogenases (GB1121) possessing only Hox1 and Hox2 enzymes. Functional studies aimed the investigation of the role of hoxI in the modulation of Hox1 enzyme. Therefore, experiments were carried out under conditions when only Hox1 hydrogenase was functional (Hox2 is silent in glucose-free medium).
The strain expressing the *hox*I gene was shown to evolve significantly higher amount of hydrogen in vivo than the negative control (empty plasmid in the same GB1121 strain). Protein interaction studies were carried out using Strep-tagged HoxI. This way C-terminally tagged HoxI and interacting proteins could be visualized either by Western hybridization or by gel staining. Two interacting proteins were identified beside HoxI, Adenylylsulfate reductase alpha subunit (AprA)

and Sulfate adenylyl transferase (Sat), which are shown to have roles in sulphur metabolism in other bacteria. It is hypothesized that the HoxI protein might modulate the Hox1 function by binding to these enzymes and by interfering the sulphur metabolism in *T. roseopersicina*.

ROLE OF *FVM*K2, THE CELL WALL INTEGRITY *MAP*K GENE IN CASPOFUNGIN TOLERANCE IN *FUSARIUM VERTICILLIOIDES*

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According to previous results of this research group, the HOG (High Osmolarity Glycerol) MAPK pathway, besides having a role in maintaining the homeostasis of the cell and attenuating the harmful effects of stress factors, regulates other events, as well including programmed cell death, secondary metabolite production and fludioxonil tolerance. To improve knowledge on the functions of signal transduction pathways in fungicide tolerance, another MAPK gene (*Fvm*k2), the CWI (Cell Wall Integrity) MAPK gene has been cloned from *F. verticillioides*, *ΔFvm*k2 null mutants were generated by targeted gene disruption and complemented strains were produced by introducing the functional wild type *Fvm*k2 gene. The *ΔFvm*k2 mutants had reduced growth rate as compared to the wild type parental strain, hydrophobicity of their surface structures became diminished and they showed increased sensitivity to chemicals interfering with cell wall biosynthesis (calcofluor white, CFW – 10-15 µg/ml, Congo Red – 5-10 µg/ml, SDS – 50-100 µg/ml). Contrary to the *ΔFph*og mutants, the *ΔFvm*k2 CWI MAPK mutants showed only negligible sensitivity to fludioxonil. Treatments with caspofungin caused an opposite effect: this chemical, added at 1-2.5 and 25 µg/ml concentration in serial conidial dilution assays and in liquid cultures, respectively reduced the growth of the Δ*fvm*k2 CWI MAPK mutants by 50-70%. The complemented strains showed sensitivity values similar to that of the wild type strain. The role of the CWI MAPK route in caspofungin tolerance was also supported by a 3.5-4-fold increase within 0.5 hour in transcription of the *Fvm*k2 MAPK gene measured in caspofungin treated wild type strains, CFW caused lower and delayed induction of *Fvm*k2. As the qrtPCR analyses were performed by using primers upstream of the insertion position of the hph-cassette (used in the gene disruption construct), expression levels of the *Fvm*k2 gene could be measured also in the *ΔFvm*k2 mutants, in these strains reduced expression was observed even in the absence of treatment, while after caspofungin treatment the induction of *Fvm*k2 was totally prevented. This finding indicates that the functional MAPK protein contributes to its own regulation, either under stressed conditions or in the absence of stress. Results of further qrtPCR assays showed that the chitin synthase gene, *Chs*1 was activated by an *Fvm*k2 MAPK-dependent manner, while in the case of *Chs*6, another chitin synthase gene no similar effect was observed. On the other hand, CFW treatment increased the *Fvm*k2 MAPK-dependent expression of *Chs*6. These results demonstrate that, besides the HOG MAPK route the CWI MAPK pathway also plays an important role in fungicide tolerance.
Caspofungin affected the MAPK-dependent expression of *Chs*1, whereas this fungicide had no effect on the other chitin synthase genes, *Chs*2, *Chs*6.

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CHARACTERIZATION OF NITRIFYING BACTERIAL COMMUNITY STRUCTURE IN FIVE DIFFERENT SMALL DRINKING WATER NETWORKS

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Protecting drinking water is essential for our health. Accepted limits for drinking water compounds - including inorganic nitrogen-forms (ammonium, nitrite and nitrate) - were tightened recently. Eliminating nitrite and nitrate from raw water is important because their presence in drinking water could cause public health problems. In addition, nitrite and nitrate could be generated from ammonia by nitrification process in drinking water network. Aim of the present study was to detect, identify and quantify microorganisms involved in nitrification and denitrification processes in five different small water networks. The organism was enumerated by most probable number (MPN) analysis. To amplify the gene of ammonia-monooxigenase (*amo*-A and *arch-amo*-A), *Nitrobacter* spp. and *Nitrospira* spp. specific fragments of 16S rDNA gene, and genes of the enzymes taking part in denitrification process (*nir*S, *nir*K) Polimerase Chain Reaction (PCR) was applied. The community structures of nitrifying organisms were mapped with Terminal Restriction Fragment Length Polymorphism (T-RFLP), the ammonia- and nitrite- oxidizing organisms were identified by clone library based amo-A gene and 16S rDNA respectively. The nitrifying plate count number was low but it was increased in line with increasing nitrite and nitrate concentrations in consecutive points of the network. The ammonia-oxidizing bacteria (AOB) were detected only a few sampling points – especially in reservior/watertower and consumer endpoints - its plate count was lower than nitrite-oxidizing bacteria (NOB) which was detected all of the sampling points. Each network had different pattern of the nitrifying bacteria community structure, wells had more similarity to each other than other points of networks. Nitrite-oxidizing bacteria – dominated by *Nitrobacter vulgaris* and *Nitrospira moscoviensis* - were identified in all network points, the difference between the community structure of the wells and the endpoints was determined by *Nitrospira moscoviensis*. Candidatus *Nitrospira defluvii* and *Nitrospira* sp. were also detected in some samples. Ammonia-oxidizing bacteria belonging to Nitrosomonadaceae and Nitrosopumilaceae were mainly detected in consumer endpoints. Denitrifying bacteria were identified from all sampling points but there were significant differences in the plate count in respect of networks. Total inorganic nitrogen was decreased by denitrification process in two drinking water networks. Nitrification was observed in all of the examined drinking water networks, while in some networks denitrification as well.

STRUCTURAL INVESTIGATION OF TRICHOBRACHINS BY MOLECULAR DYNAMICS METHODS

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Trichobrachins, as a microheterogeneous mixture of fungal peptides, belong to the peptaibol family, and among them, several molecules are the representative members of short-sequence peptaibols. In an earlier study, eight different trichobrachins, composed of 11 amino acids, were isolated from the marine strain of Trichoderma longibrachiatum, which can be differentiated in two groups according to the characteristics of their sequences. The first group (i.e. the trichobrachin A series) includes peptides with a sequence pattern of Ac-Aib-Asn-xxx-xxx-Aib-Pro-xxx-xxx-Aib-Pro-xxol, while the

second group (i.e. the trichobrachin B series) comprises peptides characterized by a sequence pattern of Ac-Aib-Asn-xxx-xxx-Aib-Pro-xxx-Aib-Val-Pro-xxol. Considering all the members of both groups mentioned above, to the best of our knowledge, any data derived either from experimental or from theoretical investigations, concerning the three-dimensional structure of these peptaibols, have not been published in the literature, so far. Therefore, in this theoretical study, a comprehensive structural characterization was carried out by means of different molecular dynamics (MD) methods, including simulated annealing (SA) and replica exchange molecular dynamics (REMD) calculations, for the eight, afore-mentioned trichobrachins. In the course of this structural investigation, the typical conformational patterns with regard to the turn and helical structures, as well as to the intramolecular H-bonds were examined. For the conformational states supplied by both types of MD simulations, the presence of various secondary structural elements (i.e. beta-turn and helical structures) was studied along the entire sequence of peptides. Nevertheless, the occurrence of different intramolecular H-bonds was investigated, which could contribute to the structural stability of a variety of the conformations of trichobrachins. Based on the conformational states obtained by the SA and REMD calculations, cluster analyses were performed, in order to determine the conformationally related subfamilies, as well as to identify their representative structures. The characteristic conformational features of the representatives of different clusters were examined in detail, especially regarding their secondary structural elements and intramolecular H-bonding patterns. As it was mentioned previously, this theoretical study is the first structural investigation carried out on the eight trichobrachins, which provided a detailed description of the three-dimensional structure, as well as of the various conformational features of these peptaibols.

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EFFECTS OF THE CIS-TRANS ISOMERISM ON THE MICELLE-BOUND CONFORMATIONS OF INDOLICIDIN

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Indolicidin is an antimicrobial peptide (AMP) isolated from the cytoplasmic granules of bovine neutrophils, which possesses a broad spectrum of antibacterial and antifungal activities, furthermore, it also exhibits hemolytic and antiviral effects. This AMP is known to be highly membrane-active, however, its exact mechanism of action is still obscure. Taking into account its primary structure (i.e. H-ILPWKWPWWPWRR-NH2), it can be seen that this peptide is characterized by a high content of aromatic (i.e. Trp) and basic (i.e. Lys and Arg) amino acids. Beside the two types of residues mentioned above, this AMP contains three Pro amino acids, and in accordance with the cis-trans isomerism about three Xaa-Pro peptide bonds, eight distinct stereoisomeric forms could be distinguished for this peptide. In the case of each stereoisomer, molecular dynamics (MD) calculations were carried out on different peptide-micelle systems, in order to study the effects of cis-trans isomerism on the micelle-bound conformations of indolicidin. After an initial energy-minimization, for all peptide-micelle systems, a 20-ns-long MD simulation was performed with fixed geometry of solute, which was followed by a 120-ns-long MD calculation without restraints. Based on the MD trajectories, the influence of cis-trans isomerism on the inserted conformations of indolicidin could be characterized by various structural features, such as, insertion depth, secondary structural elements, as well as intra- and intermolecular interactions. To describe the insertion depth regarding the stereoisomeric forms, distances were calculated between

the centers of mass, which were defined based on the heavy atoms of stereoisomers, as well as of micelles. In the case of the inserted conformations of stereoisomeric peptides, the presence of beta-turn structures was investigated, and for the side-chains of amino acids, the preferred rotamer states were identified. Since the structure of the micelle-bound conformations of stereoisomers could be stabilized by various intramolecular interactions (i.e. aromatic-aromatic, proline-aromatic and cation-pi interactions), the appearance of these interplays evolved between the certain groups of peptides was studied. Additionally, to characterize the interactions of stereoisomeric forms with micelles, the occurrence of intermolecular interplays formed between the peptides and lipid molecules were examined. The MD simulations performed on peptide-micelle systems pointed out that the micelle-bound conformations of the stereoisomers of indolicidin differed substantially from one another. It could be deduced that the appearance of structural features proved to be dependent not only on the isomerization state of Xaa-Pro peptide bonds, but also on the type of micelle.

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CHARACTERIZING THE FOLDING FEATURES OF THE PALINDROMIC SEQUENCES OF ANTIMICROBIAL PEPTIDES

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For several antimicrobial peptides (AMPs), a characteristic feature could be observed concerning their primary structures, namely, they contain palindromic sequences with different lengths. These palindromes can play a relevant role in determining the three-dimensional structure of AMPs, as well as in the biological effects of peptides. Among the AMPs possessing palindromic segments, some molecules are found to be rich in apolar residues, i.e., the decoralin and few temporin peptides. Decoralin (i.e. H-SLLSLIRKLIT-OH) was isolated from the venom of the solitary eumenine wasp Oreumenes decoratus, whereas four members of the temporin peptide family were identified in the skin of frogs belonging to the genus Rana, which were as follows: the temporin C (i.e. H-LLPILGNLLNGLL-NH2), the temporin 1Pra (i.e. H-ILPILGNLLNGLL-NH2), the temporin 1TSb (i.e. H-FLPLLGNLLNGLL-NH2) and the temporin 1DYa (i.e. H-FIGPIISALASLFG-NH2). In the present study, molecular dynamics (MD) simulations were carried out for all palindromes mentioned above, in order to characterize their dynamic behavior, as well as to explore their folding features. In the case of each palindromic peptide, starting from the same geometrically optimized conformation, 100 individual 40-ns MD calculations were performed applying random initial velocities for every single simulation. On the basis of MD trajectories, the alterations of various structural properties were investigated as a function of simulation time, and it was characterized how these conformational features change during the calculations. For the palindromic sequences, the evolution of helical structures with regard to the whole conformation, as well as to each amino acid residue was studied as a function of time. Since typical intramolecular H-bonds (i.e. so-called local H-bonds), evolved between the backbone NH donor and CO acceptor groups, play an important role in the structural stabilization of helical structures, the occurrence of these interactions was examined along the entire sequence of palindromes. Furthermore, the appearance of the other types of intramolecular H-bonds (i.e. so-called non-local H-bonds) was investigated, which could be formed either between the backbone NH and CO groups, or between the NH and CO groups of both backbone and side-chain. Similarly to the case of helical structures, the evolution of local and non-local H-bonds mentioned above was also studied as a function of simulation time. Based on the

multiple MD trajectories, the dynamic behavior and folding features were characterized for the palindromic sequences of decoralin and temporin peptides. On the whole, our theoretical study led to several observations concerning the helix and H-bond formations of the palindromes of AMPs rich in apolar residues.

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STUDYING THE INTERACTIONS OF THE STEREOISOMERS OF TRITRPTICIN WITH MICELLES

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Tritrpticin is a cationic tridecapeptide identified in the porcine bone marrow, which belongs to the family of Trp- and Arg-rich antimicrobial peptides (AMPs). Similarly to the other member (i.e. indolicidin) of this family of AMPs, tritrpticin also shows a wide spectrum of antimicrobial activity against Gram(+) and Gram(-) bacteria, as well as fungi, nevertheless, it possesses hemolytic effect too. Although, this peptide is also well-known as a membrane-active molecule, its mode of action is not clearly understood. As for the indolicidin, the primary structure of tritrpticin (i.e. H-VRRFPWWWPFLRR-OH) is found to be remarkable too, consisting of five aromatic (i.e. Trp and Phe) and four basic (i.e. Arg) amino acids, as well as of two Pro and two apolar (i.e. Val and Leu) residues. Since tritrpticin contains two Pro amino acids, this AMP exists as an equilibrium mixture of four different stereoisomers, according to the cis-trans isomerism about two Xaa-Pro peptide bonds. In order to study the interactions of the stereoisomeric forms of tritrpticin with micelles, molecular dynamics (MD) simulations were performed on various peptide-micelle systems, using both DPC and SDS micelles. For each system mentioned above, first a geometry optimization was carried out, then an MD calculation with fixed geometry of solute for 20 ns, and finally a further MD simulation without restraints were performed for 120 ns. The interactions of the stereoisomers of tritrpticin with micelles were described applying equilibrium properties, i.e., insertion depth, secondary structures, intramolecular interplays and intermolecular interactions. To characterize the insertion processes of stereoisomeric forms, the alterations of distances measured between the centers of mass, defined on the heavy atoms of peptides and micelles, were examined as a function of time. For the micelle-bound conformations of stereoisomers, the beta-turn structures appeared in certain tetrapeptide units were identified, and furthermore, the preferred rotamer states of the side-chains of amino acids were determined. Nevertheless, the occurrence of intramolecular interactions (i.e. aromatic-aromatic, proline-aromatic and cation-pi interplays) formed between the different groups of peptides was investigated, as well as the presence of intermolecular interactions evolved between the peptides and lipids were studied. On the basis of results derived from the MD simulations, it could be concluded that differences could be detected between the stereoisomers of tritrpticin, with regard to their insertion processes, as well as to their micelle-bound conformations. Thus, the four distinct stereoisomeric forms showed typical structural features, and additionally, differences could be also observed concerning two types of micelles.

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ECTOMYCORRHIZAL FUNGI OF *SALIX ROSMARINIFOLIA* ON A SEMIARID SANDY FOREST STEPPE

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The mycorrhizal associations between fungi and vascular plants play important role in ecosystem functioning. Among others, they can increase the drought stress resilience of vascular plants, therefore, these interactions have great importance in arid and semi-arid ecosystems. One of the most important mycorrhizal types is the ectomycorrhiza (EM) that is generally formed by woody plants and fungi belonging to the phyla Ascomycota and Basidiomycota. The main aim of the work presented here was to study the diversity of EM fungi of *Salix rosmarinifolia*, a native woody plant of characteristic semiarid sandy grassland close to Fülöpháza (Hungary). Fungal diversity was screened by the study of EM root samples collected from *S. rosmarinifolia*, DNA extracted from soil samples of *Salix* patches and fruit bodies of EM fungi collected in the area. Sampling was carried in 2010 and 2011. Representatives of each EM morphotypes from each soil samples were fixed and subsequently studied by molecular methods. The ITS region of the nrDNA was amplified and sequenced from both sporocarps and EM samples. We extracted total DNA from soil samples and fungal ITS was amplified using tagged ITS primers and sequenced using 454 (Roche) technique. Sequences were analyzed and clustered into "molecular operational taxonomic units" (MOTU). Altogether, EM were collected from 44 soil samples of two *Salix* patches. We gained 276 and 55 ITS sequences from EM and fruit body samples, respectively. These data were completed with the results of the massively parallel sequencing from pools of 20-20 soil samples collected from two *S. rosmarinifolia* patches. Our results revealed a much higher species richness of EM fungi then thought according to the sporocarp data. Several EM species (e.g. *Tomentella* spp., *Tuber* sp.) with no record in the area were detected. Generalist and specialist fungal taxa were identified when EM community of *S. rosmarinifolia* was compared with fungal partners of other EM plants of the area.

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HHP TREATMENT IN EGG PROCESSING

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Numerous researches have been conducted today to develop procedures replacing conventional liquid egg pasteurization technologies (heat treatment at 60-65°C for 5-10 minutes). One such procedure includes treatment of liquid egg products at high hydrostatic pressure (HHP). Previous research has shown that HHP technology is suitable for destruction of numerous pathogenic micro-organisms in egg products. Use of HHP technology allows better preservation of native characteristics of food raw material with similar antimicrobial efficacy as heat treatment, and its beneficial effect has been demonstrated with many foods sensitive to heat treatment. An additional advantage includes that in HHP procedure liquid foods are treated in the packing material to avoid potential post-infection of the product. Furthermore, for pasteurization of bulk material (exceeding 1 kg) in contrast to heat treatment, no heat shock effect induced by low warming-up ate is expected in

case of HHP procedure since antimicrobial effect occurs momentarily at the same time at all points of food. In our tests we artificially infected the liquid whole egg samples with *Salmonella* Enteritidis, *Listeria monocytogenes*, and *Staphylococcus aureus* bacteria, and then treated the samples in „Food Lab900" high hydrostatic pressure instrument for 3 – 17 minutes at 200 – 400 MPa. Subsequently, the change of the viable cell count of the specific bacteria has been tested. In addition to the samples infected with various bacteria, non-infected samples were also treated in each test and the change in viable cell count of the samples upon the effect of the treatment. In summary, it can be concluded that in each test of our investigations the viable cell count of *S.* Enteritidis critical for egg products is reduced significantly while the reduction of the total viable cell count was around 2 magnitudes. Additionally, based on our results microbial destruction was significantly affected by the pressure level only.

INCIDENCE OF CONGENITAL AND PERINATAL CYTOMEGALOVIRUS INFECTIONS IN SOUTH HUNGARY (2007-2011)

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The human cytomegalovirus (CMV) is the leading cause of congenital infections worldwide. Congenital CMV causes sensorineural hearing loss in a significant proportion of infected newborns and it is associated with mental retardation, blindness or visual deficit. In 10% of all newborns' mortality the CMV is the causative factor. Congenital transmission of CMV can occur with maternal primary infection, reactivation, or reinfection during pregnancy. The consequences of perinatal CMV infection affect mainly the premature newborns and the leading symptom is the hearing loss. The aim of our study was to determine the incidence of congenital and perinatal CMV infections in the Southern region of Hungary. From 01 January 2007 until 31 December 2011 263 patients, aged from 1 days to 3 years were admitted to the Paediatrics Department, University of Szeged, who had such symptoms, which suggested congenital or perinatal CMV infection. Urine, blood or liquor specimens of the children were investigated. The level of the CMV-DNA was monitored by real-time quantitative PCR. The CMV-specific IgM and IgG antibodies were measured using ELISAs.
Eighteen patients were diagnosed with congenital and 4 patients with perinatal CMV infection. One newborn died in the consequence of severe thrombocytopenia, anaemia and icterus despite of the intensive care. Five children suffered permanent damage (sensorineural hearing loss, retina atrophy, hypoplasy of the opticus nerve, ventriculomegaly, microcephaly). Nine patients received antiviral therapy, 2 children needed blood transfusion because of the CMV infection. Taking the results into consideration it would be important in Hungary to enlighten the women in child-bearing age, especially pregnant women, how they can avoid the CMV infection. Screening pregnant women or newborns for CMV could help diagnose congenital CMV infection early in life. The progression of permanent damages could be reduced with passive immunization or with antiviral therapy.

MOLECULAR CHARACTERISATION OF THE HOST RESPONSE INDUCED BY *CANDIDA PARAPSILOSIS*

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Candida yeasts are well known members of the normal human flora. However under specific circumstances, if the host control is compromised, these yeasts are capable of turning themselves from commensalist into pathogenic forms causing local or lifethreating systemic infections in immunocompromised patients. *C. parapsilosis* is considered to be an emerging fungal pathogen being the third most commonly isolated species of the genus outnumbered by *C. albicans* and *C. glabrata* respectively. Having the highest incidence rates, the pathogenesis, virulence factors and the host-pathogen interactions of *C. glabrata* and especially *C. albicans* are well-studied, but little is known about the infections caused by *C. parapsilosis*. To reveal the details of the immune response given to *C. parapsilosis* and to identify virulence factors an in vitro model system was created using J774.2 murine macrophage-like cell lines *C. parpasilosis* wild-type (WT) and its lipase (LIP-) deficient strains. Light-, fluorescent- and scanning electron microscopic techniques were used to follow the progress of phagocytosis and intracellular elimination. J774.2 macrophages killed LIP- strain more effectively compared to the WT in killing assays. The RNA microarray and quantitative Real-Time PCR data revealed a robust Th1 T-helper response. These results also show, that the presence of the pathogen notably induces the expression of a cell surface molecule, called TNFRSF9, suggesting its importance in the interaction. Flow cytometry data confirmed a significantly higher level of the functional protein on the cell surface as well. The TNFSRF9 protein is known to be involved in the process of CD4+ T-cell costimulation during bacterial infections, however it has never been examined in correlation with *Candida* infections. To investigate if other members of this genus can induce this response, seven other *Candida* species (including *C. albicans* and *C. glabrata*) were included in our in vitro infection experiment. All examined strains were able to trigger the overexpression of TNFRSF9 but the members of the parapsilosis sensu lato group (*C. metapsilosis, C. orthopsilosis, C. parapsilosis*) caused the most intense host response. Although killing assays, cytokyne and TNFRSF9 expressions showed significant differences in the host responses given to the WT compared to the LIP- in vitro, to reveal the exact role of this molecule in the modulation of the immune response needs further examinations in vivo and in vitro as well.

SEROLOGIC EVIDENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS INFECTION IN HUNGARY

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Crimean-Congo hemorrhagic fever virus (CCHFV) is a typical tick-borne pathogen that causes an increasing number of severe infections in many parts of Africa, Asia, the Middle East, the Balkans as well as in some other parts of Europe. The virus is transmitted primarily by *Hyalomma* spp, and the spectrum of natural hosts for CCHFV is broad including wild and domestic animals. Although, the presence of CCHFV was hypothesized in Hungary, no significant research activity has been

carried out in the past thirty years. In the present study we have developed two validated antibody detection assays, an enzyme-linked immunosorbent assay (ELISA) and an immunofluorescence assay (IFA), both using recombinant CCHFV nucleoprotein (rNP) as antigen. A total of 198 serum samples collected from European brown hares (*Lepus europeus*) in the surrounding area of Dévaványa village between 2008 and 2009 were tested parallelly with ELISA and IFA. Out of the 198 samples 12 (6%) were positive for IgG antibody against CCHF virus by both methods. Four samples were positive with IFA and one serum was positive with ELISA only. 181 samples were negative, therefore, the homology was 71% between the two tests. Our findings nicely complement historic observations that endemic foci of CCHFV are present in the country. The antibody detection methods we developed will enable us to perform large-scale surveillance that should help us to gain a clearer picture of the epidemiology, ecology and public health risk of CCHFV infections.

SCREENING OF POLYUNSATURATED FATTY ACID PRODUCING *MORTIERELLA* STRAINS

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Polyunsaturated fatty acids (PUFAs) are elemental structural components of biological membranes, where they confer fluidity and flexibility, regulate the membrane permeability and modulate the behaviour of certain membrane-bound proteins. They are also precursors of a wide variety of metabolites regulating critical biological functions, such as prostaglandins, leukotrienes and hydroxy-fatty acids. Moreover, PUFAs have 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. Besides this, docosahexaenoic acid is beneficial to infants' brain and retinal development, whilst arachidonic acid prevents alloxan-induced diabetes mellitus and protects various tissues from oxidative stress. Other ω-3 and ω-6 PUFAs such as eicosapentaenoic acid, γ-linolenic acid and dihomo-γ-linolenic acid are also used as food additives and are targets of medical research. The increasing demand for biologically important PUFAs has led to a change from conventional oil origin to the search for alternative sources, so oleaginous microorganisms have been intensively studied. Among them *Mortierella* species belonging to Zygomycetes are particularly active in PUFA synthesis. *M. alpina* is one of the most important industrial species of PUFA production, its main product is arachidonic acid, but it is able to produce other ω-3 and ω-6 PUFAs in significant amounts. The lipid production and the background of PUFA biosynthesis in *M. alpina* is extensively examined, however the diversity of the lipid production within Mortierellales is very poorly explored. The aim of our work was to investigate the lipid profiles and PUFA production of different *Mortierella* species 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 a large number of *Mortierella*-related strains were screened and their lipid yield and lipid composition were investigated. The strains were incubated in submerged cultures, 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) derivatization reagent was used to convert the PUFAs into the corresponding fluorescence derivatives. Then after the derivatization the PUFAs were separated with HPLC. In our work, a number of species related to *M. alpina* also seemed to be promising PUFA producers, however significant differences were observed in the lipid composition of the strains. Accordingly, we are planning to use the fatty acid profiles together with morphological and molecular data in phylogenetic studies.

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PROTEIN SEQUENCES: A TREASURE TROVE FOR ALL

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Amino acid sequences deduced from genome projects provide a wealth of information about the structure and function of the encoded proteins. In my talk, I will give an overview of what kind of information could be extracted from the raw sequence data even by a bioinformatics novice. Methods of sequence alignments will be covered only briefly. I will put more emphasis on protein sequence analysis and structural bioinformatics that is the prediction of structural and functional properties of proteins. I will introduce ExPASy, a bioinformatics resource portal that provide access to a large number of databases and tools that are useful in mining the treasure trove of protein sequences. It is connected to UniProt, the most comprehensive, non-redundant and expertly-curated protein sequence database that also includes abundant functional information. The first question to raise about a novel protein sequence, whether it would fold into a well defined 3-dimensional structure under physiological conditions. Over the last decade, it became evident that intrinsically disordered proteins and protein segments, a feature that can be predicted from sequence, are highly abundant in nature and they carry out critically important biological functions in all domains of life. Other predictable structural properties include secondary structure elements, coiled-coils and transmembrane helix topologies, structural and functional domains and motifs, as well as posttranslational modification sites. Protein structures can be divided into compact domains and structural motifs that can be predicted and could tell us a lot about the function of the protein of our interest. Based on comparative analysis, the structural elements and the proteins themselves can be classified in various ways, such as folds, domains, protein families and superfamilies (see e.g. the SCOP database). Ab initio structure prediction is still in its infancy, therefore 3-dimensional structures can be reliably predicted only by homology modeling (e.g. by the program SwissModel or by asking the help of an expert). It relies on the identification of one or more experimentally derived protein structures likely to resemble the structure of the query sequence. The known structures can be found in the Protein Data Bank (PDB), the official repository of the 3-D structural data of large biological molecules. The atomic resolution structures can be visualized by one of several open resource molecular graphics programs, like PyMOL or Jmol (a Java-based interactive browser applet). Proteins do not act in isolation, but interact with various ligands and other macromolecules. Understanding the intricate network of protein-protein (and protein-nucleic acid) interactions is a big challenge in systems biology. Some of the sequences that mediate these interactions (e.g. short linear motifs in protein-protein interactions) and domains (e.g. DNA-binding domains) can also be predicted. Extracting of the plentiful information hidden in protein sequences is a required step towards a system biology approach in understanding biology including microbiology.

SEWAGE SLUDGE TOLERANCE AND LIGNOCELLULASE ACTIVITY OF THERMOPHILIC MICROFUNGI ISOLATED FROM SEWAGE SLUDGE CONTAINING COMPOST

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Composting can be reduced the environment harmful materials and crop residues too under controlled conditions. This natural waste breakdown in the final product of the resulting humus-like organic matter, good nutritional value warrants to the soil nutrient supply and improvement of target zones is recovered. 21 fungi were isolated from thermophilic period of composted sewage sludge and plant debris mixtures. The fungi identified by morphological and molecular techniques belonged to ten species, six thermophilic (*Aspergillus fumigatus, Aspergillus versicolor, Rhizomucor pusillus, Thermoascus aurantiacus, Talaromyces thermophilus, Thermomyces lanuginosus*) and four mesophilic (*Aspergillus oryzae, Aspergillus terreus, Neosartorya fischerii* and *Trichoderma hamatum*) starins. *T. lanuginosus* was the most frequently occurring species, represented by eight isolates respectively (57%). The allergens of *Aspergillus* strains to the exclusion from assay, remainder 14 thermophilic strains of sludge tolerance was examined in vitro. Thirteen isolates could tolerate even 10 % sludge concentration, furthermore the growth of seven strains was stimulated by extreme concentration of the sewage sludge. The high tolerance showing sludge microfungi, 57% belonged to the *T. lanuginosus* species. The lignocellulase degrading capability of these fungi was assessed by photometric determination of their lignin peroxidase, manganese-dependent peroxidase and laccase activities. Strains of *T. lanuginosus* showed the highest activity of lignin peroxidase and manganese-dependent peroxidase activities, almost all strains laccase activity one of *T. thermophilus* showed an outstanding activity of this lignin degrading enzyme. The present study demonstrated that compost inhabiting thermophilic fungi can maintain their highly efficient lignocelluloses degrading enzyme systems even under extreme environments. The composting technologies should be adjusted to support the colonization activity of these fungi or alternatively, bulk cultures of these fungi can be used to inoculate sewage sludge containing compost piles.

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DETECTING DOBRAVA-BELGRADE AND PUUMALA VIRUS INFECTIONS IN HUNGARIAN FORESTRY WORKERS BY ELISA AND WESTERN BLOT ANALYSES

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Hantavirus infections represent a constant healthcare threat also in Hungary. Since the virus is mainly transmitted by mice living in wooded areas, most likely exposed people are professional hunters and forestry workers. The aim of the study was to survey the prevalence of human hantavirus infections among the potentially exposed population, also covering the majority of the country area. Sera collected from several regions of Hungary were tested for the two abundant hantavirus species in Hungary, Dobrava-Belgrade (DOBV) and Puumala (PUUV) viruses. Anamnesis of voluntaries regarding symptoms susceptible for hantavirus infection was recorded. For serological analyses, full capsid proteins of DOBV and PUUV viruses were produced in a bacterial expression system, while Ni-resin was used for protein purification. A total of 659 samples were tested by mixed indirect ELISA. Microtiter plate wells were first coated with full capsid antigens of

both DOBV and PUUV, followed by testing positives with regular ELISA to differentiate serotypes. Samples were then forwarded for control by Western blot assay. Out of the 34 (5%) ELISA-positive samples, 32 could be confirmed also by Western blot analysis. Results show that PUUV infection is nearly twice as frequent as DOBV (17 and 9 cases, respectively). Based on this preliminary study, the south-western (Somogy) and north-eastern wooded areas (Bükk) are the most infected regions. The average age of infected persons is around 40 years, the majority of them has 15-30 years spent in field work. Thus hantavirus infection is a significant risk among professionals in forestry.

INTERACTION OF FIV WITH HETEROLOGOUS MICROBES IN THE FELINE AIDS MODEL

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Since the emergence of HIV, there has been a great deal of interest in identifying cofactors that accelerate progresion of AIDS. Infections frequently occur in HIV infected persons might augment HIV replication consequently facilitate AIDS progression. In vitro studies demonstrated that products of immediate early or early genes of several DNA viruses such as human herpesviruses 1,2,3,4,5,6A and 8, adenoviruses as well as hepatitis B virus X gene, human T lymphotropic virus type I tax gene transactivate HIV-1 and HIV-2 LTR sequences through mediation of nuclear transcriptional factors in the same cell. Heterologous viruses infect many types of cells which are not targets of HIV but relase several immunomodulatory mediators that also transactivate HIV in carrier cells. These confounding factors act in a pleiotropic manner which is impossible to study in vitro. Feline AIDS (FAIDS) induced by FIV is the best suitable natural small animal model to study human HIV/AIDS pathomechanism. Cats and species of Felidae host several viruses that are homologues of human counterparts. Human and cat have several common opportunistic infections as well. Recent surveys on simultaneous infections were carried out not only for epidemiological purposes, but for establishing their auxiliary role in disease development. Feline herpesvirus type1 through ICP4 gene produts decreases primary humoral immune reactions, lymphocyte proliferation, cellular immunity to other parasites, but increases FIV replication in animals. Feline isolate of human adenovirus type 1 detected in both human and their cats worldwide might pose a simultaneous risk to cat owners and their pets, especially if immunocompromised. Simultanaeous infection with feline leukemia virus (FeLV) accelerate and exacerbate feline AIDS, and promote onset and progression of unusual solid tumors. Cats and related species frequently suffer from feline calicivirus, canine distemper virus, Toxoplasma gondii, Leishmania species, Bartonella henselae, mycoplasmas and fungal pathogens. These both aggravate immunosuppression and cause opportunistic infection in a vitious circle. Further studies are warranted to better delineate their molecular pathomechanism in the course of FAIDS. These help establish new prognostic markers and therapeutic intervention in human AIDS.

EFFECTS OF HUMAN PAPILLOMAVIRUS 16 E6, E7 ONCOPROTEINS ON THE EXPRESSION OF GENES INVOLVED IN KERATINOCYTE DIFFERENTIATION

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The human papillomaviruses (HPVs) infect proliferating basal keratinocytes, while virus production occurs only in terminally differentiated layers of the epithelium. The expression of genes involved in keratinocyte differentiation is tightly regulated during keratinocyte differentiation. Oncogenic HPV infection has been shown to delay the normal differentiation program of keratinocytes. The E6 and E7 oncoproteins of high-risk HPVs are responsible for the transforming activity of the virus. The aim of the present study was to investigate the effects of HPV16 E6 and E7 oncogenes on the expression of some selected genes involved in keratinocyte differentiation. Primary human foreskin keratinocytes were maintained in serum free, low calcium medium and transduced by LXSN (control) retrovirus or virus vectors expressing HPV16 E6, E7 or E6/E7 genes. These cells were induced to differentiate by culturing in high calcium containing medium for 5 days. The expression level of genes involved in differentiation in differentiating and non-differentiating infected cells was estimated using real-time RT-PCR. We selected 48 genes, which are important in keratinocyte differentiation (such as keratins, desmosomal genes, transglutaminases, genes coding for cornified envelope proteins etc.), and investigated the effects of the 16E6E7 oncogenes on the mRNA level of these genes in differentiating and non-differentiating cells. The differentiation of keratinocytes by calcium had different effects on the transcription level of these genes. The E6E7 oncogenes of HPV 16 together caused down-regulation of transcription of a subset of the selected genes. Notably, the expression of genes known to be expressed in basal epithelial cells (keratin 5 and 14) were not affected by the HPV oncogenes, while the expression of genes known to be up-regulated in differentiating keratinocytes (such as keratin 1 and 10, involucrin, etc.) were significantly inhibited by the HPV oncogenes. These results indicate that the HPV oncogenes may have a direct and specific effect on the expression of genes involved in keratinocyte differentiation.
In order to see whether the HPV oncogenes exert an effect on the activity of the promoters of these genes, transient transfection assays will be performed in primary human keratinocytes using HPV 16 E6 and E7 expression vectors and luciferase reporter constructs containing the promoters of selected genes involved in keratinocyte differentiation.

THE DEVELOPMENT OF A NOVEL, HIGHLY SENSITIVE QRT-PCR SYSTEM FOR THE DETECTION OF ASPERGILLOSIS CAUSING *ASPERGILLUS* SPECIES

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Despite of the rapid development in antifungal therapy during the past decade, the incidence of invasive mold infections, especially those caused by *Aspergillus* species remains a major cause of the infection-related morbidity and mortality in developed countries. The reasons behind this phenomenon are the use of immunosuppressive agents (corticosteroids) in recipients of an allogenic stem/bone marrow cell and solid-organ transplantation or hematopoietic stem cell transplantation. Major causative agents of the highly devastating systemic mycoses are mainly the opportunistic filamentous fungi of the *Aspergillus* genus, such as *Aspergillus fumigatus, A. terreus, A. flavus* and *A. nidulans*. The saprophytic *Aspergillus* species are ubiquitous in our environment. The exposure to *Aspergillus* spores or conidia is therefore almost constant. Due to the immunocompromised state of these individuals, aspergillosis can become invasive and in spite of the fact that in the status of the primary disease may appear to get better, the secondary evolved infections could lead to death.

The only means of survival is the antifungal therapy initiated early enough. The most reliable microbiological and histopathological methods are time consuming, signs and symptoms of systemic diseases caused by *Aspergillus* species are non-specific and patients are often unable to undergo invasive diagnostic procedures. The current, commercial aspergillosis diagnostic methods are based either on detection of DNA or being serological or the combination of these. However these hybrid methods that unite the high specificity of DNA based PCR techniques and the high sensitivity of serological methods are time consuming. We have developed species specific, highly sensitive quantitative real-time PCR diagnostic assays for detecting specific markers and identifying *Aspergillus fumigatus* and *A. terreus* species in different biological samples. The principle of our novel invention is based on the finding that the orthologous of *Streptomyces fac*C gene is present in certain human pathogenic filamentous fungi. It is worth to mention that the advantages of our quantitative real-time PCR diagnostic assays are that they are rapid, cheap, reliable, highly sensitive and reproducible. Due to the fact that detectable markers are almost exclusively found in *Aspergillus fumigatus* and *A. terreus*, the high rate of false positive results that is given from the presence of other pathogen species obtained by using other multi copy target genes in DNA based assays will be reduced to zero. The sensitivity of these tests was measured and they do not only range with those of other commercially available Q-RT-PCR diagnostic methods, but top them. They are able to detect 5 CFU and 2.5 CFU in biological samples in manual and automatic extraction systems respectively. Due to the fact that such detectable markers are not present in other fungi, the level of discrimination is maximum high (100%). The possibility of false positive results that originate from the presence of other species is neglectable.

NOVEL PICORNAVIRUS IN DOMESTICATED COMMON QUAIL (*COTURNIX COTURNIX*) IN HUNGARY

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Picornaviruses (family Picornaviridae) are small, non-enveloped viruses with single-stranded, positive-sense genomic RNA infect humans and a wide variety of animals. Picornaviruses are classified into 12 genera but compared to the thousands of bird species only 8 picornaviruses have been described in birds. The 7.2–9.1kb-long picornavirus genomes have a common genome organization pattern: between the 5' and 3' untranslated regions (UTR) there is a single open reading frame (ORF) encoding a single polyprotein, which has divided into a three regions P1 (VP4-VP2-VP3-VP1 capsid proteins), P2 (2A-2C) and P3 (3A-3D) (both for protein processing and genome replication). Some picornavirus encodes a leader (L) protein as well. This study reports the detection and characterization of a novel picornavirus in domesticated common quail (*Coturnix coturnix*) in Hungary. Fecal samples were collected from quails from a family poultry farm in July 2010 (N=1) and in April 2011 (N=10). Further 10 swab specimens were gathered from the outer surface of commercially distributed quail egg shells. Samples were screened with primers initially designed for kobuvirus 3D regions by RT-PCR. 5'/3' RACE PCR method was used to determine the complete viral nucleotide sequence. The secondary structure of the 5'/3' UTRs were predicted by Mfold, the possible polyprotein cleavage sites were determined using NetPicoRNA and phylogenetic analysis was done using MEGA5. Specific (216nt-long) PCR product for "kobuvirus" was identified from the sample collected in 2010. However, only a 74 nt-long partial sequence had 81% nt identity to human enterovirus 99 (EF015012), and the putative amino acid sequence had 58% identity to simian picornavirus 17 (YP_001718553) as the closest matches using GenBank BLAST/BLASTX.

The complete genome of quail picornavirus (QPV1/HUN/2010, JN674502) is 8159 nt long and the P1 (857 aa), P2 (458 aa) and P3 (777 aa) regions show 43%, 39% and 47% amino acid identity to the closest reference avian sapelovirus (AY563023). A long (390 aa) L protein was found which is cysteine-rich and encodes two copies of a 34-aa-long (102 nt) repeat motif. QPV1 has 494-nt-long variant type IVB internal ribosomal entry site (IRES) with a 20-nt-long apical "8"-like structure that is found as a conserved sequence in avian-origin and seal picornaviruses. Three of 10 (30%) fecal samples were positive for QPV1 in 2011. These QPV strains showed 99% nucleotide identity compared to QPV1. QPV RNA was not detected on the shell surface of commercial eggs.
Because of the species diversity and their wide migration patterns birds could be more important for spreading infectious diseases than previously believed and might be reservoirs of unknown viral pathogens. Whether the species quail picornavirus have zoonotic potential and can create an avian-origin picornavirus genus being in close phylogenetic relationship with members of genera *Enterovirus* and *Sapelovirus* are still open questions.

DIMORPHISM IN *SCHIZOSACCHAROMYCES JAPONICUS*

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The process of hyphal growth has been studied for many years in several dimorphic fungi species, as this feature can be in close connection with their pathogenesis. Well-known dimorphic fungi species are: *Ustilago maydis*, which is responsible for corn smut, *Candida albicans* and *Cryptococcus neoformans*, which are important human pathogens, or the baker's yeast, *Saccharomyces cerevisiae*. In the wild, this ability could provide a selective advantage, as it would facilitate foraging for nutrients or surviving a stress. The morphological switching is a complex process. It requires environmental sensing [1], cooperation of different signalling pathways [2], a changeover to unipolar growth [3], reorganisation of the actin and microtubular cytoskeleton, repression of cell separation [4], etc. The non-pathogenic fission yeast *Schizosaccharomyces japonicus* has proved to be an excellent model for studying dimorphism, as its cells can alternate between yeast and mycelial morphology and it has a haploid chromosome set. In this project, we have focused on the effect of certain environmental factors, which can influence the formation of mycelia. Therefore, *Sch. japonicus* cells were cultured under different circumstances. Morphological transition in *Sch. japonicus* is induced by increased temperature, $FeCl_2$, or fetal bovine serum similar to the *Candida albicans* cells [5]. Ethanol and isopropanol have inhibited the filament formation.

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ANALYSIS OF THE PHYSIOLOGICAL ROLE OF PROTEIN ADP-RIBOSYLATION IN *STREPTOMYCES COELICOLOR*

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Streptomycetes are Gram-positive, obligate aerobe saprophytic, soil bacteria, well known for their mycelial growth and complex morphological differentiation process which is coupled to the production of a wide variety of secondary metabolites with important medical, pharmacological, biotechnological importance. Complete understanding of the regulation of differentiation process and antibiotic production would require the characterization of regulatory mechanisms acting on protein level. Mono-ADP-ribosylation is a post-translational protein modification, catalyzed by mono-ADP-ribosyltranferase enzymes (mART) that transfer a single ADP-ribose moiety of β-NAD^+ to a specific amino acid side chain in a target protein. ADP-ribosylation is a reversible regulatory mechanism, it is reversed by removal of the ADP-ribose moiety by ADP-ribose-protein glycohydrolases. Our previous results suggested the involvement of protein mono-ADP-ribosylation in the regulation of morphological differentiation and antibiotic production. Here we summarize our work, focusing on protein ADP-ribosylated in *Streptomyces coelicolor* M145 in order to expand our knowledge about this elusive protein modification. Recently using bioinformatics approach we have identified the product of SCO5461 gene of *S. coelicolor* as a mART enzyme that is predicted to be a transmembrane protein with an extracellular catalytic domain. PCR targeted disruption of the gene with an apramycin resistance casette resulted in the *S. coelicolor* ΔSCO5461::apr null mutant. Disruption of the SCO5461 gene resulted in a conditional pleiotropic phenotype which was complemented by cloning the SCO5461 into the ΔSCO5461::apr null mutant. The mutant differentiated normally on complex medium, however morphological differentiation in minimal medium was significantly delayed, a phenotype that was even more pronounced on osmotically enhanced minimal medium. The normal morphological differentiation of the mutant could be restored extracellularly. In addition to the defect in morphological differentiation, the production and secretion of actinorhodin antibiotic was also affected in the null mutant. Analysis of the pattern of ADP-ribosylated proteins showed that some of the modified proteins present in the wild type were absent in the mutant, suggesting that the protein product of SCO5461 is an mART enzyme.

MULTILOCUS BASED PHYLOGENY AND MORPHOLOGICAL, PHYSIOLOGICAL ANALYSIS OF THE MORTIERELLALES

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Members of Mortierellales belong to one of the largest groups of 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 many species are widely used by the biotechnological industry as producers of polyunsaturated fatty acids (e.g. *M. alpina*). At the same time, the phylogenetic relationships of these fungi have been poorly understood. Previously, we have demonstrated the paraphyly of the genus *Mortierella* which included the genera *Dissophora, Gamsiella* and *Lobosporangium*. However, using ribosomal

genes only, deep branches could not been resolved. Here, we address the early divergences in the Mortierellales by using 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). We employed partitioned Bayesian and Maximum Likelihood approaches and paid special attention to dealing with rate heterogeneity between the different loci and partitions. The phylogeny inferred in this study demonstrates the improved power of combined protein coding and ribosomal data sets to resolve early nodes of the Mortierellales. We also tested if the former morphology based classifications meet our results. Microscopic – both light- and scanning electron microscopy - analyses of various characters (septation, structure and branching of the hyphae, sporangia, etc.) also showed that the accepted classification is strongly unnatural. In pursuance of the physiological experiments we tested the fungal utilization of 67 different carbon sources. Utilization patterns do not highly vary intraordinary but show many differences compared to the closely related Mucorales.

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INTRACELLULAR SPORULATION IN A GROUP OF WIDESPREAD MYCOPARASITIC FUNGI

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Powdery mildew fungi (the Erysiphales) are well-known pathogens of monocotyledonous and dicotyledonous plant species, including important crops. *Ampelomyces* spp. are common intracellular mycoparasites of powdery mildews worldwide producing their pycnidia inside the powdery mildew conidiophores. The position of pycnidia in the conidiophores was suggested to distinguish genetically different groups in the genus *Ampelomyces*. To investigate this presumption, we determined the localization of pycnidia of different *Ampelomyces* strains inside powdery mildew conidiophores including a special type of these structures, namely microcyclic conidiophores. To investigate the pycnidial localization in powdery mildew conidiophores, mildew-infected tobacco, cucumber, tomato and barley plants, kept in pots in a greenhouse, as well as detached grapevine leaves, collected from potted plants kept in a greenhouse, were inoculated with conidial suspensions of ten *Ampelomyces* strains. Further mycoparasitic tests were performed to determine the localization of pycnidia in artificially modified conidiophores. Tobacco and grapevine powdery mildew colonies were gently brushed every day to break the already developed conidiophores and prevent the maturation of the new ones. Two *Ampelomyces* strains with distinct patterns concerning the localization of their pycnidia in intact conidiophores were selected for these experiments. Development of *Ampelomyces* hyphae and pycnidia was also examined in the microcyclic conidiophores of six powdery mildew species using light microscopy. The results of all the mycoparasitic tests performed with five powdery mildew species and 10 genetically different *Ampelomyces* strains showed that the morpho-physiological patterns of the powdery mildew conidiophores are those factors that strongly influence the position of pycnidia in the cells of these conidiophores. However, in certain powdery mildew species strain-specific differences were also detected in the localization of pycnidia. These in vitro experiments have also confirmed the lack of any strict mycohost specialization in these mycoparasites because genetically different strains infected several different powdery mildew species in addition to their original mycohosts.

EXAMINATION OF THE BACTERIAL COMMUNITY DURING THE CULTIVATION PROCESS OF OYSTER MUSHROOM (*PLEUROTUS OSTREATUS*) BY CULTIVATION-INDEPENDENT METHODS

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The large-scale cultivation technology of oyster mushroom (*Pleurotus ostreatus*) has become well established. It is known that the autochthonous microbial community has a substantial role in substrate preparation which is the first part of the cultivation process. Recently we studied the changes in the bacterial community of the first part by sequence-aided Terminal-Restriction Fragment Length Polymorphism (T-RFLP) based on 16S rRNA gene. A marked bacterial succession was observed from characteristic ubiquitous mesophilic bacteria through *Bacillus* spp. and *Pseudoxanthomonas* spp. to the presence of Actinobacteria, Firmicutes and the members of genus *Thermus* in the mature substrate. However there is still sporadic knowledge about the microbial community of the second part of the cultivation, when the substrate is inoculated with *P. ostreatus* spawns, its hyphae colonize the substrate and the growth of fruiting bodies begins. To examine how the structure of the microbiota changes due to the presence of mushroom hyphae, the bacterial community of the second part was analysed in a model system. Mature substrate was filled into tubes, inoculated with oyster mushroom spawn at one end, and incubated at 26°C, 65% RH for one month. Control tubes without inoculation were also prepared. Before complete colonization the tubes were cut into slices and community were analysed by 16S rDNA T-RFLP. The first results showed that the bacterial community of the fronthyphae and the uncolonized regions were similar to each other. In the colonized regions a minor bacterial succession was observed toward the regions of the older hyphae. Further studies (sequence analysis of 16S rRNA gene) are required to identify the relevant members of the bacterial community.

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SYSTEMS BIOLOGY APPROACHES FOR ORGANIC ACID PRODUCTION IN FILAMENTOUS FUNGI

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Among filamentous fungi *Aspergillus niger* is a well known production host for a wide variety of enzymes (amylase, cellulose, protease) and metabolites (organic acids). Based its performance in these more traditional fermentation processes *A. niger* is already used for the production of novel proteins [1]. But even more recently this fungus is now also considered for the production of new so-called platform or building-block chemicals for the chemical industry. These chemicals, currently produced based on petrochemistry, are the starting point for the production of a wide variety of materials, such as resins, plastics, etc. Production of these compounds via biobased routes will be a major contribution towards a Biobased Economy. For the production of these bulk compounds robust host organisms are required, suitable for using low cost lignocellulose-based feedstocks, resistant against adverse conditions due to inhibitory feedstock compounds and capable of coping with high product concentrations. *A. niger* was shown to fulfill most of these prerequisites [2, 3]. Based on the extended molecular genetic toolkit systems biology approaches were developed for *A.*

niger and other fungi [4]. These approaches may be followed to produce several of these platform chemicals in *A. niger*, as demonstrated by the example of itaconic acid [5, 6].

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THE ENVIRONMENTAL SAFETY EXAMINATION OF *ACINETOBACTER* ISOLATES ORIGINATING FROM HYDROCARBON CONTAMINATED SITES

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Nowadays, some of the opportunistic microorganisms imply increasing public health problems in clinical environments, moreover, they are ubiquitous in nature, too. Regarding their human health effects the development and spread of antibiotic resistance and multidrug resistance are an urgent issue. Some of these opportunistic microorganisms have biodegrading abilities, too, and they can survive easily on the contaminated sites during remediation. In the course of our work we focused on the genus *Acinetobacter*, because the spread of multidrug resistance among its strains in clinical environment has been increasingly reported in recent years. At the same time the hydrocarbon degradation ability of the genus is widely known for environmental practice, too. Our aim was to establish and extend a culture collection of environmental *Acinetobacter* strains isolated from hydrocarbon contaminated groundwater samples. Pure cultures of *Acinetobacter* spp. were isolated and identified with traditional microbiological and molecular biological (16S rDNA based) methods. The available information on the antibiotic resistance of environmental strains was very limited, therefore our further aim was the determination of the antibiotic resistance profiles of the *Acinetobacter* isolates originating from hydrocarbon contaminated sites with E-test method. The hydrocarbon degradation ability of the genus suggested by the scientific literature was also to be examined. Therefore hydrocarbon degradation tests were done by gravimetric analysis and by the detection of gene sequences responsible for the degradation of aliphatic hydrocarbons. In the course of our experiments, 227 hydrocarbon contaminated samples of 14 contaminated sites were examined and our *Acinetobacter* culture collection was successfully extended with the development and application of a multi-step isolation method. It was determined that in the case of environmental strains, as it could be seen in the case of clinical ones, multidrug resistance may occur, and some of the environmental strains show an increasing level of resistance. Based on our results some *Acinetobacter* strains have outstanding ability to adapt to hydrocarbon contamination, they can use hydrocarbons as primary carbon source and they can be isolated from hydrocarbon contaminated sites. However, based on their pathogenic features and increasing antibiotic resistance, these strains possibly mean an environmental health risk.

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EXPERIMENTAL INFECTION OF ALBINO RABBITS WITH EPSTEIN-BARR VIRUS (EBV, HHV4)

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Two groups of albino rabbits (total 21) were inoculated with a concentrated suspension of B95-8 cells containing either $5x10^8$ or $2x10^9$ copies of EBV DNA. The sera of infected animals were tested by ELISA (before virus inoculation and on days 8, 28 and 98 post-inoculation, p.i.) and immunoblot tests. The groups differed from each other by the fact that the sera of 10 rabbits revealed non-specific binding to the viral capsid antigen (VCA) coated plates in dilutions 1:20 to 1:40 already prior to infection. No such binding was found when the plates coated with the early (EA-D) EBV antigen were used for antibody testing. Seroconversion to VCA was found in a total of 9 animals by day 8 (43 %) and in 12 of them (57 %) on day 28 p.i. At the latter interval, a similar seroconversion rate (13/21, 62 %) was found also against EA-D antigen. At same time, the immunoblot was positive in 10 out of rabbits 17 serum samples (59%) showing a good correlation with the ELISA results. Later on, at the 3 month (authopsy) interval the immunoblot test showed an increased positive rate of 100% (17/17). Noteworthy that in the sera of 10 rabbits showing nonspecific binding to VCA coated immunoplates, the seroconversion could be better demonstrated using immunoblot than ELISA. While IgG antibodies reacting with the trans-activation protein (encoded by the BZLF1 gene), were always present at immunoblot testing, the anti-EBNA1 antibodies were never detected on the strips. The presence by PCR of EBV DNA was followed at same p.i. intervals as serum antibodies in peripheral white blood cells (WBC) and in the spleen extracts (on authopsy day only). While the former never exceeded the positive rate of 18 % (3 out 17), the latter has remained as frequent as 11 % (2 out 18). Along with this, the LMP1 antigen expression was followed all the intervals tested with a similarly low positive rate result never exceeding 25% (4 out 20). The impact of these results will be discussed in comparison with available reports published.

COMPARATIVE GENOMICS OF *STAPHYLOCOCCUS AUREUS* PHAGES

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Currently, *Staphylococcus aureus* is one of the most problematic pathogens. It is a harmful infective agent causing a wide range of illnesses, which might be difficult to cure. Due to its multidrug resistance it seems as a forever - living bacterium. Among the various cell lines, the methicillin-resistant *S. aureus* (MRSA), whose occurrence dramatically increased in the last two decades both in medical patients and even in the population. Moreover, the appearance of *S. aureus* strains with reduced susceptibility to vancomycin (VISA), and the vancomycin-resistant *S. aureus* (VRSA), further raised difficulties in treatment of staphylococcal infections. These facts reflect a real need for the development of efficient new anti-staphylococcal agents. Bacteriophages were first discovered in 1915 and used as antimicrobial tools since 1919. However, the appearance of antibiotics confined their applications. Nevertheless, phage therapy entered into its renaissance in the last few years as a

consequence of frequent emergence of multidrug resistance in pathogenic microbes. The developments of newer and newer antibiotics, antimicrobial agents are very expensive and time consuming processes. The phage therapy has several advantages over antibiotics: easier and cheaper to produce, the phages are versatile, new phages can be easily isolated, moreover phage mixtures, phage cocktails can also be applied. And there is no need for long medical testing. There are several phages isolated from various environments which might be potential biocontrol agents against staphylococci infections. However, there are only few, three complete phage genomes sequenced. In this work, we isolated and characterized more than 20 *Staphylococcus* phages. They belong to the Myoviridae family. Five phages functional against methicillin-resistant *Staphylococcus aureus* were chosen and sequenced by new generation sequencing technologies. The genomes are annotated and a comparative genomic study is performed and presented.

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DATA TO THE EARLY HISTORY OF TEACHING AND RESEARCH OF MICROBIOLOGY IN HUNGARY

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The short history of professional education and also teaching of medical, veterinary and agricultural subjects will be listed. A definition of microbiology and microorganisms will be presented. Then the first explanation of plague from 1588 will be shown. Gergely Frankovith used the term of sticker, poison and pestilent substance. Around 1600 György Lencsés also wrote that feverish illnesses were caused by pestilent substances. In 1690 Ferenc Pápai Páriz thought that poison caused rabies and the use of animal experiment was proposed for the diagnosis. In 1784 Sámuel Rácz published that the cause of scarlat might be a special seed. Around the years of 1800 Ferenc I Bene and Ferenc Xavér Gebhardt delth with smallpox and Bene predicted the eradication of variola. Gergely Frankovith (1588) published about rust and János Lippay (1664) the black-rust also and they thought that these diseases of plants spreading with air. Lippay spoke about the use of "acid leaven", too. As to the use and teching of magnifiers in "Lexicon" of Albert Szenci Molnár published in 1621 the conspicillum word can be read and these instruments have been known by Hungarian persons, too. In the revised edition of "Dictionarium" of Ferenc Pápai Páriz in 1767 microscopium word can be seen and János Paterson Hain around 1671 performed micro- scopic examiations with his self-made microscope in Eperjes. The first textbook entitled „Institutiones philosophiae naturalis dogmatico-experimentalist" was published by István Tőke in 1736 from Nagyenyed in which the pictures of microscopes can be seen and about insects as well as animalcula can be read. János Keresztély Grossinger was the first real important microbiologist who lived in Komárom in the years of 1790. He wrote about the role of microorganisms in fermentation and also the importance of micro- as well as macroorganisms in animal diseases. Grossinger published the first microbiological chapter entitled „Micrographia" in his big "Encyclopaedia", too. After Pápai Páriz Ferenc's diagnostic animal test (1690), as well as György Fekés's self and animal experiments (1787), József Hoffner tought from 1827 that the verification of an infectious disease could only be done, if test-animal was infected with the questionable material. This statement was important as the first verification of the human and animal pathogenic *B. anthracis* happened only in 1850 and the postulate of R. Koch was merely borne in 1877. The first human dermato-pathogenic fungus was observed by Adolf Ferenc Láng at Nyitra in years of 1840. Ignác Fülöp Semmelweis verified in 1847 that "unknown daceyed organic substances" caused puerperal fever in years of 1840 as the hands of health service personels and that

of students were contaminated. He could stop the epidemies with the use of chlorinated water - concept of antisepsis was borne. Pathological potatos were studied with microscop by Lajos Tognio in 1847 and he saw microorganisms. Microscopic picture of mildew fungus was presented by Pál Bugát in 1853. *B. anthracis* was seen by Kálmán Balogh in 1863 in a microscopic preparation and he infected rabbit with this bacterium. Finally János Ádám Raymann performed variolation in the district of Eperjes and the results of which were published in 1717 in a book entitled "Historia medica Variolarum Eperiensini in …". Human vaccination started at the end of years of 1700 and Ferenc Schraud organized vaccination in all our country in 1804. He also helped that this immunization was ordered to be obligatory in 1812.
Ovinatio was applied at least at the beginning of the XIXth centrury. Two specialists Ignác Havas and Vilmos Zlamal almost in the same time applied vaccine against east cattle plague in 1839. Immunization against of measles was studied by Mihály Katona in 1842. István Lippthay organizad the obligatory vaccination of animals in 1881 and the malleination was also started in 1895. Endre Hőgyes prepared own vaccine to rabies in 1888 at the University of Budapest.

RESISTOMICS AND ANTI-DRUG-RESISTANCE VACCINE DISCOVERY

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Multidrug-resistant (MDR) Gram negative bacterial (GNB) pathogens have emerged as agents of serious community and healthcare-associated infections all over the world. While antimicrobial drugs used in health-care settings can contribute to the selection of drug resistant strains, there is growing evidence that a large proportion of infections caused by MDR GNB pathogens, especially extraintestinal *Escherichia coli* pathogens (ExPEC), is associated with non-clinical factors, including food. Not only does food serve as vehicles of dissemination of ExPEC, but it also contributes to the dissemination of drug-resistance genes inside saprophytic organisms on the food products. We launched a project to identify all possible drug-resistance genes in food items commonly consumed in the United States ("resistomics"). We used a functional genomics approach to identify drug-resistance genes among the microbiota on spinach and chicken. On spinach, we not only found blaCTX-M-15, the most common extended spectrum β-lactamase (ESBL) gene circulating globally, but other ESBL genes as well as new genes that resemble genes that encode other β-lactamases as well as fluoroquinolone resistance. In addition, we found a new sequence that encodes resistance to a new antimicrobial agent tigecycline. This resistomics approach may be able to identify new genes that encode resistance before new antimicrobial agents are introduced into clinical practice. A large proportion of drug resistance in GNB pathogens is mediated by β-lactamases. There are more than 950 β-lactamases expressed by bacteria, which makes development of new antimicrobial agents very complicated. A new approach to combatting these β-lactamase-producing GNBs is needed. Using a bioinformatics approach, we initiated another project to develop a multivalent therapeutic vaccine to inhibit β-lactamases in GNB. We wished to immunologically block β-lactamases of GNB when a patient gets infected with MDR-GNB so that the activity of β-lactam drugs can be preserved. In a preliminary experiment, we showed that a polyclonal antibody to a carbapenemase (KPC) used together with imipenem inhibited the growth of imipenem-resistant *Klebsiella pneumoniae* in vitro. Mouse urinary tract infection (UTI) model will be used to assess whether a mouse vaccinated with recombinant KPC can be cured with treatment with imipenem when the animal has imipenem-resistant *E. coli* UTI.

Using new bioinformatics and functional genomics approach, we may be able to develop completely new ways to address the global spread of MDR GNB infections.

INVESTIGATION OF HERBICIDES ON SOME MICROBIOLOGICAL PARAMETERS OF SOILS IN AN INCUBATION EXPERIMENT

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The intensive agricultural production is unimaginable without chemical plant protection. In the plant production the protection against the pathogens and pests, in the regulation of weeds are important technologies for growing the plant production. Among the agricultural chemicals nowadays newer herbicides are distributed, with better selectivity and these can be used in smaller concentration, than the earlier ones. Soil microorganisms are indispensable to the survival the life, because of their tasks in the matter and energy cycles and participation in the ecological functions of soil. Soil ecological indication processes can make a measurable impact and indicate the statues of ecological communities. Soil microbiological effects of herbicides are influenced by the adsorption capacity of soil. According to scientists, the herbicides can play as phosphorus, carbon and nitrogen sources for soil microorganisms. Authors reported that the number of microscopic fungi and soil bacteria increased by the application of normal field dose of herbicides. In a laboratory incubation experiment the effect of three herbicides with different agents was examined on some parameters of the soil biologial activity. Three different soil types from Debrecen surrounding was used for the experiment which took place in the soil microbiological laboratory of the Institute. Soil samples were taken one,- tree and seven weeks after setting the experiment. Along the soil microbiological investigation the number of total bacteria and microscopic fungi, quantity of nitrifying bacteria, nitrate exploration and the activity of catalase enzyme were determined. The number of total bacteria and microscopic fungi increased along the incubation time till the third sampling. In the Chernosem (Ck) the bacteria number increased in all treatments compare to the control. In Vertisol (Vp) soil the bacteria number was increased by 2,4D and thiencarbazone-methyl treatments, while was decreased by the large dose of tembotrion. Regarding the Arenosol, the bacteria number decreased in all treatments, especially 1 week after spreading the herbicides. The number of nitrifying bacteria was similar to the total bacteria number. In the calcic chernozem the number of microscopic fungi increased in all treatments and all the three sampling time compare to the control. In the Vp and Arenosol (Ql) the number of fungi decreased significantly in the first and second sampling time but by the third sampling slight stimulatory effect was experienced. The nitrate content of soil increased in the first half of the incubation period, but by the end of the experiment decreased, parallel with the number of nitrifying bacteria. Concerning to the catalase activity, the largest activity was measured in the Ck soil, and about its half in the Ql, the lowest activity was measured in the Vp about one-tenth of the Ck. Among the treatments there were no differences.

COMPARISON OF CELLULAR AND HUMORAL IMMUNE RESPONSES IN GUINEA PIGS AFTER VARICELLA ZOSTER VIRUS VACCINATION BY THE INTRADERMAL OR SUBCUTANEOUS ROUTE OF ADMINISTRATION

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The skin is a highly immunogenic organ. The cellular composition of the skin include a heavy population of resident and transient professional antigen presenting cells, such as Langenrhans cells, dermal dendritic cells, dendritic cell precursors, macrophages and B lymphocytes. The intradermal (i.d.) route of administration may increase the efficacy of vaccination. A varicella-zoster vaccine (OKA strain, live, attenuated, given subcutaneously) to prevent varicella was developed and approved for use in many countries. A zoster vaccine was approved for use in the USA in 2006, but the titer of the OKA virus is 14-fold higher than in the varicella vaccine, making the production of the zoster vaccine difficult and expensive. The i.d delivery system may reduce the dose and of the vaccine and improve the stimulation of cell-mediated immunity of the elderly to prevent the reactivation of VZV. Guinea pigs are appropriate experimental animals for testing the i.d route of administration because of the similarity of the guinea pig skin to the human skin. A needle-free device was used for the i.d application of the OKA vaccine and the guinea pigs were also vaccinated by the conventional s.c. route. The amount of the VZV-DNA in the skin biopsies of guinea pigs at different times after i.d. administration was very similar, as determined by real-time PCR, indicating the reproducibility of the vaccine administration by the cell-free device. The cell-mediated immunity and humoral immune responses were compared in the i.d. or s.c. vaccinated animals. For T cell responses Granzyme-B production, IFN-gamma mRNA expression of the stimulated splenocytes were compared, as well as IL-10 and IL-12 expression were determined. Humoral immune response was measured by immunofluorescence assay.

ISOLATION AND IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI FROM AGRICULTURAL FIELDS OF VIETNAM

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Nowadays environmentally friendly crop production technologies more and more come to the fore, which ensure the reduction of the amount of fertilizer and pesticide while maintaining crop yield and quality. In Vietnam, where more than 50% of population deals with agriculture, it is particularly important to create conditions for modern agricultural technology and to improve crop safety. The arbuscular mycorrhizal (AM) fungi, that form symbioses with the majority of land plant species including a large proportion of cultivated plants, can play a significant role in this process. In our work we investigated the AM fungal communities in the rhizosphere soils of rice (*Oryza sativa*), maize (*Zea mays*), peanut (*Arachis hypogaea*), croton (*Croton tonkinensis* Gagnep.) and tomato

(*Lycopersicon esculentum*) plants originated from Bắc Ninh, Vĩnh Yên and Hà Nội provinces in Vietnam. The presence and richness of the AM fungal community were assessed by extraction and counting of AM fungus spores and by characterization of the isolated strains based on molecular methods. Our results showed that the Vietnamese red clay sub-tropical soil (acrisol) is rich and diverse regarding to AM fungi. The dominant members of the AM fungal communities in the rhizosphere soils tested belong to the genus *Acaulospora*, except the rhizosphere soil of maize where the vast majority of AM fungal spores belong to the genus *Funneliformis*. When using AM fungi as inoculum the indigenous AM fungal community cannot be ignored, hence the results of the present work may extend our present knowledge for determining the composition of an efficient inoculum combination of AM fungi, best adapted to the environmental factors and cultivation systems.

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FROM FUNGAL GENETICS TO GENOMICS AND BEYOND. A PERSONAL ACCOUNT

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I stared working on fungal genetics exactly 49 years ago. This is perhaps why the organisers kindly asked me to open this session. The changes witnessed in the methodologies and concepts used to understand fungal biology and its multiple applications in human, animal and plant pathology and in biotechnology, can be seen through three periods. Up to the early eighties, the methodology was that of classical genetics for model organisms such as *Neurospora crassa* and *Aspergillus nidulans* and that of rounds of mutagenesis for biotechnological important species such as *Penicilium chrysogenum*. Transformation of *N. crassa* was achieved as early as 1979, but it was the transformation of *A. nidulans* in 1983, which opened the way of genetic manipulating pathogens and industrially important organisms. It is noteworthy that the work with *N. crassa* resulted in the discovery of novel, unsuspected phenomena such as RIP, Quelling and MSUD, while transformation of other fungi led to a more profound level of analysis of phenomena that were identified previously by classical genetics, such as multiple levels of genetic regulation, the cell cycle and mitosis. A collection of mutations was there, just waiting for reverse genetics to unveil the nature of each of the abstract factors proposed as long as forty year before. The sequencing of whole genomes opened a new era. 2003 could be taken as an arbitrary watershed date, as it marked both the 50th anniversary of Pontecorvo's epochal "The genetics of *Aspergillus nidulans*" and the completion of a publically available genome of the latter organism. New sequencing methods are leading to the public appearance of scores of complete genomes in the data bases, usually accompanied by collections of ESTs, with a goal of 1000 fungal genomes. An interesting reversal of the situation is taking place: while in the early days, a strong research community was a pre-requisite to the start of a genome sequencing project, organisms are now chosen by their intrinsic interest, even in the paucity or absence of such a research community, in other words, the genome will hopefully generate the community. The availability of multiple genomes and expression studies has obvious importance for applied purposes such as the production of new secondary metabolites. However, the informed use of genomic and transcriptomic data bases could open entirely unsuspected new fields such as

assessing the importance of alternative splicing in fungi, the role of transposons in evolution, and understanding the role and evolution of silencing mechanisms at genetic and epigenetic levels.

SESQUITERPENE EMISSION OF THERMOPHILIC FUNGI AND DIFFERENT COMPOST PRODUCTS

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Recently soil has been proved as considerable source of biogenic sesquiterpene (SQT) emission, contributing to the global formation of organic aerosol [1]. Mesophilic mycota representing average 40% of total soil biomass must be responsible for SQT emission of soil origin [2]. The SQT emission of three composts of different origin was measured in an aseptic flow-through apparatus designed for solid phase microextraction in our laboratory. The SQT emission of the pure cultures of 8 thermophilic fungal species most common in composting processes and compost products also were measured. The amount of 7.2×10^5-5.1×10^6 CFU g^{-1} of thermophilic fungi were cultured on malt extract agar from the tested compost products and the level of SQT emission (14.5-1392 ng m^{-2} h^{-1}) could be measured from these media. Fungal isolates were identified on the basis of morphological characteristics and ITS DNA sequence analysis. The pure cultures of the most common thermophilic fungal species in compost materials produced 2-10 different molecules of SQT substances at 40°C. All of the tested strains, with an exception of *Thermomyces lanuginosus* Tb116 emitted the secondary metabolite SQTs in remarkable amounts: 370 ng h^{-1} g^{-1} as maximum by *Myceliophthora thermophila* Tb085 and 3.2 ng h^{-1} $^{-1}$ as the minimal value by the strain *Malbranchea cinnamomea* Tm34. Considering that composting technologies have spread in waste management of both the agriculture and the environmental protection, furthermore that the intensive role of thermophilic fungi in the decomposition of organic materials in warm climate territories, their SQT production contribute to the global SQT emission.

[1] Horváth, E. (2012) J Geophys Res 117: D15304.
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SYSTEMS ANALYSIS OF LACTOSE METABOLISM IN *TRICHODERMA REESEI*

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Lactose (1,4-0-ß-D-galactopyranosyl-D-glucose), a by-product from cheese and whey processing industries, induces the formation of cellulases and other plant biomass hydrolyzing enzymes in the fungus *Trichoderma reesei*. These enzymes are used today to break-down cheap plant material to their monomers which can be further fermented to bioethanol or used as feedstock for other biorefinery products. Here, we use a systems analysis approach to understand induction by lactose and the underlying mechanism. Analysis of the *T. reesei* transcriptome during growth on lactose

shows that the respective CAZome encodes glycosyl hydrolases specifically tailored for the attack of monocotyledon xyloglucan. In addition, genes for a high number of putative transporters of the major facilitator superfamily were also induced. Systematic knock out of these upregulated transporters identified a gene whose knock-out completely impaired lactose utilization and cellulase induction in *T. reesei*. Our data shed new light on the mechanism by which *T. reesei* metabolizes lactose and illustrates the key role of ß-D-galactosides for this fungus.

ISOLATION AND CHARACTERIZATION OF SMALL AND LARGE PLASMIDS OF *BACTEROIDES* SPECIES

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Isolates of *Bacteroides* species may harbour three kinds of small plasmids (2.7, 4,1 and 5,6 kb) very frequently (30-50%). Earlier we have characterized a representative of the 5.6 kb type by nucleotide sequencing. From genomic sequencing studies we also know the sequences of other three large plasmids (33, 36.5 and 40.3 kb) but have not data about their functions. Therefore we aimed to study the small and most importantly the large plasmid content of a collection of *Bacteroides* strains (n=34) from Hungary. Out of the 37 examined strains 15 (44.1%) harboured small and 6 (17.6%) harboured large (>15 kb) plasmids. Based on the molecular weights the large plasmids were 18 kb, in the range of 30-50 kb, ~70 kb or ~160 kb. We compared the homologies of these latter large plasmids to the one from *B. fragilis* NCTC9343 (p9343) by Southern blotting. Three highly homologous and three plasmids bearing low homology were found. No correlation was observed between the plasmid profile and the clinical origin (normal microbiota and clinical isolates) of the harbouring strains. The plasmids of two selected strains were subjected to Ion Torrent next-generation sequencing. We could determine the novel sequence of the 4.1 kb small plasmid type, the sequences of other 8, 10 and 11 kb plasmids and the sequences of two, 39 and 68 kb, large plasmids. The former was a variant of p9343 and the latter of a novel one. Our investigations revealed that large plasmids can also be frequently found among Bacteroides isolates, and further studies are needed to clarify their roles.

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EFFECTS OF Ag-FUNCTIONALIZED TiO_2 NANOPARTICLES ON THE DNA

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The TiO_2 has an antimicrobial effect based on its ability to generate highly reactive free-radicals from water and based on this ability, it can be used for enviromental sterilization in several areas. However, the biochemical pathways mediating this effect are not very well examined. We aimed to study the effect(s) of Ag-functionalized TiO_2 on DNA. Different concentrations of plasmid (pUC19 – bla β-lactamase gene of *Escherichia coli*) or chromosomal (*B. fragilis* BF8 – cfiA carbapenemase gene) DNA was streaked on the surface of Ag-functionalizedTiO_2 coated glass slides illuminated with LED light (General Electrics Hungary, λ=405 nm) for different time intervals. The distance of the light source was 35 cm. The remaining DNA was detected in Real-Time PCR amplifications, or

the effect of superhelicity (changes caused by nicking of the sugar-phosphate backbone) of the plasmid DNA was studied in agarose gel electrophoresis. The LED light activated nanoparticles caused significant changes of across several orders of magnitudes in the amplifyability of the tested DNAs in concentration and time-dependent manner. Accordingly, the superhelicity of the pUC19 DNA was also affected. Our studies demonstrate that the antimicrobial effect of the Ag-functionalized TiO_2 nanomaterial could be mediated by DNA damage.

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REACTIVATION OF LATENT ADENO- AND HERPESVIRUS INFECTIONS IN THE ELDERLY

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Ageing is regulated by intrinsic factors, among them telomeres. Accumulating effects of physical, chemical, biological, societal factors through lifespan exert negative or positive effects on ageing. Profound changes in the immune system of elderly people determines life expentancy. Declination of the cellular immunity dominates over changes in the native and humoral immune reactions. Diminished ability to recognise pathogenic microbes, abnormal pattern of proinflammatory mediators, decreased production of naive T cells, accumulation of anergic memory and regulatory T lymphocytes promote virus infections in the elderly population. Replication of carried viruses deterioriates immune functions in a vitious circle. Reactivated cytomegalovirus infection exerts the most deleterious effect on the ageing immune system inducing stress anergy. Antiviral therapy is ineffective in such conditions. Epstein-Barr virus production abrogates natural killer cell supply contributing to its tumourigenesis. Infection of NK cells by HTLV-I renders them unresponsive to IL-2. Clinical manifestion of HSV-1 or 2 reactivation might result in neurological damages, while reactivation of VZV can be prolonged, multiple, repetitive. Reactivation of latent and integrated HHV-6 elicits severe neuropsychiatric symptoms. Sporadic Kaposi's sarcoma cases attributed to HHV-8 were seen in the aged. HIV infection leading to AIDS is regarded as an extremely rapid ageing process. Reactivation and partial expression of human endogenous retroviruses (HERV) contribute to severe debilitating diseases (e.g. multiple sclerosis) and malignancies. Antibody production against viruses caused primary infection at young age is retained (>90 years after the Spanich flu). Reactivation of latent viruses simultaneously or in a cascade (HHV-6 and HIV or HERV) drastically reduces immune responsiveness to vaccines. Not only antimicrobial (e.g. influenza, pneumococcus) but cancer vaccines are largely ineffective due to dimished activity of CD8+ lymphocytes. New strategies are required to restore immune exhaustion. Adenovirus vectors containing immunomodulating mediator genes such as IL- 2, IL-4, IL-7, IL-10 have already been applied. Improving efficacy of vaccination in the elderly requires new formulations among them „oil in water emulsion" adjuvant for influenza shot. Regular vaccination against emerging, re-emerging and biological warfare microbes is also considered.

BIOGAS PRODUCTION FROM CELLULOSIC SUBSTRATES

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Alternative energy carriers should be implemented in large scale because of both economic and environmental considerations. Biogas production is one of the sustainable technologies with the considerable benefit of being able to generate useful energy carrier from various raw materials of biomass origin including plants and plant residues. The largest amount of biomass on Earth is plant biomass and it carries vast energy potential. Plant tissues consist of cellulose as the major component. Cellulose is a complex polymeric carbohydrate, cellulases are needed for its efficient decomposition. Cellulases are divided into three major groups: endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). For the utilization of substrates having high cellulose content the biogas producing microbial community should contain a significant number of bacteria, which produce cellulases and they should break down cellulose to easily utilizable sugar monomers. An adaptation strategy to acclimatize the community to lignocellulosic substrate has been developed. The experiments were carried out under thermophilic conditions at 55°C. α-cellulose was used as substrate for the adaptation and the control fermentors received glucose as carbon and energy source. The changes in the concentration of volatile fatty acids were followed by HPLC, the activities of β-glucosidase and exoglucanase enzymes were regularly monitored. Samples were withdrawn for DNA purification and subsequent metagenomic analysis. The adapted microbial community was also used as inoculum in the next set of biogas experiments. The preliminary results show positive effect on biogas productivity.

METHANE PRODUCTION FROM DIFFERENT AGRICULTURAL BY-PRODUCTS IN THERMOPHILIC LABORATORY FERMENTATION

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One of the renewable energy-resources of supporting our daily life is the biogas, which option can be efficiently considered nowadays. Agriculture can generate lots of by-products, applicable potentially for the biogas production. The aim of this study was to determine the best substrate mix and mixing ratios of various by-products from agricultural origin. The applied substrates were pig slurry and corn silage or pig slurry and pig litter manure mixtures, sampled from different places of new and an old storage-reservoir. Temperature of the fermentation was in the thermophilic range, at 55 ºC, by using OxiTop® OC110 system. At first, we have examined, what are the differences among the best pig slurry samples and among the mixing ratios. Thereafter the methane and carbon dioxide amounts were determined from the finally selected two types of substrate mixtures. When the biogas production stopped, KOH solution was injected to absorb the carbon dioxide and to estimate the ratio of methane in the system. The results showed that the best ratio was 1:1 (V/V) in both substrate mixtures (pig slurry with corn silage and pig slurry with pig litter manures). The best slurry sample came from the new storage, due to the higher content of degradable substrates and the greater microbial activities in them. At pig slurry and corn silage mixtures the fermentation time was shorter (2 days) in contrast with the pig litter and manure mix, where the fermentation time was 5 days. The chopped silage can be better for the bacterial communities then the pig litter manure, due to the bigger surface areas, and therefore the shorter fermentation time.
Results related to 1 gram of substrate mixtures, however showed 3,5 times higher methane amount at the pig litter manure mixtures in comparison with the corn silage mix. Up-scaling of results needed for the agricultural scale of utilization.

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EFFECTS OF SUBSTRATE CONCENTRATION AND ORGANIC-AQUEOUS BIPHASIC MEDIA ON FRUCTOSYLTRANSFERASE ACTIVITY OF PECTINEX ULTRA

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Oligosaccharides are a major class of naturally occurring carbohydrates consisting of 3 to 10 monosaccharides. These biomolecules play a fundamental role in many important biological processes and are commonly found in nature as glycoconjugates. Although there are some oligosaccharides, which have received particular attention because of their favourable features, being low in calories and noncariogenic, and acting as selective energy sources for beneficial microorganisms in the intestinal flora. Recently, oligosaccharides can be produced by transglycosyl activity of enzymes. Due to hydrolytic activity of these enzymes, the yield of synthesis process may be enhanced by decrease of the water activity that can be done by several methods such as increasing of substrate concentration or by adding of organic solvent. In this work, the effects of substrate concentration and some organic agents on transfryctosyl activity of Pectinex ultra from A. aculeatus were studied. The transfer reaction was carried out at 60^0C and pH 5.5 with about 1U hydrolytic activity of enzyme preparation. Based on our previous studies maltose and sucrose were selected as acceptor and donor, respectively. The ratio of maltose and sucrose was 1:9. Samples were taken at time intervals and boiled for 10 minutes for stop the reaction before analysis. Bioconversion was monitored by measurement of amount of carbohydrates using HPLC technique. Effects of carbohydrate concentration on transferase activity were investigated in range from 20 to 70 (w/v)%. The highest oligosaccharide content (4% DP3) was achieved on the 5th day of bioconversion with 60 % carbohydrate content and 9:1 ratio of sucrose and maltose. This value was two times higher than in case of control sample. Effects of organic-aqueous biphasic media on synthesis of oligosaccharides were also studied. Different organic solvents (acetone, acetonitrile, tri-ethyl-phosphate, ethanol, ethyl-decanoate and glycerol) were applied in combination with buffer system in ratio 1:2. Ethyl-decanoate and acetone induced the oligosaccharide synthesis. In the cases of ethyl-decanoate and acetone, the yields were increase in 203% and 102%, respectively, comparing with control sample. Generally, about 72 hours are needed to form the highest contents of oligosaccharides. Adding of acetonitrile, tri-ethyl-phosphate, ethanol and glycerol strongly inhibited the transfer reaction. Summarising, the optimal substrate concentration was 60 (w/v)% for transferase activity of Pectinex ultra. Organic-aqueous biphasic media with acetone and ethyl-decanoate induced the fructosyl transferase activity of the enzyme. These results are very promising for development of technology for synthesis of certain oligosaccharides.

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PURIFICATION AND PARTIAL CHARACTERIZATION OF INULINASE FROM *THERMOMYCES LANUGINOSUS*

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Inulinases are carbohydrolases (β-fructan fructanohydrolases, EC 3.2.1.7, EC 3.2.1.80) which have two types that depolimerase their substrate, the inulin polysaccharide. Chemically, inulin is a fructan and consists of linear β (2,1) linked polyfructose chains displaying a terminal glucose unit. One type is exo-inulinase (EC 3.2.1.80), which hydrolyses inulin to produce high fructose syrup (full hydrolysis). This product is usable as sweeteners and can be used for energy drinks and therapy. Exo-inulinases can be used for bioethanol production from artichoke as well. The other type is endo-inulinase (EC 3.2.1.7), which acts randomly at the internal glycosidic bonds of inulin producing fructooligosaccharides (partial hydrolysis). These products are usable as sweeteners and prebiotics. Inulinase preparations are commercially available and all of them are from mesophilic *Aspergillus*. *Thermomyces lanuginosus* is real thermophilic fungus and none pathogen as well as secretes numerous thermostable extracellular enzymes. Hence very few data are available in literature dealing with inulinase, in this study, purification and partial characterization of extracellular inulinase from Thermomyces lanuginosus was focused. As a result of an earlier selection procedure and medium optimisation by Response Surface Method (RSM) - *Thermomyces lanuginosus* IMI 140524 strain was found to be the most promising one to produce inulinase. After fermentation in shaken flasks the mycelia were removed by filtration. Extracellular proteins were precipitated by iso-propanol and collected by centrifugation at 8000 rpm for 15 minutes. The precipitated proroteins then were disolved in minimal amount of sodium acetate buffer pH 5.5 and the isopropanol was removed by ultrafiltration. Enzyme purification was executed by combination of numerous chromatographic steps. Ion exchange chromatography (DEAE-Sepharose, Q-Sepharose) followed by gel filtration (Superose 12). All chromatographic steps were performed with fast performance liquid chromatography system (FPLC) at 4°C. Protein contents were monitored by determination of absorbance at 280 nm, meanwhile the inulinase activity was assayed by monitoring released reducing sugars by bicinchoninic acid method (BCA). Purified inulinase enzyme exhibits optimal pH and temperature at pH 7 and 60 °C, respectively. The effects of different metal ions were also investigated. Based on results obtained presence of Na^+, K^+, Ca^{2+} and Mg^{2+} ions acted as inhibitors. Detailed characterisation of this enzyme is still in progress, but it can be summarised that *Thermomyces lanuginosus* IMI 140524 strain was capable of producing extracellular inulinase, which is very promising to stable at higher temperature and broaden range of pH.

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CYTOLETHAL DISTENDING TOXIN V (CDT-V) IS CARRIED BY A P2-LIKE PROPHAGE IN AN ATYPICAL BOVINE *ESCHERICHIA COLI* O157:H43 STRAIN

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Cytolethal distending toxin V (CDT-V) is an emerging virulence factor of enterohaemorrhagic *Escherichia coli* (EHEC), Shiga-toxigenic *E. coli* (STEC), and pathogenic *E. coli* strains with atypical pathotype. Earlier we found that atypical *E. coli* O157 strains also produced this variant of CDT. In this study we sequenced the flanking regions of the cdt-V operon of a bovine *E. coli* O157:H43 strain. Sequence analysis revealed that the cdt-V operon is harboured by a 32.3 kb long

prophage, with strongest homology to the P2-like bacteriophage L-413C. The cdt-V operon is integrated into the TO region of the P2-like prophage. The average GC content of the prophage sequences is 54%, while that of the cdt-V operon is 43%, suggesting that the acqusition of the toxin genes by the prophage might be a recent evolutionary event. The prophage is integrated into the chromosome of the host strain between the housekeeping genes *cpx*P and *fie*F, which is a known integration site for P2-like prophages in *E. coli*. We performed PCR screening for characteristic structural genes and genetic regions of this P2-like prophage in a collection of CDT-V-producing (n=7) and CDT-V-negative *E. coli* strains (n=14). The monitoring of P2-like genes revealed that they are strongly associated with the presence of the cdt-V operon. CDT-V producing O157 strains of atypical pathotype (n=4) were positive for the majority of the investigated genetic regions, while the CDT-V producing O157:NM STEC strains (n=3) showed some sequence differences, and it seems that in these strains the integration sites of the prophages are different. Phage induction experiments revealed that some of the CDT-V producing strains carried lytic phages, but none of them proved to be cdt-V-positive. When compared to the available P2-like prophage sequences, our results also show that so far cdt-V is the only established virulence gene cluster known to be integrated into the TO region of P2-like prophages.

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MODELLING THE G1 AND G2 SIZE CONTROL MECHANISMS IN FISSION YEAST

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Size control maintains size homeostasis in cell populations, it enables that size distribution does not change in consecutive generations. The most important requirement is that there must be at least one cell cycle event, which cannot be performed unless the cell has reached a critical size. Generally, two events are known to be controlled by cell size, namely initiating DNA replication (S phase) and the onset of mitosis (M phase). As a consequence, two size checkpoints exist in the eukaryotic cell cycle, one in G1 phase, and another one in G2. Since the late 1970s fission yeast is an attractive model organism in size control studies. The cylindrically shaped fission yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. In wild-type fission yeast cells, G2 phase is long and size-dependent, meanwhile G1 is short and constant, therefore, size control seems to operate in G2. The wee1 mitotic inhibitor was found to be mainly responsible for this size checkpoint. The general view is that in small cells wee1 keeps the cdc2/cdc13 complex (also known as M-phase promoting factor or MPF) in an inactive form. After reaching a critical size, wee1 itself becomes inactivated by some mitotic activator(s) and the cell starts to prepare for mitosis. Recent experimental data seems to give a new clue how this size control mechanism might operate. Namely, a spatial gradient of a mitotic inhibitor (pom1, indirectly acting positively on wee1) is generated along the cell cortex, having a maximal value at the cell tips, and a minimum at the centre. As the cell grows, local pom1 concentration at the cell centre decreases and finally in late G2 it drops below a critical level, which is no more able to delay mitotic onset. In wee1 mutants, the G2 size control is abolished. By contrast, these cells are viable (although small, hence the Scottish name wee) and their population proliferate with a generation time similar to that of wild-type. However, in a typical wee1 mutant cell of a steady-state exponential phase culture, G2 is much reduced and

G1 is much extended, indicating that size control acts in G1 rather than in G2. There is unfortunately no known experimental data suggesting any idea on how this G1 size control might operate. We hypothesize that an inhibitor of the G1/S transition might be diluted in the cell centre as a consequence of the increase in cell size, i.e., spatial gradients may cause both size controls in the cylindrical cells of fission yeast. In the case of the G1 mechanism, our candidate to have this role is the ste9 protein, which is responsible for ubiquitination of cyclins in G1, thereby keeping cyclin-dependent kinase activity nearly at 0.

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DETERMINATION OF FECAL CONTAMINATION SOURCES IN RIVER TISZA USING ANIMAL VIRUSES

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Many microbes shed into natural waters with the untreated or not properly treated wastewater. These microbes can cause several diseases by water consuming and water recreational activities. The determination of the origin of these contaminations is the very first step to preserve the quality of our waters. Our aim was to introduce a new method in Hungary to determine the source of wastewater contamination using animal viruses. In this study porcine adenovirus and bovine polyomavirus were detected. These viruses do not cause any specified diseases, but they are constantly presented in their host populations. These viruses are collectively known as enteric viruses, because they are excreted via faces or urine. Samples were taken biweekly from January 2011 to March 2012. The samples were originated from raw sewage, secondary treated effluent and four surface water sampling points along the River Tisza. Seven new sampling points were added later, for tracking the source of contaminations. Altogether 123 surface water and 57 wastewater samples were concentrated by direct flocculation. Taxon- specific real-time polymerase chain reaction was applied for the quantitative detection of porcine adenovirus and bovine polyomavirus from the virus concentrates. Results were confirmed by nested PCR, when the result was close to the detection limit. The efficiency of the concentration method and the nucleic acid extraction method, as well as the presence of animal viruses in surface waters and urban sewage water was verified in this study. The porcine adenovirus was detected between 7.55×10^2 - 1.99×10^7 genome copies/L and the bovine polyomavirus was presented between 2.04×10^2 - 6.09×10^5 genome copies/L in the surface water samples. The measured values in the sewage waters were larger by 2 to 3 magnitude compared to the river water samples.

Sewage treatment reduced the viral titers and was more efficient for adenovirus elimination. General temporal and spatial tendencies have been observed by the alteration of the viral load. Despite the low number of the analyzed contamination sources, there was a remarkable correlation between the contamination of the inflows and the presence of the nearby farms.

DISTRIBUTION OF ANOGENITAL HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES IN CERVIX SAMPLES AND IN ANUS OF MSM SAMPLES IN HUNGARY

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Human papillomavirus (HPV) is one of the most common sexually transmitted infections. HPV is causally linked to the development of anal and cervical cancer. 90-99% of the world's cervical cancer cases and 90% of the world's anus carcinoma cases are caused by HPV. Over 100 human papillomavirus (HPV) types have been identified, of which 40 infect the anogenital tract. Prevalence, distribution and diversity of HPV types are different in countries and in parts of body. We examined prevalence, distribution and diversity of HPV infection in cervix samples from United Saint István and Saint László Hospital and from a private clinic, and in anus samples of MSM (men who have sex with men) from a 2011 finished cross sectional study. HPV was detected and genotyped by the Roche Linear Array HPV Genotyping Test. Medical and behavioural risk factors were evaluated. HPV genotype distribution was assessed by geographic region and in comparison with published data. HPV was detected in 78 (97,5%) of 80 HIV infected patients, in 7 (58,3%) of 12 HIV negative patients and in 103 (66,5%) of 155 cervix samples. High risk genotype was detected in 88,8% of HIV positive, in 33,3% HIV negative and in 80,6% of cervix samples. 75,0% of HIV positive, 58,3% of HIV negative and 47,6% of cervix samples were positive for low risk HPV type. The most common HPV types in descending order of frequency were HPV 16 (43,6%), 6 (24,4%), CP6108 (24,4%), 18 (23%), 59 (23%), 51 (21,8%) in more than 20% of HIV infected patients' anus samples, HPV 16, 84, 61, 53 in HIV negative patients' anus samples, HPV 16 (27,2%), 31 (15,5%), 51 (14,5%), CP6108 (24,4%), 58 (10,7%) in more than 10% of cervix samples. Infection with multiple genotypes in HPV positive cervix samples compared with HPV positive anus samples multiple genotypes were detected in less than half (49,5%) of cervix specimens and in 84,6% of HIV infected MSM's anus samples. Multiple genotypes infection isn't too frequent in cervix samples but it's absolutely common in HIV infected MSM patients' anus samples, 2-12 genotypes in one sample. These results are essential for planning prevention by HPV vaccines, for HPV public information and for screenings program based on HPV testing. Distribution and diversity of HPV types are different in HIV infected patients' anus samples and in cervix samples in Hungary. Typical MSM's anus HPV types have started to appear in some cases of female samples because of spreading of anal intercourse and prostitution. There are more common HPV types in Hungarian population than in vaccination HPV types. It should be other types in vaccine in Hungary. Early male vaccination besides female is absolutely recommended.

CONTROL PLANT-PARASITIC NEMATODES WITH *TRICHODERMA* SPECIES: CHITINASE AND PROTEASE GENE EXPRESSIONS DURING NEMATODE EGG-PARASITISM

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Plant-parasitic nematodes have a substantial impact on human welfare and economy either by attacking root systems, stems, buds or by acting as vectors of plant viruses. Despite the propensity of

nematicides to be lethal to a broad range of soil organisms, and to induce the development of resistant strains among nematodes, they are still widely used to control plant-parasitic nematodes. *Trichoderma* species are able to antagonize plant pathogens and they have also potential to control plant-parasitic nematodes without negative impact on the environment. Since plant-parasitic nematodes and *Caenorhabditis elegans* eggs share many essential features, *C. elegans* was used as a model system in this study. In vitro comparison of eighteen strains of five *Trichoderma* species revealed that *T. harzianum* strains possess the strongest egg-parasitic ability. The microscopic observation of egg-fungus interactions revealed clear evidence that all characteristic events of egg-parasitism ie. coiling hyphae around eggs, formation of appressorium-like structures, trophic hyphae growing inside the eggs occur in a time period of the ex utero development of *C. elegans* (9-11 hours). Quantitative RT-PCR analyses revealed that chitinase chi18-5 (log2 fold change: 3.60) and chi18-12 (log2 fold change: 10.32), acidic serine peptidase pra1 (log2 fold change: 3.44), sedolisin serine proteinase p5216 (log2 fold change: 5.20) and aspartic peptidase p6281 (log2 fold change: 3.70) genes were highly induced by the presence of nematode eggs. Upregulation of aspartic peptidase p9438 (log2 fold change: 2.62) and metallo-endopeptidase p7455 (log2 fold change: 3.21) genes were also characteristic, however fold changes were lower than other proteases. Our results can contribute to a better understanding of the complex host-pathogen interactions.

HUMAN PAPILLOMAVIRUS TYPE 16 E7 ONCOPROTEIN EXPRESSION IS ASSOCIATED WITH ACTIVATION OF SRC-FAMILY KINASES IN KERATINOCYTES

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Src-family tyrosine kinases (SFKs) are cellular regulatory proteins that influence cell adhesion, proliferation, invasion and survival. In light of recent publications indicating that elevated activity of SFKs contributes to the increased cell proliferation and invasivity in human papillomavirus (HPV)-associated malignancies, we investigated whether it is a down-stream effect of papillomaviral oncoproteins. We investigated the expression and activity of ubiquitously expressed SFKs, namely Src, Yes, and Fyn in human keratinocytes expressing HPV 16 E6, E7 or both oncoproteins. Furthermore, we studied whether the effect of HPV 16 E6 and E7 oncoproteins can be affected by cellular differentiation. Western blot analysis showed that Src and Yes protein expression is influenced differently by HPV oncoproteins and differentiation, whereas Fyn protein levels were not affected by them. However, phospo-kinase array revealed that ubiquitously expressed SFKs are activated by phosphorylation in the presence of E7 oncoprotein. Taken together, our study identified an important mechanism of HPV oncoproteins which might contribute to the development of malignant host cell phenotype in HPV-associated malignancies.

IDENTIFICATION OF SURFACTIN HOMOLOGUES FROM A *BACILLUS SUBTILIS* STRAIN USING ION-TRAP MASS SPECTROMETRY

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Lipopeptides, such as iturin, surfactin and fengycin are non-ribosomal oligopeptides synthesised by large multienzyme complexes of several *Bacillus* species. Lipopeptides possess a number of remarkable biological actions such as plant diseases control, anti-tumour and anti-viral activities as well as a wide anti-microbial spectrum. The anti-microbial effects are due to their exceptional surface activity that allows modifications of the bacterial surface hydrophobicity and can consequently interfere with the microbial adhesion to solid surfaces. Surfactin family is a mixture of cyclic lipopeptides built by variants of a heptapeptide and a linked β-hydroxy fatty acid with chain length of 13–15 carbon atoms. A lactone bridge between the β-hydroxyl function of the acid and the carboxy-terminal function of the peptide confers a cyclic structure to the molecule. In our study a *Bacillus subtilis* strain was selected, which is able to produce surfactins in relatively high amounts according to the results of our earlier investigations. From the ferment broth the lipopetide mixture was precipitated with 6 N HCl and extracted by methanol. To decrease the possibility of the formation of artifacts, the surfactins were analyzed by reversed-phase high-performance liquid chromatography with electrospray ionization ion trap mass spectrometry (RP-HPLC/ESI-IT-MS) immediately after the extraction of the culture material without any additional sample cleaning. In full scan ESI-MS spectra the sodium adduct molecular ions of various surfactin molecules were detected at m/z 1016.9, 1030.9, 1044.9 and 1058.9, which represent the cyclic lipopeptide backbone and the linked fatty acid chains with various length (C12-C15). The further MS-MS analysis of the molecular ions allowed the elucidation of the structures and the identification of the surfactin types, because the appeared m/z values at 707 and 693 resulted from the loss of the fatty acid and the linked glutamine residue characteristic for the type A and B, respectively.

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ANALYTICAL MEASUREMENTS OF PROSTAGLANDINS FROM PATHOGENIC YEASTS

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Together with the closely related thromboxanes and leukotrienes, prostaglandins are present at trace levels in nearly all mammalian fluids and tissues. The members of these substances are active metabolites of an unsaturated fatty acid, arachidonic acid, and may be derived through several different biochemical pathways in different organisms. Recently, some fungal strains such as *Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans* have been reported to produce prostaglandin (PG)-like substances. The production profile and kinetics of these fungal eicosanoids and their role in the biological processes are however still unknown. Thus, their quantification in the microbiological matrices is important to monitor their PG producing ability as well as to understand their biological relevance. For the simultaneous analysis of prostanoids, the

precolumn tagging methods utilizing fluorescence detection after the HPLC separation provide a highly selective and sensitive tool. In this case, the derivatization reagents act on the carboxyl group of PGs resulting detectable esterified compounds. In our study, the halogenoalkyl type 3-Bromomethyl-6,7-dimethoxy-1-methyl-1,2-dihydroquinoxaline-2-one (Br-DMEQ) was use to convert the prostaglandins into the corresponding fluorescence derivative in the presence of potassium hydrogen carbonate and 18-crown-6 as base catalyst and phase transfer agent, respectively. During the research work the derivatization parameters were optimized using prostaglandin (PG) F2α as selected reference compound including the reaction temperature, amount of reagents, and the incubation period. Than a chromatographic separation were developed for derivatives of number of prostaglandins, prostaglandin metabolites and cyclopentanose prostaglandins including 6-keto-PGF1α, PGF2α, PGF1α, PGE2, PGE1, PGB2, PGJ2, 15-deoxy-12-14-PGJ2, PGA2, PGD2, 13,14-dihydro-15-ketoPGD2, 13,14-dihydro-15-ketoPGE2, 11β-PGF2α, 13,14-dihydro-15-ketoPGF2α, PGF2 β. These compounds are separated within 60 min on a reversed phase column (Phenomenex Kinetex) with mixture of acetonitrile, water and acetic acid.

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IDENTIFICATION OF BIOSURFACTANTS FROM A POTENTIAL BIOCONTROL *BACILLUS AMYLOLIQUEFACIENS* STRAIN

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A large number of microorganisms generate a variety of biosurfactants and can exist in a variety of environments, including soil, marine, desert, and aqueous environments. Biosurfactants are biologically surface active agents that can be biosynthesized by diverse microorganisms, including bacteria, fungi, and yeasts. These compounds are amphipathic molecules with both hydrophilic and hydrophobic residues. The bacteria belonging to the *Bacillus* genus are well known as producers of lipopeptide biosurfactants. A strain of *Bacillus amyloliquefaciens* used in this study has been isolated in our laboratory from tomato rhizosphere in the south Hungary and identified by 16S rRNA gene analysis and partial *gyr*A gene sequence analysis. It showed high levels of antagonistic properties against a number of microorganisms that attack tomato plants both in seedling and in developed foliar state, maybe due to the production of bioactive peptides. In our study, these compounds were extracted and identified using analytical techniques. Initially, the chromatographic run was developed for crude extract by reversed-phase high-performance liquid chromatography (RT-HPLC) with UV detector, than the optimized separation was used for the electrospray ionization ion trap mass spectrometry (ESI-IT-MS) investigations. After the tuning of the mass spectrometer with the biosurfactant iturin A standard, the optimal ion source and mass filter parameters for the lipopeptide measurements were determined. During the full scan measurements the lipopeptid components were identified as mainly the members of the fengycin families based on the acquired m/z values. For structural elucidation, MS-MS fragmentations of selected molecular ions were carried out and the sequences were determined using the characteristic fragment ions.

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INVESTIGATION OF MULTIDRUG RESISTANT *ACINETOBACTER BAUMANII* STRAINS OF ICU ORIGIN

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Acinetobacter baumannii is responsible for nosocomial infections in critically ill patients in intensive care units. This organism has ability to adapt to the hospital environment and to take up resistance genes, which help to survive for a long time and cause epidemic outbreaks in hospitals. We compared the virulence factors and resistance patterns of *A. baumannii* strains isolated from samples of patients and staff in intensive care units between 2008-2011. Biofilm formation, serum resistance, siderophore production were investigated and resistance patterns were compared. PCR method was used to detect integrase genes. Most of the strains produced biofilm and siderophore. Only few strains were sensitive to serum. The multidrug resistant (MDR) *A. baumannii* strains belonged to three different resistance patterns and each strain carried the integrase 2 gene. Multidrug resistant *A. baumannii* strains originated from ICU may occur due to the selection effect of broad-spectrum antibiotic therapy. MDR *A. baumannii* strains with three different types of resistance patterns have circulated for years. Biofilm formation may play role in surviving and in the virulence process. The serum resistance helps the bacteria to cause bloodstream infection. These MDR *A. baumannii* strains have adapted to hospital environment and became more virulent.

GENETIC VARIABILITY OF BLACK *ASPERGILLUS* ISOLATES ORIGINATED FROM CEREALS IN HUNGARY

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Black Aspergilli (*Aspergillus* section Nigri) are commonly found as soil organisms decomposing dead plant residues and they are pathogenic on several crops. The plant pathogens are of concern not only for their ability to destroy several agronomically important food crops, but also due to their ability to produce several mycotoxins including ochratoxins and fumonisins. Black Aspergilli associated with any plant pathological problem are usually mentioned as *A. niger* in early publications. However, *Aspergillus* section Nigri is one of the more difficult groups concerning classification and identification and closely related species belonging to this taxa are difficult to be assigned based solely on their phenotypic characters, but all species can be distinguished from each other using calmodulin or β-tubulin sequence data. In this study 57 isolates originated from cereals (corn, wheat, barley) and soil were identified at the species level based on partial calmodulin sequence data. 47 isolates were found to belong to the *A. niger*, while 10 isolates to the *A. tubingensis* species. The genetic variability of the isolates was analysed using the UP-PCR method using eight primers. All of the isolates were found to belong to unique haplotypes. Distribution of the mating type genes was examined using a diagnostic PCR approach and 74.4% of the *A. niger* isolates were found to belong to the MAT1-2, while 90% of the *A. tubingensis* isolates belonged to the MAT1-1 mating type. It indicates a clonal population structure, what was also supported by the population genetic analysis of UP-PCR data (index of association and parsimony tree-length permutation tests). Studies are in progress to examine the mycotoxin producing abilities of isolates.

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FILOGENETIC DIVERSITY OF BACTERIAL COMMUNITIES INHABITING A COOLING RESERVOIR USED FOR STORAGE OF THERMAL WATERS

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Lake Therm-Organ located near Szarvas is a cooling reservoir used for the temporary storage of thermal waters before being redirected into the river Hármas-Körös. Water of this lake is anaerobic, alkaline, rich in phenol and is of high salinity. The aim of this research was the examination of the chemical composition as well as the phylogenetic diversity of bacterial communities inhabiting the lake water and sediment, the thermal water of the well and the utilized thermal water. The bacterial phylogenetic diversity was examined by cultivation based and molecular biological denaturing gradient gel electrophoresis (DGGE) and clone library methods. Depending on the time of residence and season, the chemical composition and biological quality of the thermal inflow transformed in the reservoir. Significant reduction of the concentration of some chemical parameters was observed. Compared to the thermal inflow, the concentration of COD, BOD5 and total phenol decreased significantly in the reservoir in summer. Regardless of the date of sampling, bacterial communities of water samples showed the highest similarity to each other on the basis of the results of 16S rDNA DGGE analysis which indicates a uniform distribution of planktonic bacteria in the water body. Sediment samples formed distinct similarity groups from the water samples. In the structure of water and sediment bacterial communities, temporal changes were more pronounced than spatial differences among the sampling sites. Bacterial communities of the thermal inflow each time had a unique band pattern which differed from the water and sediment samples, as well. Members of a total of 13 phylogenetic phyla, such as: Proteobacteria, Deinococcus-Thermus, Aquificae, Nitrospirae, Firmicutes, Caldithrix, Chloroflexi, Cyanobacteria, Tenericutes, Bacteriodetes, Actinobacteria, Spirochaetes, Deferribacteres were detected by the applied cultivation-based and cultivation-independent methods. The greatest diversity was revealed using the clone library method. Altogether 82 ARDRA representatives of molecular clones from the influent water, 54 from the lake water, and 60 from the sediment samples were sequenced. By cultivation, the presence of strains belonging to Alpha- and Beta-Proteobacteria was detected. Members of the following genera were the most abundant: *Spirochaeta, Thauera, Rhodobacter, Alkaliflexus, Dehalogenimonas, Desulfobacterium, Achromobacter*. Based on literature data, the majority of microorganisms detected in Lake Therm Organ is haloalkaliphilic, and able to use a wide range of different carbon sources including aromatic hydrocarbons, as well.

ELECTRICITY GENERATION BY SINGLE-CHAMBER MICROBIAL FUEL CELL USING NICKEL ON AIR-CATHODE

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Microbial fuel cell (MFC) is a biochemical-catalyzed system which generates electrical energy through the oxidation of organic matter in the presence of fermentative bacteria, thus it provides an alternative method for simultaneously producing renewable energy while treating wastewater. Basically, the potential developed between the bacterial metabolic and electron acceptor conditions separated by a membrane leads to generation of bioelectricity. Design (electrodes, membrane, etc.), substrates, microorganisms, operating conditions (pH, temperature, etc.) are important factors in efficiency of MFC. In this study, the electricity generation by single-chamber MFC using nickel on air-cathode was focused. The *Geobacter sulfurreducens* DSMZ 12127 strain was selected as biocatalyst in MFC that is known as strict anaerobic microorganism. Pure Na-acetate was applied as substrate for production of exoelectrons. The electric potential (voltage) generated by MFC was monitored by VC-820 multimeter connected to PC. Metabolism of G. sulfurreducens was followed by analysis of samples using HPLC technique. Single-chamber microbial fuel cell was designed and constructed in our laboratory. Graphite electrode was used in anode chamber with 150 cm^2 of total surface. The working volume of anode chamber was 825 mL. Cathode was formed by grid coating with nickel. Nafion N-117 was used as proton selective membrane. Operation of MFC was modeled using Na-acetate as substrate in different concentration from 0.5 g/L to 1.5 g/L in steps of 0.25 g/L. In all cases of acetate concentrations, after the inoculation the voltage of MFC was increased rapidly. Generally, the highest level of the potential difference between the electrodes reached about 150 mV - 210 mV after two days (50 h) operation. In this stage, Na-acetate concentration in anode chamber decreases to 2/3 of initial concentration. Increase in Na-acetate concentration resulted decrease of the maximal voltage in MFC. In the case of 0.5 g/L Na-acetate concentration the potential difference was the highest (0.212 V) which provides the highest powerdensity (7.35 mW/m2). Meanwhile in the case of 1 g/L Na-acetate the voltage was below 0.195 V and in case of 1.5 g/L the maximum voltage was less than 0.15 V. The electrical potential was kept in constant in the time period from 50 to 150 hours of operation and then decreased slowly. In this stage, very low change in amount of Na-acetate was detected. Summarizing, the single-chamber MFC with nickel air-cathode using *G. sulfurreducens* DSMZ 12127 strain was designed, constructed and able to generate electrical potential on Na-acetate as substrate. These results can serve as good base for development of suitable technology for treatment of wastewater.

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A COMPARATIVE STUDY OF THE INFECTIVITY AND THE FUNCTIONAL ASPECTS OF INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI ORIGINATED FROM ORGANIC AND CONVENTIONAL AGRICULTURE SOILS

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Nowadays more and more attention is paid to environmental friendly and cost effective technologies for soil management and crop production. In contrast to the conventional agricultural practice, the new technologies - such as organic farming systems with low input of fertilizers, biocides, minimized tillage and monocultures - can contribute to long-term stability and productivity. Soil microbial community is a key component of soil functionality and resilience and it is also an important indicator of the state of natural and agricultural ecosystems. One of the most widespread kind of soil-borne fungi is the arbuscular mycorrhizal one (AMF, Glomeromycota), which forms mutualistic associations with 80-90 % of terrestrial plants. The beneficial effects of AM fungi on host plant nutritional status, vitality, fitness and tolerance to stress conditions were proved. Our aim was to compare the status and functionality of AM fungi indigenous in an organic and a conventional agricultural ecosystem in Martonvásár. In a pot experiment a strongly mycorrhiza-dependent vegetable (pea, *Pisum sativum*) and a less dependent crop plant (an ancient wheat species, *Triticum spelta*) were used to analyse potential differences in the functionality and the fungal composition of AM in relation to host type and agricultural practice. AMF propagule density of the two differently managed soils were modified by applying a 5 dilution MPN (Most Probable Number) method. The parameters of AMF root colonisation were determined by staining the roots and microscopic analysis using the five class system method (F%: frequency, M%: intensity of colonisation, a% and A%: relative and absolute arbuscularity). Samples were tested for AMF functionality realised in plant growth, nutrient uptake and photosynthetic efficiency.

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SILVER FUNCTIONALIZED TiO_2 COATED LAMP ACTIVATED WITH LED LIGHT SOURCE HAS AN ANTIMICROBIAL EFFECT IN INDOOR AIR SAMPLE

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The development of sterilizing methods is an important step to prevent the spread of pathogens in hospital and health care settings. Varied disinfectants have been used to kill pollutants and microorganisms on the surface and in the air. It is already known that TiO_2 can be activated under UV-light irradiation and has a pronounced antimicrobial activity because of producing highly reactive radicals. The photocatalytic effect of noble metal functionalized TiO_2 has been presented in many studies. The wavelength of light source of photocatalysis can be extended to the visible light region with plasmonic catalyst so the noble metal functionalized surface can be widely used in air sterilization. In this study we present the antimicrobial effect of silver functionalized TiO_2 coated lamp (reactive lamp) in indoor air sample. The reactive lamp was developed by General Electric Hungary. The inner surface of the reactive lamp was prepared using the spray coating technique. The silver functionalized TiO_2 was activated with LED-light source ($\lambda = 405$ nm) inside the lamp to express the antimicrobial effect of the lamp when it is turned on. The antimicrobial activity of the reactive lamp was carried out in a closed photo reactor using RCS PLUS air sampler. Parallel experiments were carried out with a photocatalyst-free lamp and without lamp. Colony forming units were counted on nutrient agar (HYCON Agar Strips TC) and compared to the null-point

sample. Two parallel experiments were carried out in each case and the counts of different adhering bacterial/fungi colony forming units were averaged. Gram positive cocci and different molds were isolated from air samples. We found an antimicrobial effect by using reactive lamp, the percent reduction of the colony forming units was 69.7% after 24h illumination and 96.3% after 48h illumination. Using photocatalyst-free lamp, the percent reduction of the colony forming units was 23.3% after 24h illumination and 23% after 48h illumination. According to the experiment without a lamp the percent reduction of the colony forming units was 26.9% after 24h illumination and 38.5% after 48h illumination. After 16 days light activation the reduction of the colony forming units was still 92.5% after 48h illumination in the photo reactor. The results showed that the antimicrobial effect of the reactive lamp was sufficiently high, the reactive lamp was able to kill a wide spectrum of bacteria and fungi in indoor air sample after a 48h operation.

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COMPARATIVE PHYLOGENETIC ANALYSIS OF THE ALKANE-1-MONOOXYGENASE, GYRASE B AND 16S RRNA GENES OF THE GENUS *RHODOCOCCUS*

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Species of the actinomycete genus *Rhodococcus* are of interest for their enormous metabolic abilities. Many of them can degrade a wide variety of environmental pollutants including petroleum hydrocarbons which are the main groundwater pollutants in Hungary. The classification of the genus has considerably changed in the recent years as some species were reclassified while the description of new species is permanent. The molecular taxonomy of the genus is based on the conventional rule that bacteria with 16S rDNA similarities of 97% and above may be the same species and where there is doubt, DNA-DNA hybridization values can be obtained. Nevertheless, some *Rodococcus* strains, which share 99% or even 100% 16S rDNA similarity with formerly described species have been accepted recently as new members of the genus. Notably *R. percolatus* has 99.3% 16S rDNA sequence identity with *R. opacus, R. baikonurensis* shows 99.2% 16S rDNA homology with *R. erythropolis, R. qingshengii* shows 99.8% 16S rDNA homology with *R. baikonurensis*, while *R. jialingiae* shows 100% 16S rDNA homology with *R. qingshengii*. In most of these cases the low DNA-DNA hybridization value was the basis of species distinction. However, the routine identification of environmental *Rhodococcus* isolates is still based on the determination of the 16S rDNA sequence. Therefore the description of new species showing even 100% 16S rDNA homology with other species of the genus makes the 16S rDNA sequence based identification of environmental *Rhodococcus* isolates ambiguous. In order to address this problem, we tried to find marker genes, which are harbored by each member of the genus *Rhodococcus* and may have evolved faster than the rrna operon, providing larger phylogenetic resolution. We have chosen the gyrase B (*gyr*B) and the alkane-1-monooxigenase (*alk*B) genes to investigate their possible role as phylogenetic marker genes. It was found that *R. qingshengii* has 100% *alk*B sequence identity with *R. jialingiae*, and they are only distinguishable based on their *gyr*B sequences, by showing ~99.5% *gyr*B sequence identity. Besides, *R. qingshengii* is readily distinguishable from *R. baikonurensis* both upon the *gyr*B and *alk*B gene sequences by showing 98.5% and 96.8% sequence identity respectively.

Similarly *R. baikonurensis* can be differentiated from *R. erythropolis* by showing 98.5% *gyr*B and 97.2% *alk*B gene sequence homology. Interestingly *R. erythropolis* shows 100% *gyr*B sequence homology with *R. globerulus*, while they have 98.9% 16S rDNA and 85% *alk*B sequence identity. Results of this genotyping approach may help us to better reveal the phylogenetic relationship of *Rhodococcus* species and of closely related environmental isolates.

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COMPLETE GENOME SEQUENCE OF THE HYDROCARBON-DEGRADING *CUPRIAVIDUS BASILENSIS* OR16

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Until now, five *Cupriavidus* genome projects have been completed for representatives of the species *C. necator, C. metallidurans, C. pinatubonensis*, and *C. taiwanensis*. The sizes of these genomes range between 6.5 and 8.5 Mbp, thus the genus belongs to a group of microbes which have a large genome. The subject of our genome project, *C. basilensis* strain OR16, was isolated from a Hungarian pristine soil sample. It was identified by molecular taxonomy as *C. basilensis*. Regarding its metabolic properties, it was characterized as a good petroleum hydrocarbon-degrading strain. A unique feature of this isolate, in contrast to all known *Cupriavidus* strains, is that it can use glucose as the sole carbon and energy source for growth. Genome sequencing of *C. basilensis* OR16 was performed by combining the cycled ligation sequencing on the SOLiD 4 system (Life Technologies) with 454 FLX pyrosequencing (Roche). We generated 205,522,122 mate-paired (2 by 50-bp) reads on SOLiD along with 165,651 (360-bp) reads on 454 FLX, which altogether yielded 1,500-fold coverage. Assembly was performed using the Genomics Workbench 4.8 de novo plug-in and the Omixon Gapped SOLiD alignment 1.3.2 plug-in provided by CLC Bio and Omixon, respectively, which generated 416 large (200 kbp) contigs. Automatic annotation of the genome was performed with the NCBI Prokaryotic Genomes Automatic Annotation Pipeline which utilizes GeneMark, Glimmer, and tRNAscan-SE searches. The uncompleted draft genome of *C. basilensis* OR16 consists of 8,546,215 bp, with a GC content of 41.2% and 7,534 putative coding sequences. The genome sequence of *C. basilensis* OR16 reveals an impressive catabolic potential, since several ring cleavage pathways for aromatic compounds were found, including catechol and protocatechuate ortho ring cleavage pathways, a catechol meta ring cleavage pathway, gentisate and homogentisate pathways, a hydroxyquinol pathway, a hydroquinone pathway, and a benzoylcoenzyme A pathway. Its survival under heavy metal stress conditions is ensured by genes encoding heavy metal transport/ detoxification proteins, such as copper-zinc-cadmium-chromate resistance proteins (copCD, a copper chaperone) and heavy metal efflux pumps (copper/heavy metal efflux P-type ATPases and CzcA family heavy metal efflux pumps). Interestingly, besides an *Opr*B glucose porin, a putative membrane-bound PQQ-dependent glucose dehydrogenase gene (OR16_10529) was also identified, which catalyzes gluconate production from glucose. The presence of this gene is unique in strain OR16, as none of the other known *Cupriavidus* genomes encode it or its homologues, and this may account for the unusual glucose metabolism of strain OR16.

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HUMAN PAPILLOMAVIRUS CARRIAGE OF THE HEALTHY ORAL MUCOSA IN EASTERN HUNGARY

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It is well known, that human papillomaviruses (HPVs) are able to infect the mucosa of the head and neck region and induce benign, premalignant and malignant disorders in the oral cavity. A proportion of the infections, however, remain subclinical and the virus may be present in healthy individuals. To reveal the exact role of HPV infection in oral carcinogenesis, the prevalence and viral load of HPV DNA were examined in the healthy oral mucosal samples of individuals from Eastern Hungary. Exfoliated cell samples from buccal and lingual mucosa of 196 healthy individuals (63 male, mean age 39 years, range 16-77 years, 133 female, mean age 39 years, range 10-77 years) were collected. HPV DNA was detected by MY/GP consensus nested PCR and virus genotype was determined using restriction fragment length polymorphism or sequencing. As HPV16 is the most frequent genotype, HPV16-specific PCR was also performed. Virus copy numbers were determined by real-time PCR assay using the MY consensus primers or HPV16 specific primers with SYBR-Green as a detection method. HPV DNA was demonstrated in 11.7% of the individuals (23/196), all positive individuals carried the virus in the buccal mucosa, one individual had viruses in both samples. Age and gender of the persons did not influence significantly the carrier rate, 13.0% and 10.4% in persons below and above the age of 35, respectively, 11.1% of males and 12.0% of females were carriers. Out of the 23 positive samples six carried low-risk (three HPV6, and one each of HPV11, HPV55, HPV61), while seven high-risk HPV genotypes (six HPV16 and one HPV56), in case of ten samples genotype determination was not possible sue to low virus copy numbers. Both males and females harboured low-risk and high-risk genotypes in roughly equal proportions (two HPV6s and one HPV61 vs. three HPV16, one HPV was not genotyped in males and (one each of HPV6, HPV11 and HPV55 vs. three HPV16 and one HPV56), though in females the majority of HPVs (nine cases) could not genotyped. HPV copy number average was $3.9x10^2$ ($10-9.8x10^2$) copies/1 μg total cellular DNA, and copy numbers were comparable between the two age groups as well as between males and females. These copy numbers are significantly lower (independent samples t-test, $p=0.002$) than in oral squamous cell cancer as determined in a previous study ($2.4x10^5$ copies/1 μg total cellular DNA). In summary, frequency of HPV carriage is cca. 10% in the Eastern Hungarian population and is not influenced by age or gender. These data confirm that the well-documented significantly higher prevalence of HPVs in premalignant and malignant lesions of the head and neck region point to the etiological role of HPVs in these diseases.

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THE EFFECT OF ADDITION OF HARDLY DEGRADABLE SUBSTRATES TO BIOGAS PRODUCTION IN A WASTE WATER SLUDGE DIGESTER

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Investigating the microbial communities of biogas reactors is a highly relevant area of microbiological studies, since biogas is one of the primary alternative energy sources of today. Biogas is a mixture of CH_4, CO_2 and other trace amount gases produced during the anaerobic degradation of organic matter. Hydrolysis of monomers to organic acids, CO_2 and H_2 and production of methane from these compounds is the result of the cooperation of different microbial communities. The aim of the present work was to reveal the microbial community structures of waste water sludge digester before and after feeding with hardly degradable substrates. Two laboratory scale mesophilic (35°C) methane producing bioreactors were fed with 6-6 g lignocellulose (milled corn cob) and cellulose (MN300), in addition to 100-100 ml of sterilized raw wastewater sludge. A control reactor was fed only with 100 ml raw sludge. The experiment took 20 days and four feedings occurred during this time. Gas production was measured continuously, and samples were taken from the biomass of the reactors to chemotaxonomic (PLFA analysis) and molecular biological studies (T-RFLP analysis). Cellulose feeding led to a relatively fast gas yield increase at the first time, but during the four feedings the yield gradually dropped under the control level (at the end). Lignocellulose needed a longer adaptation period to get utilized, but in the third week the treated reactor showed a considerable yield increase. Chemotaxonomic and DNA biomarkers of the communities show shifts in the ratio of eubacterial community members, while DNA markers show a moderate change in case of the methane producing Archaea.

CONNECTION BETWEEN SULFUR METABOLISM AND HYN HYDROGENASE OF *THIOCAPSA ROSEOPERSICINA* BBS

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The purple sulfur phototrophic bacterium, *Thiocapsa roseopersicina* BBS preferably utilizes sulfur compounds as electron donors and carbonate as inorganic carbon source for growth. *T. roseopersicina* contains modified Sox cycle which has an indispensable role in the assimilation of thiosulfate, few sulfide oxidoreductases such as flavocytochrome C and sulfide quinone oxidoreductases. DSR complex likely linked to oxidation of sulfur. All these processes release electrons which might be converted to H_2 via the hydrogenases and/or nitrogenase of the strain. Hydrogenases belonging to the group of metalloenzymes are capable of oxidation of molecular hydrogen and proton reduction. *Thiocapsa roseopersicina* BBS has four active NiFe hydrogenases. Hox1, Hox2 are cytoplasmic NAD^+ reducing hydrogenases, while the other two enzymes (Hyn, Hup) are bound to the membrane. The Hup and Hox1 hydrogenases are likely connected to the central quinone pool. The main electron transport routes to/from the hydrogenases are not fully understood. In order to disclose these metabolic pathways, we cultivated single hydrogenase containing strains in the presence of various kinds of electron donors and the amounts of H_2 and various sulfur compounds were followed. Hyn hydrogenase can produce hydrogen in the absence of carbonate. Under these conditions, sodium thiosulfate could promote hydrogen evolution. It was also shown that the oxidation of zero-valent sulfur can donate electrons to Hyn. Under these conditions, sulfur is an exclusive electron donor for both hydrogen evolution of Hyn and hydrogen sulfide formation, which are consequently competitive processes. Sulfide couldn't be used as an electron donor for

hydrogen evolution of Hyn. These results suggest that Hyn hydrogenase has a role in the elimination of extra electrons released from sulfur oxidation. Sulfite eliminates the formed sulfide which is produced from elemental sulfur as sole electron donor. Sulfite could also increase the hydrogen evolution of Hyn under the same conditions, which confirms our previous finding sulfide is not a direct electron donor for the hydrogen evolution of Hyn. Hydrogen evolution of Hyn hydrogenase was found only under illumination Moreover, the oxidation of various sulfur compounds was also blocked in darkness, and therefore the light dependency of hydrogen evolution might be an indirect consequence of the light requirement of sulfur oxidation. Oppositely, in the presence of elemental sulfur, hydrogen addition increased the Hyn related hydrogen sulfide formation, thus the connection between Hyn hydrogenase and sulfur metabolism was proved to be bidirectional. Glutathione amide forms were shown to be potential redox carriers in purple sulfur bacteria. Their role was investigated in the electron transport between sulfur metabolism and Hyn hydrogenase. It was also pointed out that the two electron transport subunits of HynSL – Isp12 - were indispensable.

COMPARISON OF THE DISTRIBUTION PATTERNS OF DIFFERENT IRON UPTAKE SYSTEMS AMONG *KLEBSIELLA PNEUMONIAE* STRAINS FROM SEWAGE TREATMENT PLANTS AND DIFFERENT CLINICAL SAMPLES

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At the last conference of the Hungarian Society for Microbiology we already presented data on the distribution of different iron uptake systems of *K. pneumoniae* strains isolated from different clinical samples such as bloodstream infections (BSI), urinary tract infections (UTI) and wound infections (WI). In our present study we collected data on iron acquisition systems of *K. pneumoniae* strains isolated from wastewater (WW), and compared to those available for the UTI, BSI and WI strains. 88 each of BSI and UTI isolates, 110 strains of WI and 113 WW isolates were included into the study. The WW isolates were collected from 47 geographically different sewage treatment plants. The "cross feeding" method with indicator strains specific for the respective siderophore types was used to screen enterobactin and aerobactin phenotypes. The presence of *Klebsiella* ferric iron uptake (KFU) and yersiniabactin systems were tested by means of PCR technique detecting *kfu*B and *irp*1-*irp*2 genes in the isolates. Enterobactin showed the highest prevalence (94-83%), the second most frequent was the KFU system (14-47%) followed by the yersiniabactin system (11-24%), while aerobactin presented with the lowest prevalence (0,9-9%). The frequency of enterobactin production was significantly higher among WW isolates ($\chi 2$, $p<0,01$) compared to the clinical isolates. In case of aerobactin prevalence WW isolates showed significantly lower values compared to UTI ($\chi 2$, $p<0,05$) and BSI isolates ($\chi 2$, $p<0,05$) while the difference was not significant to WI isolates. The WW isolates possessed significantly less frequently with the KFU system ($\chi 2$, $p<0,01$) while the occurrence of yersiniabactin did not show significant difference compared to the clinical isolates. The WW isolates held the significantly lowest prevalence of carrying double iron uptake systems (15%) followed by the WI (30%), BSI (34%) isolates, and the highest frequency was found in the UTI (48%), isolates. The occurrence of the single iron uptake systems showed inverse pattern i.e. WW isolates presented the highest frequency (77%), followed by WI (60%), BSI (52%) and UTI isolates showed the lowest value (37%). These data suggest that *K. pneumoniae* may adapt to the iron levels of the milieu. In the iron abundant free environment fewer strains carry multiple iron

binding systems while the clinical isolates adapt to iron limited conditions in the host by accumulating of plural iron scavenger set-ups.

INDUCTION OF HUMAN DEFENSINS BY OPPORTUNISTIC PATHOGENIC *CANDIDA ALBICANS, C. KRUSEI, C. TROPICALIS* AND *C. PARAPSILOSIS*

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Opportunistic pathogen yeasts can modulate the host immune function by inducing defensins, the natural antimicrobial peptides. Investigation of the effects of *Candida albicans, C. krusei, C. tropicalis* and *C. parapsilosis* on human beta-defensin 2 (HBD2) production in Caco-2 intestinal cell line, and on alpha-defensin production (human neutrophil peptide, HNP1-3) in peripheral blood cells. ELISA technique was used to determine HBD2 peptide levels in cell supernatants, Western blot analysis and confocal immunofluorescence staining within the cells. The mRNA relative expression of inducible HBD2 peptide was measured using RT-PCR. ELISA kit was applied to detect HNP1-3 in peripheral blood induced with Candida spp. The increase in HBD2 mRNA was observed in Caco-2 cells following infection with the *Candida* spp, with the highest rate following the *C. albicans* infection. Similarly, the secretion of HBD2 was significantly higher following in vitro *C. albicans* infection, (13.5 ± 5 pg/ml), than following infection with *C. krusei, C. tropicalis* and *C. parapsilosis*. Intracellular HBD2 was detected in the greatest amount in cells infected with *C. albicans* by the use of Western blot analysis or with immunofluorescence staining. Both *C. albicans* and *C. parapsilosis* induced significantly higher amount of HNP1-3 secretion compared to *Staphylococcus aureus* in peripheral blood (600 ± 50 ng/ml, 300 ± 25 ng/ml vs. 150 ± 25 ng/ml).
Our investigations prove the important role of intestinal epithelial cells in the antifungal defence against *Candida* infections. In addition, *Candida* infections may play a central role in induction of alpha-defensin (HNP1-3) secretion by neutrophils.

COMPARATIVE STUDY OF TWO HOMOLOGUS ENDOMANNANASE ENZYMES (MAN5A) FROM DIFFERENT *THERMOBIFIDA* SPECIES

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Thermobifidas are aerobic thermophilic lignocellulose decomposing actinomycetes. *T. fusca* YX is far more the best characterized strain from this taxon, and lots of cellulolytic hydrolase coding genes of this strain have been cloned and characterized. However in general terms our knowledge for the hemicellulase system is very poor, although the high percentage of hemicelluloses fraction in the cell wall of higher plant makes this material the second most abundant biopolymer in nature. *T. cellulosilytica* known as a distinguished lignocellulose degrader from *Thermobifida* genus with almost uncharacterized cellulolytic and hemicellulolytic enzyme system. Unfortunately based on the known *T. fusca* mannanase (man5A) we could not design useable degenerate primers to amplify the targeted mannanase gene from *T. cellulosilytica* TB100. For identifying the mannanase gene de novo genom project data of *T. cellulosilytica* TB100 accomplished by ABI's SOLID and Roche 454

sequencing system was used. Based on the genomic data we cloned, expressed and biochemically characterized two homologus glycoside family 5 endomannanase enzyme (mannan endo-β-1,4-mannosidase, EC 3.2.1.78) from *T. fusca* TM51 and T. cellulosilytica TB100 strains. The homology between the amino acid sequences of the two enzymes is 79 percentage, and have a similar protein size about 45 kDa. Both of the mannanase have a CBM2 domain, and a secretion signal sequence indicating the extracellular localization of these enzymes. Despite the homology there are number of differences in important biochemical features. The thermal optimum of Man5A of *T. cellulosilytica* is lower, and the thermal stability of this enzyme is much lesser. After boiling Man5A was totally inactivated, compared with the very robust, renaturable mannanase of *T. fusca*. The activity studies of the *T. cellulosilytica* Man5A also revealed a minor β-mannanase activity compared to the total inactivity for that substrate of the homologous *T. fusca* enzyme.

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GENOME SEQUENCE OF *THERMOBIFIDA HALOTOLERANS* TYPE STRAIN (YIM 90462^T)

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Thermobifida halotolerans YIM 90462^T is the type species of the four membered genus *Thermobifida* of the actinobacterial family Nocardiopsaceae. The actinomycete strain YIM 90462^T was isolated from a salt mine sample collected from Yunnan province, south-west China. Representatives of the genus *Thermobifida* are of special interest for their ability to degrade cellulose and hemicellulose. Their robust thermostable hydrolases (cellulases, xylanases, mannanases) are recently being used in lignocellulosic biofuel processes. Here we describe the complete genome sequence, and annotation of this strain. Genome sequencing of *T. halotolerans* YIM 90462^T was performed by combining the cycled ligation sequencing on the SOLiD 4 system (Life Technologies) with Roche GS Junior pyrosequencing. Whole-genome pyrosequencing using double run on the Roche 454 GS-Junior sequencer yielded 101,179 high-quality reads with an average read length of 447 bp, providing 10-times genome coverage. Assembly was performed using the Genomics Workbench 4.8 de novo plug-in and the Omixon Gapped SOLiD alignment 1.3.2 plug-in provided by CLC Bio and Omixon, respectively, which generated 87 large contigs. Automatic annotation of the genome was performed with the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP), which utilizes GeneMark, Glimmer, and tRNAscan-SE searches. The uncompleted draft genome of *T. halotolerans* YIM 90462T consists of 4,123,689 bp, with a GC content of 66,7% and 3, 227 putative coding sequences. Despite *T. halotolerans* has the largest known *Thermobifida* genome we could identify smaller set of glycoside hydrolases (cellulases, xylanases, mannanses and amylases , 23) compared to the multiple glycoside hydrolase system (29 enzymes) of *T. fusca* YX.

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SOLID AND TEMPORAL DISTRIBUTION OF INDICATOR BACTERIA IN SEWAGE TREATMENT TECHNOLOGIES USING DIFFERENT BIOLOGICAL CLEANING METHODS

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The effective operation of modern wastewater treatment plants (WWTPs) has a basic importance from the aspect of the possibly less load of the receivers. The number of bacteria in the treated wastewater is determined parallelly with physical, chemical and biological properties and environmental conditions by the applied treatment technology. The quantitative and qualitative analysis of *Escherichia coli* and *Enterococcus faecalis* indicator bacteria by RT-PCR molecular diagnostic method enables the analysis of the untreated sewage and effluent of WWTPs using different biological treatment technologies. The quantity of bacteria refers to the quality of water, and the change of the number of bacteria depending on the chemical parameters shows, how effective the treatment of a given technology is, with the help of this, an alternative, quicker monitoring system can be established, that contributes to the optimization and increase of the treatment effeciency of WWTPs. During the examinations, three WWTPs (Nyírtelek, Tiszalök and Nyíregyháza) of different capacity and operation were compared with each other. The change of the number of bacteria depending on the chemical paramaters shows, how effective a method under given circumstances may be. During the examinations, analyses of wastewater arriving to the WWTP and treated wastewater leaving the WWTP after running through the technological line were performed. While *Escherichia coli* bacteria are characterized by a value of 46.690-326.355 GU/ml in untreated wastewater, by 25-280 GU/ml in treated wastewater, *Enterococcus faecalis* is characterized by a value of 4.093-84.240 GU/ml in unterated sewage, meanwhile in the effluent between 13-198 GU/ml. From the aspect of the removal of indicator bacteria, the average efficiency of the operation of the WWTP in Nyírtelek is >98,5%, WWTP in Tiszalök is >99,75%, WWTP in Nyíregyháza is >99,9%. The typical values of the chemical parameters of WWTPs are the following: chemical oxygen demand (untreated: 439-1450 mg/l, effluent: 17-206 mg/l), biochemical oxygen demand (untreated: 220-1300 mg/l, effluent: 5-48 mg/l), total phosphorous content (untreated: 2,45-32,5 mg/l, effluent: 0,14-3,23 mg/l), total suspended solids (untreated: 75-790 mg/l, effluent: 5,4-58 mg/l) and ammonium-nitrogen content (untreated: 12,9-112 mg/l, effluent: 0,18-25,5 mg/l). The examination of the efficiency of the indicator bacteria removal may be applied as a complementary method to check the treatment effectiveness of WWTPs and to notice occurent failure situations early, quicker intervention and troubleshooting are important from the aspect of the receiver (of the treated wastewater), and through this, the protection of the environment.

PRESENCE OF SAKAI PHAGE (SP) GENES AND LYTIC PHAGES OF *ESCHERICHIA COLI* COLLECTION OF REFERENCE (ECOR) STRAINS

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Bacteriophages are the most abundant replicons of the biosphere. Phages either integrate into the genome of their bacterial hosts (prophages) or occur as lytic phages. Phages are frequent vectors of virulence genes and are playing fundamental role in the evolution of bacteria. The objectives of the

present study were to investigate the lysogenic state of *Escherichia coli* collection of reference (ECOR) strains by monitoring the presence of *E. coli* O157-„specific" prophage genes, to isolate lytic phages and investigate their bacterial host specificity and morphology. The ECOR strains were screened for the presence of 12 *E. coli* O157:H7 Sakai phage (SP) marker genes. SP genes were detected in 92 % (66/72) of the strains by multiplex PCR. The number of SP genes varied between one and five. We were able to isolate fifteen lytic phages by using *E. coli* C600 as an indicator and propagating strain. Strains harbouring lytic phages represented all the main phylogenetic groups. The host specificity of the lytic phages was different. All the isolated phages could be propagated on a *S. sonnei* strain and several phages also lysed enteropathogenic- and uropathogenic *E. coli* prototype strains as well as the attaching effacing model *Citrobacter rodentium* ICC169 strain. Phages showed Myoviridae or Podoviridae morphology. Adherence and penetration of a lytic phage was demonstrated by transmission electron microscopy. The lyosgeny of ECOR strains is notable. Our data suggest the diagnostic and phage therapeutic potential of the isolated lytic phages.

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CHANGES IN THE SEROTYPES OF CARRIED PNEUMOCOCCI AFTER THE FIRST 3 YEARS OF WIDE-SPREAD PCV UPTAKE IN HUNGARY

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Carriers of *Streptococcus pneumoniae* – especially children - are often the source of infections. Conjugate vaccines (PCVs) are available for children to prevent not just infections, but also carriage. In Hungary, Prevenar (first PCV7, later PCV13) became part of the national vaccination calendar in April 2009. The aim of the present study was to observe the effect of PCV vaccination on pneumococcal carriage of children attending DCCs in Hungary. Nasal specimens were collected from 1121 children (aged 3-6 years) from 23 DCCs in different parts of Hungary, from February 2009 to December 2011. Serotyping of the isolated pneumococci was done using antisera and PCR, antibiotic sensitivity was determined by agar dilution, and the genetic relatedness of the strains was examined by PFGE. Out of 1121 children, 411 were carriers (=36.7%). 27.3% of all children (=306) were vaccinated with PCV7, and 125 of them were carriers. The most prevalent serotypes among the vaccinated children were: 11A (n=36), 19A (n=15), 47 (n=13) and 6B (n=13), while among the non-vaccinated ones: 19F (n=22), 6B (n=21), 15B (n=20), 23F (n=16), 14 (n=16). The strains [especially the new types (except 19A)] were generally very sensitive to antibiotics, except for macrolides (R=18%). All serotype 19A strains showed high resistance to macrolides and clindamycin. The close genetic relatedness of the isolates from a given DCC group was obvious by the PFGE results, indicating the intensive exchange of bacteria between children. During the examined period the PCV vaccination rate increased, but there were differences between nurseries. The carriage rate was nearly the same in the vaccinated and non-vaccinated population, but there was a clear difference in the serotype distribution. For example, while serotypes 14 and 23F were found almost entirely in the non-vaccinated group, rare types such as 11A or 19A emerged rapidly in the entire population. It seems so that even if only a certain percentage of children within a group is vaccinated, it helps in the elimination of the PCV types. The coverage of PCV13 for the non-

vaccinated carriers is 52.5%, while for the vaccinated ones only 31.2%. This could be explained with the emergence of the new types, and proves the efficiency of PCVs.

HEPATITIS C VIRUS GENOTYPES AND SUBTYPES AMONG INJECTING DRUG USERS IN HUNGARY

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Hepatitis C virus (HCV), currently the most commonly spread illegal drug injection-related virus worldwide, can cause chronic hepatitis, cirrhosis and hepatocellular carcinoma. It has at least six confirmed genotypes and several subtypes. Although each genotype and subtype demonstrates a certain geographical distribution, subtypes 3a and 1a are widely distributed among injecting drug users (IDUs) throughout the world. The genotypes differ in their susceptibility to antiviral therapy, and these differences, serve as important information guiding the treatment of the patients. Prior to our investigation, genotype data relating to HCV in Hungarian IDUs were not available. The use of dried blood samples facilitated our analysis of roughly a third of the estimated population of Hungarian IDUs, from 15 towns throughout the country. The identification of genotype 3 and subtype 1a among IDUs demonstrated that Hungary is involved in the worldwide epidemics of HCV among IDUs. Genotype 3 was significantly more prevalent among those who had been injecting drug for a longer period or were members of older age groups than among those who had started injecting only recently, or belonged in younger age groups, suggesting future changes in genotype distribution. Genotype 3 was significantly more prevalent in the provincial towns than in the capital, Budapest. It was significantly more frequent in district 13 than in other districts of Budapest. These observations suggest the existence of different IDU communities with different genotype frequencies in Hungary, even within Budapest. Phylogenetic analysis of the NS5B nucleotide sequence of genotype 3 viruses did not indicate any clear clustering of the Hungarian sequences. However, some clear sub-clusters with strong bootstrap values were apparent, suggesting that these individuals were infected by each other, probably through unsafe drug injection practices.Genotyping is not compulsory before treatment in Hungary, and is strongly recommended only for foreigners living in Hungary. Our results draw attention to the need to modifying the Hungarian treatment protocol, and underline the importance of genotyping before treatment, especially among drug users.

PHENOTYPIC AND GENOTYPIC CHARACTERISATION AND COMPARISON OF BOVINE *PASTEURELLA MULTOCIDA* STRAINS

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Bovine respiratory disease (BRD) is one of the most important health problem of cattle causing great economic loss to cattle breeding all over the world. It is a multifactorial disease resulting from interaction of bacterial and viral agents, usually in combination with various stress factors. The association of *Pasteurella multocida* with BRD has been well known since the early 1950s. *P. multocida*, as a part of the normal upper respiratory flora of cattle, is typically considered only a secondary invader. However, the increased use and the efficacy of vaccines against other respiratory

pathogens suggest that it may have a more pronounced role in the aetiology of BRD. A major epidemiologic question is whether there are strains of the resident flora that are able to overwhelm the host immune system with the help of risk factors or there are virulent clones that cause disease in itself. For answering this question, we should know details of the diversity and structure of *P. multocida* population in cattle. The aim of our study was the characterisation of 39 bovine *P. multocida* strains from different Hungarian cattle herds with traditional microbiological and molecular methods. *P. multocida* could be classified into 5 serogroups (A, B, D, E, F) and 16 somatic serotypes (1-16) based on capsule structure and lipopolysaccharide composition, respectively. The studied bovine strains were highly homogenous in this respect, being mostly A:3 or A:3,4. The combination of a wide range of fermented sugar sources and enzyme activities could separate *P. multocida* strains in 13 biovars (1-14). Nine of them were detected in our collection, however, biovar 2 and 3 were the most frequent finding (67%), demonstrating further the limited heterogeneity of our strains. Genotypic characterisation with PCR fingerprinting using M13 core primer and ERIC (enterobacterial repetitive intergenic consensus) PCR analysis revealed inherent differences between the stains. There were some considerable correlations with adequate biovars or serological features and generated DNA banding patterns. In conclusion, the phenotypic and genotypic comparison of *P. multocida* strains from cattle suffering from BRD showed moderate diversity, supporting the view of the possible existence of pathogen subpopulations. However, additional work is needed to characterise the diversity of strains isolated from healthy animals.

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FUNGICIDE RESISTANCE IN GRAPE POWDERY MILDEW: OBSERVATIONS FROM FIELD EXPERIMENTS

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Powdery mildew, caused by *Erysiphe necator* Schw. economically is the most significant disease of viniferous grapes worldwide. In grapevine this massive occurrence of this fungus before harvesting will result in serious loss of fruit and a deterioration of wine quality and is already resistant to most of the fungicides. It is known from the relevant scientific literature that resistance to modern fungicides might emerge quite frequently and rapidly. Knowledge of the presence and of the amplitude of fungicide resistance, as well as about its dynamics in the season, might clearly help the development of more efficient plant protection strategies and would certainly reduce fungicide use during the season. Thus, it is important to study this phenomenon in Hungary because there is absolutely no information about fungicide resistance in Hungarian populations of grape powdery mildew. The filed experiments were carried in 2009, 2010 and 2011 - in different experimental sites of Hungarian wine-growing areas - on shoots sampled in different dates during the vegetative growth of grapevines. During this experiments variants of multistep resistance management technology (DMI and/or strobilurin resistance, fungicide rotation and -mixture strategy) were compiled which were performed in field experiments according to fungicide resistance features of the grapevine. According to the degree of fungicide resistance, first step of the strategy was focused on prevention and delay of fungicide resistance development, the second step was aimed to keep the observed level of sensitivity reduction, and the third step was determined an annual technological research plan which will exclude the resistant active ingredient (or the active ingredient group).

Inoculum and symptom was assessed regularly and seasonally and technological modification was performed according to seasonal resistance change. In the base of our investigations could be told that in all different optioned experiments (strobilurin-dominant, DMI-dominant and strobilurin+DMI-dominant technologies) the infection of grape powdery mildew increased in the majorities of experimental fields compared with everyday used grape protection technology. The highest infection rates in occurrence of foliage and berry symptoms were found in strobilurin dominant technologies, and in several experiments connected to this technology were completely inefficient. The results shows clear that independent fungicide resistance data and technological modifications are necessary for a more effective plant protection against powdery mildew.

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POTENTIAL BIOTECHNOLOGICAL APPLICATIONS OF ZYGOMYCETES

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Fungi belonging to the order Mucorales are widely distributed in soil and plant debris, on dung and other organic substrates. These fungi have practical importance from various medical, biotechnological and agricultural aspects. Some of them are known as opportunistic pathogens causing zygomycosis, while others are used as model organisms in various genetic and physiological studies. Several species are subjects of biotechnological studies and developments mainly due to their effective extracellular enzyme (such as amylase, lipase and protease) and metabolite (such as carotenoids, sterols and unsaturated fatty acids) production and ability to selectively transform sterols and other chemical compounds. Here, two field of research of our department related with the potential application of these filamentous fungi will be presented: (i) our studies to analyze the β-glucosidase and lipase enzymes by zygomycetes in order to find new strains and enzymes potentially useful for industrial applications, and (ii) our efforts to investigate the biological requirements of the application of Mucorales (primarily *Mucor circinelloides* as a model organism) as carotenoid producers. Our studies provided new data in reference to the β-glucosidase and lipase production of zygomycetes fungi. A number of new producer stains have been identified. Moreover, significant theoretical and practical knowledge about the β-glucosidases from *Rhizomucor miehei, Mucor corticolus, Rhizopus niveus* and *Gilbertella persicaria* has been obtained for the biotechnological applications. We suggest that the *Rhizomucor miehei* enzyme is applicable for liberation and enrichment of phenolic antioxidants from plant-derived compounds and for synthesis of oligosaccharides and different glycoconjugates. The first study about a filamentous fungal β-glucosidase, which has good ability to catalyze both transglucosylation and transgalactosylation reactions, will also be presented. In the frame of the carotenogenic studies, on Mucorales, strains overexpressing the endogenous isoprene biosynthesis genes and strains expressing bacterial astaxanthin biosynthesis genes were constructed and characterized. Potential of these genes to improve the carotenoid production of *Mucor* and achieve xanthophyll, primarily astaxanthin and canthaxanthin, production in these fungi will be discussed.

NEW APPROACHES IN THE DEVELOPMENT OF GENETIC TOOLS FOR *THERMOPLASMA ACIDOPHILUM*

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The extremophile *Thermoplasma acidophilum* has become an important model organism of visual proteomic studies due to its low genome and proteome complexity. To be able to study macromolecular complexes in their natural environment and biochemical processes we have been developing genetic tools for *T. acidophilum*. We have established the basis of a working gene manipulation system however, further fine tunings were still needed. In genetic manipulation experiments it is essential to work with cell lines originating from a single colony. Regarding the difficulties that *T. acidophilum* cells often can't grow after transferring them from solid medium to liquid medium we aimed to overcome of this hurdle by using the solidifying agent Gelrite. Indeed, the cells were able to grow in liquid medium after clonal selection on this medium. Furthermore our clonal selection method was optimized to accelerate cell growth and to obtain more colonies. A suitable growth promoting substance was extracted from yeast cells and added to the solid medium by which the cells could grow significantly faster. The effectiveness of the clonal selection was increased with mineral oil overlay which gave constant temperature and humidity to the colonies resulting in decreased generation time and increased colony number. We also screened for additional selection markers to separate transformants from wild type cells and surprisingly rifampicin proved to be a good candidate. A new generation of shuttle and KO vectors were constructed based on the rifampicin resistant gene *arr*2 from *Pseudomonas* sp. These constructs produced similar results like the novobiocin resistant gene based constructs: most of the time illegitim recombination events happened. To change strategy and to find a more susceptible *T. acidophilum* strain, recently we are transforming environmental isolates of *T. acidophilum* (strains JCM 17946-17956) with our constructs in the hope that one of the strains will be able to integrate foreign DNA into the designed position and/or maintain extrachromosomal elements.

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HETEROLOGOUS EXPRESSION OF *NEOSARTORYA FISCHERI* ANTIFUNGAL PROTEIN (NFAP) IN *PICHIA PASTORIS*

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The *Neosartorya fischeri* antifungal protein (NFAP) is a small, ~6,6 kDa, basic, cysteine-rich protein, that effectively inhibits the growth of several filamentous fungi. Unfortunately, there are some limitations that have to be overcome to make the NFAP useful for a future application. One of the main difficulties is that the presently used strain (*N. fischeri* NRRL 181) produces this protein in relatively low amount despite of the available knowledge of the nature of its 5′-upstream transcriptional regulation elements. Therefore there is a substantial demand for easily fermentable, "generally recognized as safe" yeasts or filamentous fungi, which can produce the NFAP in high amount. A *Pichia pastoris* heterologous expression system would be appropriate for this purpose. In

this study the heterologous expression of the nfap gene was carried out in *Pichia pastoris* KM71H by using the pPICZαA vector (EasySelect Pichia Expression Kit, Invitrogen). After the purification (ultrafiltration and cation exchange chromatography) of the heterologous NFAP (hNFAP) the purified protein was identified with N-terminal amino acid sequencing by Edman degradation method (Innsbruck Medical University, Innsbruck, Austria). The antimicrobial effect of hNFAP was investigated on *Aspergillus niger* (Szeged Microbial Collection, University of Szeged, Szeged, Hungary, SZMC 601) and *Micrococcus luteus* (SZMC 0197) with agar diffusion test. hNFAP exerted potent antimicrobial activity against both of the tested microorganisms. Surprisingly, the hNFAP can inhibit the growth of *M. luteus* compared to the NFAP produced by N. fischeri NRRL 181, which can inhibit the growth only the filamentous fungi.
Our vector construction would be appropriate for the production of high amount of NFAP in the near future after the optimization of the fermentation. Furthermore it is hypothesised that the antimicrobial spectrum of the hNFAP differs from that of the NFAP.

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LENGTH GROWTH PATTERNS IN THE MITOTIC CYCLE OF LARGE FISSION YEAST CELLS

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The fission yeast *Schizosaccharomyces pombe* is an excellent unicellular model organism, because the cylindrical cells grow only at the ends with a constant diameter, and they divide symmetrically. Time-lapse microphotography is a classical method to study the growth of individual cells. In this method the cells are fixed on the surface of an agar plate in a microscope, and photos are taken in every 5 minutes. Later on the growth pattern of the cells can be studied by a projector. If we examine several generations of the cells, we can notice that all cells aspire to reach nearly the same size at division. This critical size is provided by a size-control mechanism. We can observe this mechanism in temperature sensitive (ts) cdc mutants. When a cdcts mutant is shifted up to its restrictive temperature, division stops, but growth continues and produces oversize cells. If the culture is then shifted down to the permissive temperature, the cells go through a series of rapid synchronous divisions as they return to normal size. This culture is called an induction synchrony (IS) one. Three different models (linear, exponential, and bilinear) are generally used to describe the growth pattern of single fission yeast cells. Bilinear model means two linear segments with a rate change point (RCP). Selection of the most adequate model to describe a given data-set requires the use of quantitative model selection criteria, like AIC. This statistical criterion can compare differently parameterized models, which is necessary in this case, because the linear and exponential models have 2 parameters, while the bilinear model has 5. We have analyzed the length growth patterns of ~240 cdc2-33 mutant fission yeast cells (~170 from an IS and ~70 from a steady-state culture). The measured data-sets were smoothed and linear, exponential and bilinear models were fitted. The above mentioned model selection criteria were used for selecting the most adequate one in the case of all these cells' length growth patterns. In the steady-state culture, more than 50% of the cells showed a bilinear pattern, while in the case of the IS culture the ratio was only about 40%. Cells having bilinear length growth pattern were further analyzed: the slopes of two linear segments were compared by t-tests. In the steady-state culture, size control seems to act before the RCP. In the case of the cdc2-33 IS strain we found that the smaller cells showed a size control up to

a critical size, whereas the larger cells lack such size compensation and the cycle time is reduced to a minimum time. We investigated whether the cycle time or the birth length of the cells affects its growth pattern, i.e., the most adequate model fitting the data. We have found that larger cells have a tendency to grow linearly with a higher probability than smaller cells, meanwhile small cells generally grow in length in a bilinear way.

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CHARACTERIZATION OF A BIOGAS-PRODUCING MICROBIAL COMMUNITY BY SHORT-READ NEXT GENERATION DNA SEQUENCING

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Renewable energy production is currently a major issue worldwide. Biogas is a promising renewable energy carrier as the technology of its production combines the elimination of organic waste with the formation of a versatile energy carrier, methane. In consequence of the complexity of the microbial communities and metabolic pathways involved the biotechnology of the microbiological process leading to biogas production is poorly understood. Metagenomic approaches are suitable means of addressing related questions. In the present work a novel high-throughput technique was tested for its benefits in resolving the functional and taxonomical complexity of such microbial consortia. It was demonstrated that the extremely parallel SOLiD™ short-read DNA sequencing platform is capable of providing sufficient useful information to decipher the systematic and functional contexts within a biogas-producing community. Although this technology has not been employed to address such problems previously, the data obtained compare well with those from similar high-throughput approaches such as 454-pyrosequencing GS FLX or Titanium. The predominant microbes contributing to the decomposition of organic matter include members of the Eubacteria, class Clostridia, order Clostridiales, family Clostridiaceae. Bacteria belonging in other systematic groups contribute to the diversity of the microbial consortium. Archaea comprise a remarkably small minority in this community, given their crucial role in biogas production. Among the Archaea, the predominant order is the Methanomicrobiales and the most abundant species is Methanoculleus marisnigri. The Methanomicrobiales are hydrogenotrophic methanogens. Besides corroborating earlier findings on the significance of the contribution of the Clostridia to organic substrate decomposition, the results demonstrate the importance of the metabolism of hydrogen within the biogas producing microbial community. Both microbiological diversity and the regulatory role of the hydrogen metabolism appear to be the driving forces optimizing biogas-producing microbial communities. The findings may allow a rational design of these communities to promote greater efficacy in large-scale practical systems.

The composition of an optimal biogas-producing consortium can be determined through the use of this approach, and this systematic methodology allows the design of the optimal microbial community structure for any biogas plant. In this way, metagenomic studies can contribute to significant progress in the efficacy and economic improvement of biogas production.