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MODELLING THE EFFECT OF HEAT STRESS ON SURVIVAL OF *LISTERIA MONOCYTOGENES* 4AB IN TRYPTIC SOY BROTH AND MILK

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The majority of present-day milk-borne illnesses is attributed to *Salmonella* spp., *Campylobacter* spp., and *Listeria monocytogenes*, among others and is associated with the consumption of raw milk or pasteurized milk that has either received an inadequate heat treatment or has been contaminated after heating.
The aim of thermal processing is to deliver sufficient heat to a food to reduce the chance of survival of an organism that is capable of growth. Severe thermal stress may eliminate sizable portion of the cell population and the adaptive response in the small fraction of the population that survives the treatment may not be measurable. Response to a mild heat shock is readily detectable when cells are treated at sublethal or minimally lethal temperatures.
In this study, the heat stress response of *Listeria monocytogenes* 4ab (avirulent strain) was characterized in pure cultures in tryptic soy broth and milk. We examined the effect of heat stress (55ºC, 60°C, 65°C) by itself and in combination with mild heat treatment (46°C, 48°C, 50°C for 30 and 60 min), and the D-values were determined. The susceptibility of strains to injury upon sublethal treatments was determined based on the organism's increased sensitivity to 5% NaCl compared to TSA media not supplemented with NaCl.

GATEKEEPERS OF THE BIOSPHERE: STUDYING MARINE MICROORGANISMS WITH ENVIRONMENTAL GENOMICS

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The planet Earth is only habitable for mankind due to the indispensable activities of the invisibly small, but incredibly abundant microorganisms. One millilitre of marine water contains about one million bacteria, one millilitre of marine coastal sediment houses roughly as many bacteria as there are humans on Earth, 6 billion. Overall, there are many more bacteria in the biosphere then stars in the universe. Only few of them are pathogenic, but the vast majority is beneficial. They catalyze essential reactions of the global biogeochemical cycles of C, O, N, P, or S, such as nitrogen fixation or waste mineralization. Environmental microbes are extremely versatile and very diverse, but they are also difficult to isolate by standard microbiological methods. Only by molecular fingerprinting microbiologists discovered the presence of thousands, if not millions of microbial species in relatively small samples. Indeed, the application of molecular biology - and especially genomics - has in the last decade resulted in a new phase of discovery which can only be compared to the Golden Age of Bacteriology at the end of the 19th century when Pasteur, Koch and their colleagues described in a few years many of the important human pathogens.
New identification methods such as fluorescence in situ hybridization with rRNA-targeted oligonucleotide probes allow the visualization of the microorganisms behind the genetic fingerprints. They facilitate quantifications of specific species over space and time which is an essential prerequisite for ecological models. Thereby abundant marine key species have been identified. For some of those pure cultures are available. Full genome sequencing of these “gatekeepers of the

biosphere" is now ongoing at high speed. */Rhodopirellula baltica/* and */Gramella forsetii/* are just two examples of aerobic heterotrophic bacteria which directly influence the marine biological pump that removes carbon dioxide from the atmosphere.
Another example of environmental genomics is a study done on the fully anoxic deep oceanic waters of the Black Sea. This is a microbial world devoid of animal or plant life. With the help of submarines reef structures have been discovered which are built by another kind of "gatekeepers of the biosphere". Two different species of microorganisms together catalyze the oxidation of the climate gas methane that would otherwise pollute our atmosphere. Genomics was here essential to reveal the mechanisms used by this unique microbial symbiosis in catalyzing an anaerobic oxidation of methane.
Although environmental sequencing proceeds at high speed with stunning results, microbiology will, nevertheless, continue to rely on work conducted on live organisms. Not only does the interpretation of new environmental genes strongly rely on knowledge obtained from pure cultures, it is just not possible to fully predict the function of a living cell from its molecular blueprint.

SUPERBUGS: THE RECENT EXPANSION OF MULTI-ANTIBIOTIC RESISTANT BACTERIA

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Although antibiotic resistance became a problem shortly after the introduction of these drugs in the 1940s, it was not a major problem as it was usually spontaneous and there were sufficient drugs to overcome it. Even the identification of transferable resistance in the 1960s, with the ability of bacteria to share resistance genes, did not cause alarm. During the 1980s, a series of multi-antibiotic resistant (MdR) bacteria emerged; the acquisition of aminoglycoside resistance with meticillin resistance in *Staphylococcus aureus* (MRSA), the emergence of the extended-spectrum β-lactamases (ESBLs) in *Klebsiella* spp. and the transfer of chromosomal *ampC* genes into the Enterobacteriaceae. The introduction of genotyping techniques revealed that the increase in resistance was often due to the spread of a few MdR clones, which have now been termed "Superbugs". In most bacterial species, there is considerable genomic variation but, in the MdR strains, this diversity had been lost. In the 1990s this trend continued with the emergence of MdR clones of *Salmonella typhi* in the Indian subcontinent, and vancomycin-resistant enterococci (VRE) and *Acinetobacter baumannii* in hospitals in Europe and the USA.
What has changed that triggered this shift towards multi-resistant clonality? Firstly, it had been occurring unnoticed for years. Our use of antibiotics has progressively removed sensitive strains of each species from the clinical environment, so the selective pressure ensured the rise of strains more capable of surviving the antibiotic challenge. The acute lack of new drugs guarantees this remains an ongoing process. In some case, the emerging strains were able to mutate and acquire resistance mechanisms much more quickly than their sensitive counterparts. Secondly, many health services in the developed world changed the way patients were managed. There is now much greater movement of patients within the healthcare system, promoting the opportunity for MDR bacteria to spread. The redesigning of many modern hospitals ensured that patients were closer to each other and caused increasing problems with infection control. Thirdly, in the last 25 years, medicine has radically changed. Many patients are now deliberately immunosuppressed and antibiotics are employed to prevent and eradicate bacterial infections in patients who lack a function immune system, a task that many drugs were not designed to do. In this environment, the pathogenic nature of an organism is

less important and bacteria with a predisposition towards resistance often thrive. All these problems are usually associated with hospitals; however clonal MDR bacteria are also found in the community and show a capability to spread over large geographic distances, particularly in the developing world. This may be promoted by the reduction or lack of controls on antibiotic supply in many countries, particularly allowing the administration of sub-optimal concentrations of drugs.
Some MdR bacterial clones are now virtually pan-resistant and cannot be controlled with antibiotics. Indeed any antibiotic use will ensure that they prosper in the clinical population. Their management and possible eradication will require a major revision of current health policies.

MOLECULAR CHARACTERIZATION OF 3-KETOSTEROID 9 ALPHA-HYDROXYLASE IN *MYCOBACTERIUM SMEGMATIS* MC2155

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The majority of the chemical synthesis of steroid drugs is based on intermediates gained by selective microbial degradation of sitosterol side chain. Insertional mutant library was generated by Tn*611* transposon mutagenesis to study the sterol degradation pathway in *Mycobacterium smegmatis* mc2155. Out of 10,000 insertional mutants screened, a strain was identified that has altered activity of the sterol degrading enzymes. Mass spectrometric and 1H-NMR analysis of the sterol degradation products revealed that a mutant, *M. smegmatis* 10A12, accumulated intermediates 4-androstene-3,17-dione and 1,4-androstadiene-3,17-dione suggesting the inactivation of a key enzyme responsible for the hydroxylation of 9α-position of ring B of the steroid skeleton. The transposon and flanking genomic DNA sequences were recovered from the insertional mutant strain using *Not*I restriction enzyme. A genomic DNA library of *Mycobacterium smegmatis* mc2155 was generated and screened with the flanking DNA sequences of Tn611 transposon rescued from the insertional mutant as a probe. A positive clone carrying a *Not*I genomic DNA fragment expected to encode the 9α-hydroxylase was isolated and sequenced.
The exact location of the putative 9α-hydroxylase gene was determined by deletion mutagenesis, followed by complementation experiments and sequencing. The open reading frame encodes the 383-amino-acid terminal oxygenase of 3-ketosteroid 9α-hydroxylase in *M. smegmatis* mc2155 and has domains typically conserved in class IA terminal oxygenases.
E. coli expressing the recombinant protein could hydroxylate the steroid ring at the 9α position.

SUCCESSION OF FUNGAL COMMUNITY IN WHEAT STRAW COMPOST

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The oyster mushroom (*Pleurotus ostreatus*) is one of the most important, world-wide cultivated mushrooms. The large scale industrial production of this mushroom is performed on agricultural wastes such as composted wheat straw.

Like other basidiomycetous fungi, *P. ostreatus* prefers organic nitrogen for growth. For this reason, an important advantage of substrate composting is that the produced microbial cells can serve as nitrogen sources for *P. ostreatus*. Furthermore, the secreted microbial extracellular enzymes are loosening and modifying the plant cell wall building biopolymers in a manner that the mushroom will be able to utilize them more easily. As fungi play important roles in degradation of complex substrates such as hemicellulose, cellulose and lignin during composting, the study of fungal community in compost throughout the composting procedure could improve process management by exploring the roles of specific microorganisms. Accordingly, the aim of the present study was to investigate the changes of fungal community that occur in straw compost before the pasteurisation step, and to explore the possible roles of dominant fungal species.

The composting process of wheat straw lasts for 6-7 days with a temperature increasing to 50-65ºC, and consists of grinding as the first step, and two rotations for aeration during composting days before the sterilization step. The succession of fungal community was investigated by the isolation of dominant strains from straw samples directly after grinding as well as on the third and sixth days of composting. This was followed by microscopical investigations and ITS sequence analysis based identification of the isolates. In the initial phase, different *Penicillium, Fusarium, Aspergillus, Cladosporium* and *Trichoderma* strains were detected most frequently, the presence of which could not be demonstrated after three days of composting, probably because of the suppressing effect of bacteria and rising temperature. Interestingly, after the third day, a substantial part of the fungal community in the compost consisted of two mesophilic yeast species, *Rhodotorula mucilaginosa* and *Trichosporon moniliiforme*. In the succession process, when the temperature was between 40-65ºC, groups of thermophilic filamentous fungi (*Thermomyces lanuginosus, Myceliophthora thermophile* and *Rhizomucor miehei*) started to colonize the straw. A human pathogenic thermophilic fungus, *Aspergillus fumigatus* was also present but with a low CFU number.

Although many of the microorganisms present in composted straw are already known, their interactions and biochemical capabilities are yet to be elucidated in order to improve the quality of the composts for higher mushroom yields.

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INVESTIGATION OF A BACTERIAL COMMUNITY INVOLVED IN BTEX DEGRADATION TROUGH THE ANALYSIS OF 16S rDNA AND CATECHOL 2,3-DIOXYGENASE GENE BY T-RFLP

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Benzene, toluene, ethylbenzene and xylene (BTEX compounds) are common soil and groundwater contaminants. These aromatic hydrocarbons can serve as sole sources of carbon and energy for several microorganisms. Microbial communities involved in the degradation of these highly toxic contaminants are often investigated trough the analysis of 16S rDNA with well known molecular genetic techniques (e.g. DGGE, cloning and T-RFLP). Detection of catabolic genes, involved in the metabolism of aromatic hydrocarbons, is in focus today. Catechol 2, 3-dioxygenase (C23O) plays a key role in the aromatic ring cleavage and can be used as a marker gene to monitor functions and activities in bacterial communities of BTEX contaminated environments. Hence, the detection of this functional gene can serve additional information about the investigated bacterial community and can

help us to choose the right bioremediation process. Several microbes possess the genes of aromatic ring cleavage enzymes like catechol dioxygenases, but the diversity of these genes is so high, that universal primers, or primer sets targeting higher taxa are not always applicable for the detection of these genes.
Gasoline contaminated groundwater samples were taken in 2007 in Hungary, from 3-3, 5 m depth. Non contaminated sample from the sampling site was used as control during the study. The groundwater samples were used for direct DNA extraction, and the community DNA was used as template for 16S rRNA gene and for C23O gene specific PCR. For the detection of C23O gene a primer pair was designed especially for the specific detection of *Comamonas* and *Delftia* related C23O genes. The PCR products amplified from community DNA were cloned and clone libraries were generated and grouped by T-RFLP and one representative of each unique T-RFLP type was sequenced. The most frequently occurring 16S rDNA clone types were related to the beta-Proteobacteria, dominated by sequences almost identical to *Malikia spinosa*, to *Rhodoferax antarcticus* and to several uncultured bacteria. The C23O clone types show homology to beta-Proteobacteria (like *Delftia acidovorans*, *Comamonas testosteroni* or *Ralstonia eutropha*) related C23O gene sequences, but have only 75-85 % similarity to them.

TRANSMISSION OF A MODIFIED LIVE VIRUS VACCINE FROM VACCINATED TO NONVACCINATED PIGS

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Porcine reproductive and respiratory syndrome (PRRS) is a widespread disease of swine characterized by reproductive disorders in gilts and sows, and by respiratory disorders in all ages, leading to death mostly in neonatal, suckling, and weaned piglets.
Analysing the ORF5 sequence data of 37 Hungarian PRRSV strains we found strains in nonvaccinated animals that were more than 98% identical to modified live virus (MLV) vaccine strains used in the herd. According to our findings the transmission can occur both vertically and horizontally. Two different MLV vaccines are authorised in Hungary both containing PRRSV strain belonging to the European genotype. The analysis of the putative ectodomains and their N-linked glycosylation sites of the vaccine strain and its variants suggested selective pressure on the first ectodomain, by a consistent amino acid change on epitope B and by loosing a glycosylation site in the otherwise conserved N-46 position.
To verify and prove the transmission of the MLV, live animal experiment was performed. Fifteen conventional, PRRSV free weaned piglets were housed together. Ten of them were randomly chosen, and treated with MLV intramuscularly according to the manufacturer's instructions, and five nonvaccinated pigs were kept in the same cage as contact controls. Five animals were kept elsewhere as negative controls, and five (also kept elsewhere) were intranasally infected with a wild, European type Hungarian PRRSV isolate. On day 0, and every 7th day PI, blood, nasal swab, and oral swab samples were obtained from each of 25 piglets. RT-PCR tests on each sample, ELISA on the sera samples, and sequence analysis of selected RT-PCR samples were performed.
According to the results of the RT-PCR, four animals out of the ten vaccinated became viremic at least for five weeks, and one out of five contact controls became viremic for three weeks. The MLV was recovered only from the nasal swabs of this contact control piglet.

The piglets infected intranasally with the wild type virus all became viremic for the whole period of the experiment (6 weeks), and the virus was extensively shed throughout this period.

FUNCTIONAL AND TRANSCRIPTIONAL STUDY OF THE HUP GENES IN *THIOCAPSA ROSEOPERSICINA* BBS

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Thiocapsa roseopersicina BBS, a purple sulfur photosynthetic bacterium utilizes reduced sulfur compound for its anaerobic photosynthesis. The cells can grow under photochemolitotrophic, photomixotrophic and microaerophilic conditions in the presence of organic substrates and - under proper conditions - it can fix molecular nitrogen. *T. roseopersicina* BBS, as world recorder, contains at least five NiFe hydrogenases having various physiological roles. The maturation of the NiFe hydrogenases is a complex process requiring concerted action of numerous accessory proteins encoded by genes being scattered along the genome. Accessory genes (*hupCDHIR*) had been identified downstream of the hydrogenase structural genes (*hupSL*) in *Thiocapsa roseopersicina* BBS. These genes code for proteins that are likely linked to the biosynthesis and the *in-vivo* function of the Hup hydrogenase. Some of these proteins are well characterised, however, there are still putative genes coding for enzymes with unknown physiological role in the hydrogen metabolism. For example, very little is known about the HupI and HupH proteins, which are present in *T. roseopersicina* as well as in most of the bacteria harbouring *hup* genes.

The genomic context of the *hupH* and *hupI* genes suggested, that the corresponding gene products had Hup hydrogenase related function. This was further confirmed by RT PCR analysis showing that the *hupSLCDHI* genes belong to the same operon.

On the basis of in silico analyses, the HupH might have a role in the transport processes, while HupI is a rubredoxin-type, consequently electron transferring protein. Frame mutant strains were created in order to investigate the function of the HupH and HupI proteins. Effects of the mutations were checked by measuring the *in-vivo* hydrogenase activity. The results showed that the absence of neither HupI nor HupH caused a significant decrease in the overall Hup uptake activity *in-vivo*. Furthermore, expression cassettes were constructed for homologous expression of the HupH and HupI proteins C-terminally fused to Flag/Strep II oligopeptides. Using this system, it is possible to purify the HupH and HupI proteins with their interaction partners which can be identified by mass spectrometry.

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EFFECT OF CPG METHYLATION ON BINDING OF NUCLEAR PROTEINS TO THE EBER 1 PROMOTER OF EPSTEIN-BARR VIRUS

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Epstein-Barr virus (EBV) encodes two protein-noncoding RNAs (EBER 1 and 2) which are constitutively expressed in latently infected host cells. Transient transfection and *in-vitro* binding assays identified a series of regulatory elements in the RNA Polymerase III (Pol III) transcribed EBER promoters and their 5' sequences containing elements of typical RNA Polymerase II (Pol II) promoters. In our earlier studies we observed that *in-vitro* DNA methylation of the EBER 1 and 2 gene resulted in an almost complete inhibition of its transcription in transfected B- and epithelial cell lines. These results indicated that the highly unmethylated epigenotype of Pol III transcribed EBER 1 and 2 genes is an important factor in maintaining their constitutive activity. To find out whether the DNA methylation directly or indirectly blocks the transcription of EBERs by affecting the protein binding pattern at their promoter, we made *in-vitro* footprinting assay on methylated and unmethylated EBER1 promoter sequence. In the Pol II region one of the two c-Myc the ATF proximal Sp1 site and the ATF binding sites showed an altered footprint on the methylated sequence, while the other Sp1 and TATA binding has not changed. Around the initiation point and in the Pol III region minor differences were observed. These suggest that DNA-methylation blocks EBER transcription through blocking the binding of important transcription factors.

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IDENTIFICATION OF NEW GROUP C ROTAVIRUS STRAINS IN PIGLETS

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In humans, infection by group C rotaviruses (GCRVs) has been associated with sporadic episodes or large outbreaks of gastroenteritis in all age groups, and GCRVs are generally regarded as important enteric pathogens. Evidence for a possible zoonotic role of animal GCRVs has been recently provided and this sets the bases for the study of GCRVs in animals. During a passive surveillance study for virus enteric pathogens in piglets with gastroenteritis, GCRVs were detected in 32/102 fecal specimens (31.3%) from 12/12 swine herds. GCRVs were also identified from a large collection of samples collected between 2003 and 2005 at the Istituto Zooprofilattico of Brescia, Italy. Out of 3453 samples examined in this time span, rotavirus-like particles were detected by electron microscopy in 411 samples (11.9%), with marked yearly variations (6.4% to 18.8%). A subset (n=86) of this collection was screened for GCRV, that was detected in 22 (25.5%) samples. GCRVs were detected rarely alone and mostly in mixed infections with group A rotaviruses and enteric caliciviruses. To gain information on the genetic relationships between human and animal GCRVs, we sequenced the VP7 gene of 10 porcine strains detected from different outbreaks. Four GCRV strains were genetically related to the prototype GCRV porcine Cowden strain. A completely new VP7 genotype included 4 strains (344/04-7-like) that shared 92.5% to 97.0% aa identity to each other, but <83% to human GCRVs and <79% to other porcine and bovine GCRVs. A unique 4-aa insertion (SSSV or SSTI), within a variable region at the carboxy-terminus of VP7, represented a distinctive feature for these 4 unique strains. An additional strain, 134/04-18, was clearly different from all human and animal GCRVs (<85% aa identity) and likely accounts for a distinct VP7 genotype. The VP7 of a

unique strain, 42/05-21, shared similar ranges of aa sequence identities with porcine and human strains (88.0-90.7% to porcine GCRVs and 85.2-88.2% to human GCRVs). These findings suggest the genetic/antigenic heterogeneity of GCRVs and provide a baseline for future epidemiological studies.

THE LAST 3 YEARS OF NATIONAL ROTAVIRUS STRAIN SURVEILLANCE ACTIVITY IN THE PREVACCINE ERA (2003-2006): FIRST DETECTION OF SEROTYPE G8 AND G12 ROTAVIRUSES IN HUNGARY

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Group A rotaviruses are the main cause of acute dehydrating diarrhoea in children, and are responsible for high mortality in developing countries and a significant socio-economic burden associated with treating the disease in developed countries. Two vaccines, a monovalent vaccine and a pentavalent vaccine are now available in Europe, including Hungary. In anticipation of future routine vaccination programs against rotavirus in our country, the antigenic diversity of rotaviruses collected from Budapest between 2003 and 2006 was investigated by characterization of the main antigenic determinant, the outer capsid protein VP7 (G typing). A total of 1981 strains were tested and 93.4% were successfully G typed. Eight distinct G types (G1, 21.7%; G2, 4.3%; G3, 2.2%; G4, 18.1%; G6, 0.5%; G8, <0.1%; G9, 40.8%; and G12, 3.4%) were identified but only 2 serotypes were predominant over consecutive seasons (G4, 2003-2004; G9, 2004 to 2006). Mixed infections with different serotypes occurred at low frequency (2.3%). Interestingly, the incidence of G1 rotaviruses, the most common serotype worldwide, was relatively low in Hungary in the study period, demonstrating dynamic changes in the epidemiology of rotaviruses. The emergence of serotype G12 rotaviruses in 2005 and the relative abundance of serotype G9 after its first detection in Hungary raise concerns on the efficacy of current vaccination strategies. It is hypothesized that live attenuated rotavirus vaccines might alter the forces and balances that drive rotavirus evolution and provoke the spread of novel strains by mechanism of antigenic escape. Therefore, it will be important to continue rotavirus surveillance and to monitor the dynamics of circulating rotavirus strains after the introduction of rotavirus vaccines.

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OCCURRENCE OF CYTOMEGALOVIRUS INFECTION IN THE MURES COUNTY HOSPITAL LABORATORY'S MATERIAL

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The human herpes virus 5, known also as *Cytomegalovirus* (CMV) is one of the major causes of fetal malformations due to its capacity of traversing the placenta following mainly the mother's primary infection. This virus is also known as an important pathogen for immunocompromised individuals, especially after marrow- and organ- transplantations, chemotherapy in cancer-treatment and other infectious diseases, such as the human immunodeficiency viral (HIV) infection. Clinical manifestations can appear even after reactivation of an earlier infection or after reinfection with another genotype of CMV. Serological demonstration of the specific immun response, by appearance of IgM and IgG antibodies are frequently used for laboratory diagnosis. The ELISA method is a very sensitive way of diagnostics.

Between 2006 and 2007 the authors, in the Mures County Hospital's Bacteriological Laboratory (Tg. Mures, Romania), examined the sera of 829 patients using the ELISA method (Diasorin kit) for the presence of CMV. The age of the patients ranged between 5 weeks and 56 years. Most of the sera, 638 (76%), were only IgG- positive, demonstrating a latent infection; these results correlate with the literature data; 24 (3%) patients had specific IgM and IgG antibodies, showing an acute infection. 113 (14%) patient's sera had no antibodies against the CMV, meaning that they did not have CMV infection. The remaining 54 cases (7%) were only IgM-positive. The authors could not follow up the evolution of these patients. In the adult patients the manifestations of the acute disease were: interstitial- and bronchopneumonia, encephalitis, jaundice, hepatosplenomegaly, thrombocytopenia. The complications of the intrauterine infections in the newborn infants were: micro- and hydrocephaly, cerebral calcifications, epilepsy, mental retardation, intellectual impairment, chorioretinitis and deafness. In two newborns the CMV infection was associated with *Toxoplasma gondii* (one case) and rubella- virus (one case) infection.

In conclusion, most of the tested patients underwent an asymptomatic CMV infection. The congenital infections were reduced by number, but were severe. They could have been prevented by an early diagnosis of the primary or recurrent maternal infection. The authors recommend the introduction of serological screening of the mother in early pregnancy and following up the evolution of the IgG antibodies, including the measurement of the IgG-avidity.

SURVIVAL OF *ESCHERICHIA COLI* AND *PSEUDOMONAS AERUGINOSA* IN WINES

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Although wine is believed one of the most safe food stuffs, the regulation of its microbiological quality is contradictory. The Hungarian regulation (4/1998 EüM) contains limits for *Pseudomonas aeruginosa* but the latest EU regulation (2073/2005 EC) doesn't whereas there is very weak scientific evidence for both sides.

To assess the extent of hazard caused by bacterial contamination of wine with two facultative pathogens *Escherichia coli* and *Pseudomonas aeruginosa*, we investigated their survival in six different wines: Debrői Hárslevelű, Tokaji Hárslevelű, Gróf Nagyrédei dry white cuvée, Egri Bikavér, Gróf Nagyrédei dry rose, and Nagyrédei Zweigelt. All of the wines killed both test bacteria within 10 min when applied as cell suspension mixed into the wine. Among them Tokaji Hárslevelű and Egri Bikavér exerted the strongest effect. The tests of alcohol, pH and sulphite as antimicrobial agents alone or in combination revealed no significant role in the bactericidal effect.

The survival of bacteria living in biofilm was also tested because the increased environmental tolerance of biofilm bacteria is well known. Biofilms were developed on two surfaces (glass or plastic) at 3 different temperatures (10, 25, 38ºC) were applied. During the development they were

stressed with draught and starvation. The tolerance of bacterial biofilm to Nagyrédei Zweigelt wine was tested with 24, 48, 72 hours exposure. Weak tolerance was observed when bacteria were incubated at 10 ºC, and 38 ºC incubations gave the best survival. There was no marked difference between biofilm holding materials. The applied stresses slightly increased the tolerance of both bacteria but they were totally killed after 72 hours of wine exposure in every combination. Because the temporary storage time of bulk or bottled wines generally exceeds this period, the hazard of contamination with the investigated bacteria seemed rather low, however, it cannot be excluded.

FINGERPRINTING LEGIONELLAE – CHALLENGE AND ALTERNATIVES

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Legionellosis is one of today's emerging infectious diseases. In Hungary, it is mandatory notifiable disease since 1998, 10-50 cases are reported annually. In Europe, most of known *Legionella* infections are acquired via aerosol from colonized artificial water environments. Monitoring legionellae in drinking or bathing waters, however, is not required by the Hungarian regulation.
Methods for detection and identification of *Legionella* species have been evolving in the past 30 years. Though culture independent techniques are on the rise, standard method of detection still is cultivation on buffered charcoal media, and identification based on morphology and cystein autotrophy. Commercially available kits for DNA based detection are mostly targeting the clinically most relevant *L. pneumophila* serotype 1.
Species and sub-species identification is routinely done by serotyping, while the golden standard for differentiation at a sub-serotype level is multi locus sequence typing. The latter is the only method to date that is generally accepted to provide sufficient discrimination for infection source-tracking. However, it is too expensive and time consuming for routine environmental monitoring, where such fine typing is not required. Serotyping, however, by commercial sera is also costly, and often yields highly ambiguous results due to cross-reactions.
Two fingerprinting methods are presented as potential alternative to serotyping: genotyping by rep-PCR using ERIC primers and chemotaxonomic profiling by intact cell MALDI-TOF MS. Methods were tested on over a 100 environmental isolates and type strains of *L. pneumophila* and other species. Both methods are rapid, reproducible, provide unambiguous result with a discriminatory power equal to or higher than serotyping, while running costs are substantially lower.

THE EFFECT OF TECHNOLOGICAL STRESS ON BACTERIAL SPORES AND VEGETATIVE CELLS

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The aim of this work was to study the combined effect of heating at 95°C for 0-20 min at pH (7.0) and the NaCl concentration (0-5%) of the medium during heat treatment on the survival and growth of *Bacillus cereus* T spores and vegetative cells using conductance (MALTHUS instrument) and redox potential measurement. Correlation was found between the initial cell counts and the measured detection times and also between the measured conductance values and the actual cell counts. Based

on this correlation a calculation technique of the growth rate and lag-phase was developed. When evaluating the survival/growth of the treated bacteria using the calibration curve made with untreated bacteria, a difference occurs in the survival rate obtained with conductance measurement and plating methods. This difference can be attributed to the injury of the surviving bacteria. At heating beyond the decrease in the cell count the injury of the surviving spores has also increased with the increase of the duration of the treatment. The heating time had no effect on the growth rate of the surviving microbes, however, the lag-phase was 5 hours longer after 20 min heating. The NaCl content of the medium during heat treatment of spores had a protective effect and also the germination of the surviving spores was promoted. NaCl independent of the concentration has increased the growth rate by 20%, and decreased the length of the lag-phase by 2 hours. An increase of the NaCl concentration of the medium during treatment up to 2% has increased the D-value by 20 min. The further increase of the salt content had no additional effect up to 4%, and at 5% the D-value returned to the initial level.

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STRUCTURE AND DYNAMICS OF THE ANTIFUNGAL PROTEIN PAF BY NMR

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The small molecular mass (6.2 kDa) cysteine-rich and basic antifungal protein of *Penicillium chrysogenum* (PAF) induces multifactorial detrimental effects in sensitive fungi. A deeper understanding of the mechanism of action of PAF is likely to lead to the development of novel antifungal drugs. Standard 2D and 3D double echo-antiecho TOCSY and NOESY **N**uclear **M**agnetic **R**esonance spectra of the 1.6 mM 15N labelled native PAF were collected. SPARKY analysis resulted in full backbone and 80% side chain assignment of the signals. 3JNH,Ha couplings were obtained from 2D 15N-HSQC and 13Ca assignments were determined at natural abundance. NOE assignments and structure calculation was performed using ATNOS/CANDID in combination with CYANA 2.0. We attempted the concerted use of NOE and S2 restrained molecular dynamics simulation (MUMO) [1] for generating realistic ensemble structures. In spite of the 47% sequence identity with respect to the antifungal protein 1AFP, PAF and AFP exhibit striking similarity: five antiparallel β strands form β barrels in both proteins that are fixed by three or four disulfide bridges. Classic 15N relaxation experiments yield 3 ns global correlation time and suggest a compact monomeric structure. ^{1}H and ^{15}N CSA/DD cross-correlated relaxation and water saturation transfer experiments also corroborate the proposed structure of PAF.

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HIV-1 INFECTION CAN BE PREVENTED BY DRUGS AND ADJUVANTS THAT WILL PREVENT THE VIRAL EVASION OF THE IMMUNE RESPONSE AND RESTORE THE ADAPTIVE IMMUNITY

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HIV-1 pandemic has killed 25 million people and currently involves 41 million infected people worldwide. Attempts to develop a vaccine had failed, but anti-viral drugs have prolonged the longevity of infected individuals. The drugs have eradicated the sensitive virions, while an increased amount of drug resistant viruses appears which continue to do damage.

The initial aim of the study was to explore the reasons for the unsuccessful attempts to develop a vaccine against HIV-1 infections and to understand the mechanism for the immune evasion.

The approach was to scan the literature for studies that had been done since 1983 with HIV-1 infected individuals and had been neglected by the mainstream researchers. It was found that three research groups had noted that in the patients' blood, a gradual increase of IgE was reported, a phenomenon that was described in allergic individuals exposed to different types of allergens. More then twenty years ago, Mosman and Coffman presented evidence that the adaptive immune response is composed of two CD4+ T cells. Th1 cells, producers of IL-2, IL-12 and IFN-G, and Th2 cells, producers of Th2 cytokines IL-4, IL-5, IL-10 and IL-13. In healthy individuals, the two cytokine systems are balanced, but in allergy, the Th2 cytokine level is induced and damages the Th1/Th2 cytokine balance.

HIV-1 is introduced into the body of an uninfected individual sexually and the virus infects epithelial cells at the virus entry. The tissue dendritic cells rush to the infection site, interact with the virions and move through the lymph vessels to the draining lymph node. There the dendritic cells present the viral antigens to the naïve CD4 T cells, inducing them to differentiate into Th1 and Th2 cells, the latter cells are infected by HIV-1. Replication of HIV-1 release the virions to act as allergens that induce the Fc epsilon R1+ hematopoietic cells, mast cells, basophils and monocytes, to release large amounts of the Th2 cytokines, shifting the Th1/Th2 balance toward Th2 cytokines. Of the Th2 cytokines, IL-4 is the cause of the inhibition of Th1 cytokine synthesis, preventing the activation of the CD8+ T cell precursors to develop into CTLs. In addition, IL-4 inhibits B cell synthesis of IgA and IgG and induces them to synthesize IgE, non neutralizing antibody to HIV-1 antigens. Several years ago a third helper T cell was identified designated Th-17,a producer of IL-17. The involvement of this cell type in allergy had been reported.

To stop HIV-1 infection: it is necessary to decrease the level of Th2 cytokines in the patients by IL-4 antagonist IL-4 delta2 splice variant or synthetic IL-4 like molecules that will bind to the IL-4 Ra on T and B cells. In addition, introduction of adjuvants that bind to Toll like receptors TLR9 and TLR4 could inhibit HIV-1 replication. Currently, the TLR9 ligand CpG ODN will bind to TLR9+ plasmacytoid DCs and induce them to release large amounts of IFN a/β, inhibitors of HIV-1 replication. The TLR4+ Th1 cells will interact with the ligand monophosphoryl lipid A (MPL) which reactivates the Th1 cells to release the Th1 cytokines that will reactivate the CD8+ T cell precursors to become antiviral cytotoxic T cells. The combined treatment with cytokine antagonists and the two adjuvants may reactivate the patients' adaptive immunity that will clear HIV-1.

STUDY OF HISTOCYTOPATHOLOGICAL CHANGES OF *PISTACIA VERA* ROOT CELLS, INOCULATED WITH *VERTICILLIUM DAHLIAE IN-VITRO*

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Soil born pathogenic fungus *Verticillium dahliae*, is involved in *Verticillium* wilt of many plant hosts. *Pistacia* trees are one of its hosts. *Pistacia* product is one of most important export goods of Iran. Therefore in this study, we focused on structural changes of cells in *Pistacia* root, after mycelium penetration. *Verticillium* spp. isolated from infected trees were identified, and *V. dahliae* strains were inoculated on *Pistacia* roots. Then these roots were prepared for observation with light and electron microscopy, after 3,5,7,9 days, with classical protocols. The results showed that in wounded roots, mycelium penetration and damage is more severe than in intact roots. In the first 3 days, fungi penetrated the epidermis and cortex. 5 days after inoculation, pathogen mycelium was observed in all of tissue layers of root. After 7 and at the end 9 days, all of the root cells were dead and destroyed. Changes in organelles such as cell wall, nucleus, mitochondria and vacuole was compared in control and infected roots.

COMPARISON OF MULTIPLICATION ABILITY OF POULTRY-RELATED CAMPYLOBACTERS DURING ENRICHMENT

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The intention of selective enrichment is to increase the proportion of target microorganisms to allow successful detection. Enrichment is particularly needed when low numbers of pathogens are the target. If more than one strain of the target bacteria were present, one strain might multiply more rapidly than others during enrichment, thus possibly yielding misleading results concerning the predominant species and/or strains in the samples examined. In this study we investigated this possibility by comparing the ability of four strains each of *Campylobacter jejuni* and *C. coli* to multiply when inoculated in varying proportions in selective enrichment broth. The test strains had previously been characterised using molecular techniques and their hippurate reaction checked (*C. jejuni* positive and *C. coli* negative). Bolton enrichment broth was used, incubated at 41.5°C for 48 h in air with tightly-closed lid and small headspace. The tubes containing Bolton broth were inoculated with pairs of *C. jejuni* /*C. coli*, using stationary phase cells with varying proportions of the two species, such that one ml of enrichment broth contained approximately 50 *Campylobacter* cells. After incubation the broth was streaked onto mCCD agar and incubated under microaerobic conditions at 41.5°C for 48 h. By the use of the hippurate hydrolysis test twenty colonies were identified from each plate. The *C. coli*/*C. jejuni* ratios at the beginning and at the end of enrichment were significantly different for each of the four comparisons. These experiments showed that all four *C. coli* isolates were able to multiply and dominate the population in Bolton selective enrichment broth even when initially inoculated in a relatively low ratio. This was true for all combinations of the test strains. *C. jejuni* was isolated in detectable numbers only when initially inoculated in a very high ratio. As five or fewer colonies of *Campylobacter* are often picked from the selective agar when examining foods or other samples, this study indicates that if the sample contains a mixture of *C. jejuni* and *C. coli*, the latter is much more likely to be isolated than *C. jejuni*.

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1. EN ISO 10272-1/2006.

INVESTIGATION OF THE STRUCTURE AND TRANSFER OF THE *YERSINIA* HIGH PATHOGENICITY ISLAND IN *KLEBSIELLA PNEUMONIAE*

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The structure and function of the *Yersinia* High Pathogenicity Island (HPI) is well-characterised in human pathogenic *Yersiniae* and in extra-intestinal *E. coli*. In spite of the fact that the island is widely distributed in further members of the family Enterobacteriaceae its genetic organisation and transfer mechanisms have not yet been studied in detail. A recent discovery of a unique type HPI in *E. coli* ECOR31 resembling integrative-conjugative elements (ICEEc1) indicates the possible role of conjugative transfer in dissemination.

From a collection of HPI-positive enteric bacteria isolated from extra-intestinal infections we identified five *K. pneumoniae* strains bearing an unusually structured, ICE-like HPI. Although they share common features, they are not identical since they suffered several truncations and deletions. Furthermore, our sequencing data reveal that their most downstream region is not only completely different from the one of ICEEc1 but there is an individual variation among the distinct *K. pneumoniae* strains. Episomal interspecies transfer of the ICE-like HPI of *Klebsiellae* has been observed, however, the island displayed instability in its new hosts.

REGULAR DYNAMICS OF PLANT-MICROBIAL COLONIZATION IN SEWAGE-SLUDGE TREATED SOILS

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The use of sewage sludge in arable fields is an increasing agricultural practice and necessity nowadays. After a long-term use, however, several adverse effects on the abundance and functioning of the soil (rhizosphere) microbiota are known to develop, which is due to the accumulation of heavy metals. Concern is also to be raised about potential pathogens and food safety [1].

The effect of various sewage sludge doses (0, 2.5, 5.0, 10.0 and 20.0 $g.kg^{-1}$ dry soil, i.e. at the 0, 7.5, 15, 30 and 60 $t.ha^{-1}$ rates, respectively) of municipal and industrial origin (enhanced Zn and Cr content, 2845- and 5225 $mg.kg^{-1}$ dry sludge, respectively) was investigated on four Hungarian representative soil types (calcareous sand- [CS] and chernozem [CC], or the acidic sand- [AS] and forest soils [AF]) in four-year periods. Parameters, such as the dry-matter production of green pea (*Pisum sativum* L.), macro- and micro-element accumulations and the rhizosphere colonization by symbiotic bacteria and fungi, were assessed at the flowering periods of the pea in each consecutive year. A shortest circadian daily periodicity of the plant-soil ecophysiological interaction was measured by a quadrupole mass spectrometer.

Increasing doses of sewage sludge resulted in a fertilizer effect on the soils studied, according to the awareness on a short-term basis and at the lower ratios applied. Soils, particularly the sandy types (CS, AS), responded quite positively to the sludge addition by improved physical and chemical soil

characteristics. In the case of the acidic soils, the translocation of the heavy metals towards the shoot biomass was found to be a more critical issue. The seed production, however, was protected from the accumulation of toxic metals independently from the negative effects of the high Cr content on the green yield of pea found already after the 2nd year of applications. Such adverse effect of the accumulation of Zn could be developed in the 4th year only. Colonization of microsymbionts proved to be highly affected both by the increasing nutrient availabilities and by the increasing accumulation of heavy metals in later periods. Sensitive or tolerant adaptation of some nitrogen-fixing bacteria was found as a function of metal doses of the Zn-containing municipal sludge on a long-term basis [2].

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REGULATION OF SPORULATION IN *STREPTOMYCES*: THE ROLE OF FACTOR C

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Bacteria of the Gram-positive filamentous *Streptomyces* are a well-known model system for the study of prokaryotic multicellular differentiation, with a complex life cycle culminating in spore formation. The onset of development is triggered by nutritional signals and temporally relates to the production of antibiotics and other secondary metabolites. Autoregulatory molecules play a key role in controlling both the onset of cellular differentiation and secondary metabolism in these microbes. The best studied autoregulator is A-factor (2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone), a small microbial hormone-like molecule (243 Da) that induces both morphological and physiological differentiation in *Streptomyces griseus*.

Another interesting autoregulator is the secreted signaling protein Factor C (Mw 34.555 Da), originally isolated from the culture fluid of "*Streptomyces griseus* 45H" but recently shown to be identical to a laboratory strain known as *Streptomyces flavofungini*, itself a member of the *Streptomyces albidoflavus* species group. The Factor C producer strain like S. griseus readily sporulates in submerged culture. Similarly to A-factor, Factor C also plays a key role in cellular communication and cytodifferentiation. Here we report our results on the study of factor C and the possible interconnection of the A-factor and Factor C regulatory pathways.

THE EXPANDING REALM OF *CRYPTOCOCCUS NEOFORMANS*

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Cryptococcus neoformans is a basidiomycetous human pathogenic yeast with a predilection for the central nervous system. For a long time a single species divided into three varieties and four serotypes (A-D) was recognized, but various genotyping approaches have yielded a more complex genotypic and intraspecific substructure. *C. neoformans* contains two serotypes A, D, and the AD hybrids. *C. gattii*, previously considered to be a variety under *C. neoformans*, comprises serotype B and C isolates. Nine different genotypes can be discerned. Six of them represent haplotypes, two in

C. neoformans and four in *C. gattii*. Three genotypes represent diploid or aneuploid hybrids between the haplotypes. Demographic and geographical differences occur between the different genotypes. *Cryptococcus neoformans* var. *grubii* is known to be a risk for immunodeficient patients, in particular those infected with HIV. Estimates of UNAIDS/WHO show that in Africa, in particular sub-Saharan Africa, more than 28 million people are infected with HIV (www.who.int/hiv/; data 2002), and in the S. African province of Gauteng, the incidence of cryptococcosis has been reported to be 3.117 per 100.000 HIV-infected people. *C. neoformans* var. *neoformans* seem to have a preference to infect Caucasian elderly (patients >60 years of age), and those receiving corticosteroid therapy.
C. gattii, until recently considered to have a tropical or subtropical distribution is mainly involved in infections of immunocompetent humans. Since 1999 a major outbreak caused by *C. gattii* serotype B is responsible for infection of immunocompetent patients, and domestic and wild animals on Vancouver Island (Canada). The fungus could also be isolated from a number of temperate trees as well as air surrounding these trees. Detailed genotyping demonstrated the presence of two genotypes. As almost no genetic divergence was observed within the two genotypic lineages involved in the outbreak, it is assumed that the expansion of the population size is due to clonal propagation. The three other genotypes of *C. gattii* occur both in the environment (viz., trees) and are able to infect humans as well.
Here, we will discuss current views on the systematics of the complex, the clinical relevance of the various genotypes, and the application of comparative genome hybridization using microarrays in order to understand the complexity at the genomic level.

STUDY OF THE BACTERIAL COMMUNITY OF INDUSTRIAL COOLING WATERS BY MOLECULAR METHODS

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Many industrial institutions suffer from the microbial contamination of cooling water though they use ultra pure water. Microbes exist not only in the water but can also form biofilms on different surfaces (ion exchange resins, pipelines, tanks) of the system. Due to the biofilm formation metals are subjected to microbially-influenced corrosion (MIC). In our study, cooling waters were examined in front of and behind different storage tanks both in the primer (boyler) and the secondary (turbines) cycle of a Hungarian power plant. The volume compensatory tank (TK) stores and gives the regenerated saltless water to the primary cycle. The production of ultra pure waters was performed with mixed bed ion exchange resins in all cases. Three different water cleaning tanks (VT) were examined: I.VT treats the water from the primary cycle with non-regenerated resin; IV.VT cleans waters from both cycles, containing a long time ago regenerated (2-year-old) resin; V.VT treats the water from the secondary cycle and has been regenerated 1 year ago. For studies, 5-9 litres of water samples were filtered, then total DNA isolation and community fingerprint was carried out with T-RFLP. Shannon-Weaver diversity indexes were calculated based on T-RFs. To identify the dominant bacteria in the samples, a previously constructed clone library was used. According to the T-RFLP analysis diverse profiles could be detected in many cases. The calculated Shannon-Weaver diversity indexes varied from 3.18 to 4.45. In the case of compensatory tank (TK) on contrary there was a slight decrease in the diversity index. Changes in the taxon composition could also be detected: while in the inlet water α-proteobacteria (*Sphingomonas* sp., *Novosphingobium hassiacum*) dominated, in the outlet water the bacterial community shifted to β-proteobacteria (*Rhodoferax* sp., *Polynucleobacter* sp., *Sterolibacter* sp.), CFB (Bacteroidetes) and Firmicutes. Regarding I.VT,

increasing diversity and shift in the bacterial community structure could be detected. Taxa belonging to β- and γ-proteobacteria which have been found in inlet water disappeared, except *Methylosinus* sp. of that amount increased in the outlet water. In addition, new phylotypes appeared such as Firmicutes, Chloroflexi, division Verrumicrobia, and *Rhodoferax* sp. It indicates not only the microbial enrichment in tanks but also the possibility of the resin originated contamination. Concerning the tanks IV.VT and V.VT, diversity indexes were similar (around 3,39), changes in the T-RFLP profile could be detected in decreased T-RF ratios. Using the clone library, some peaks could be identified, but for the majority of the peaks, no match with the sequences was found. β-proteobacterial T-RFs (*Sterolibacter* sp*., Methylibium petroleiphilum, Polaromonas rhizosphaerae*) were abundant in the profiles. Similarly, the abundant T-RFs belonged to members of α-proteobacteria (*Sphingomonas* sp., *Novosphingobium hassiacum*) CFB (Bacteroidetes) and Firmicutes could also be detected.

HERPESVIRUS-MEDIATED DELIVERY OF FLUORESCENT ACTIVITY MARKER GENES TO NEURONS

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In this study we have constructed recombinant pseudorabies virus (PRV) vectors for delivery of fluorescent activity marker genes to various neurons across synapses. Changing of calcium level in neurons indicates their activity. We have constructed recombinant viruses expressing various genetically encoded fluorescent Ca^{2+} indicator proteins (FCIP), including cameleon, troponeon and G-CaMP. The Ca^{2+} sensitivity of the fluorescence readout of FCIPs is achieved either via changes in the efficiency of fluorescence resonance energy transfer (FRET) between two spectral variants of fluorescent proteins (cameleon and troponeon) or via changes in the pKa of circularly permuted spectral variants of GFP (G-CaMP). We have also constructed fluorescent pH sensor (synapto-pHluorin) expressing PRV strains, which can indicate neurotransmitter release. PRV exerts detrimental effects on the infected cells, therefore, we have eliminated several genes contributing to cytotoxicity.

THE B12 RIBOSWITCH-MEDIATED CONTROL OF METABOLIC PROCESSES IN *STREPTOMYCES*

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Riboswitches are metabolite-sensing elements present in the 5'-untranslated regions of mRNAs of bacterial genes that control gene expression in response to binding of small molecules in the absence of proteins. B12 (adenosylcobalamin) riboswitches are widespread in bacteria and are commonly present in front of genes responsible for cobalamin biosynthesis and transport. In some bacteria B12 riboswitches occur in the regulatory regions of genes or operons encoding non-B12 related functions, the best characterized examples of which are the riboswitches associated with genes encoding the B12-independent class I NrdAB and class III NrdDG ribonucleotide reductases (RNRs), and the B12-independent methionine synthases MetE. These bacteria invariably contain genes encoding a second,

alternative, B12-dependent class II NrdJ RNR and methionine synthase MetH isoenzymes, respectively. The B12-dependent NrdJ and MetH are normally the more efficient of the isoenzymes.
We show that *Streptomyces* possess B12-independent and B12-dependent RNRs and methionine synthases either of which is sufficient for normal vegetative growth. We identified the presence of consensus B12 riboswitches in the 5'-UTRs of the class Ia *nrdABS* and the *metE* mRNAs. The effect of B12 on growth and on transcription of the class Ia *nrdABS* RNR genes was examined in a mutant in which the *nrdJ* gene, encoding the class II RNR, was deleted. B12 concentrations of 50 $\mu g.L^{-1}$ completely inhibited growth of the NrdJ mutant and significantly reduced *nrdABS* transcription. Similarly, B12 repressed transcription of *metE* and inhibited growth of a mutant in which the *metH* gene was disrupted. Riboswitch insensitive mutants were isolated by their ability to grow in the presence of B12. Mutations were found to map in highly conserved regions of the respective riboswitches and are likely to affect B12 binding. These studies demonstrate that in *Streptomyces* B12 controls RNR and methionine synthase expression in two fundamentally different and reciprocal ways, via riboswitch regulation of the B12-independent isozymes and by serving as an essential cofactor for the class II RNR and MetH enzymes.

PRODUCTION AND PURIFICATION OF EXTRACELLULAR PHYTASE FROM *ASPERGILLUS NIGER*

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Phytase (myo-inositol hexakisphosphate phosphohydrolase EC 3.1.3.8) hydrolyzes phytic acid to myo-inositol and inorganic phosphate. Phytic acid is the major storage form of phosphate in plants, which is biologically unavailable to non-ruminant animals, including humans and pigs. Phytic acid is a strong chelating agent and forms complexes with several divalent cations of major nutritional importance (e.g. Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}), and reduces digestibility of proteins, starch and lipids. On the other hand, inositol-6-phosphate is considered to exert an anticancer function not only through its antioxidant effect but also through its conversion into lower inositol phosphates (InsP1–5), suggesting that other mechanisms like its simple chelating power is involved.
Phytase can be produced from several sources including plants (oilseeds, legumes, and cereal grains), animals and micro-organism. Although several strains of bacteria, yeasts and fungi have been used for production of phytase under different conditions, especially those originating from filamentous fungi such as *Aspergillus niger* and *Aspergillus ficuum* have most commonly been employed for commercial production of extracellular phytase.
Eleven strains of *Aspergillus* have been screened for phytase enzyme activity on rice flour medium. The *Aspergillus niger* F00735 was found to be the most promising one. The composition of medium for the production of phytase enzyme was optimized. Effects of various nitrogen sources were investigated and sodium nitrate seems to be the best one. Among the tested natural and syntetic substrates, the highest activity was measured on medium containing rice flour. The ratio of inoculum size to the volume of fermentation media was also investigated.
The enzyme production was carried out under optimized conditions: medium with 6% rice flour at 28°C on rotary shaker (200 rpm) in 1000 cm^3 Erlenmeyer flasks containing 150 ml medium. The maximal activity was 700-800 $U.L^{-1}$. After 6-day fermentation the mycelia were collected by filtration. Extracellular proteins were fractionally precipitated by ammonium-sulphate.
Enzyme purification was achieved by a combination of concentration by ultrafiltration followed by chromatographic resolution using ion exchange (DEAE-Sepharose CL-6B), gel filtration (Sephacryl

S200 HR) and chromatofocusing (PBE94) steps. All chromatographic steps were performed with fast performance liquid chromatography system (FPLC) at 4°C. After chromatofocusing two peaks with phytase activity were detected. Phy-I and Phy-II were purified to homogeneity on hydrophobic interaction chromatography (Phenyl Sepharose high-sub) to approximately 21 and 4.71-fold, with a recovery of 10.5% and 4.82%, respectively. By SDS-polyacrilamide gel electrophoresis the molecular masses of the purified enzymes PhyI. and Phy II. were estimated to be 65-67 kDa and 117-120 kDa, respectively. Phytases are used as an animal feed additive to improve phosphate bioavailability, and reduce phosphate excretion and environmental pollution.
Acknowledgement: This work was financially supported by OTKA grant T042653.

BACTERIAL DIVERSITY OF THE RHIZOSPHERE OF *ELYMUS ELONGATUM* GROWN IN THREE DIFFERENT SOILS, TREATED WITH FERTILIZERS AND WOOD ASHES

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In a plot-experiment the effect of different composts and wood ashes on the rhizosphere of the grass *Elymus elongatum* (*Agropyron elongatum*) cv. Szarvasi-1 has been studied in Baranya county.
Three characteristic Hungarian soil-types (meadow soil [Bf18], Raman-type brown forest soil [M9], and loamy brown forest soil [M16]) have been investigated. *Elymus elongates* cv. Szarvasi-1 was planted as energy-plant in the spring of 2005.
Soil samplings were carried out in autumn 2005 and in spring 2006 from the 0-20 cm layer. Physical, chemical and microbiological analyses were done. Total count of aerobic heterotrophic bacteria (on nutrient-plates) and counts of free living N-fixing bacteria (on Ashby-plates) were determined.
The bacterial diversity of the soil was analysed by nucleic acid-based methods. After DNA extraction, fragments of the 16S rRNA gene were amplified by PCR using universal Bacteria specific primers with GC-clamp. A molecular fingerprinting method, the denaturing gradient gel electrophoresis (DGGE) was used and results were evaluated by cluster analysis. Dendrograms were created by using the neighbour-joining algorithm based on S_{SM} values.
The total count of bacteria in the control, untreated soils was high in all soil types: 1 - $2{,}4.10^8.g^{-1}$ dry soil. There weren't any changes detected in the count of bacteria in soil Bf18 applying either fertilizers or wood ashes, only in the case of compost, originated from sewage sludge. The N-content was high in soil type M16, so the inorganic N supplementation did not influence the germ counts, only the addition of organic compost had a positive effect. In the case of M9 total count of bacteria increased by adding fertilizers, but the wood ashes plus compost treatment gave an opposite result.
There weren't any detectable differences in the bacterial diversities of soils Bf18 and M16 without plant and with energy grass. The diversity in the M9 soil type was different in the rhizosphere and bulk soil. In this soil type together with M16 the biggest changes in bacterial diversity were detected as the effect of the treatment with wood ashes mixed with compost. Such changes were seen at the soil Bf18 only in the case of the treatment with "green compost" and wood ashes.
The soil microbial monitoring will be continued in the next year. Analysis of the biomass values of the energy grass, together with soil chemical parameters and the changes in bacterial diversity could give an answer on the effect and optimum use of wood ashes as fertiliser.
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RIBONUCLEASE RNE/RNG FAMILY PROTEIN FROM *MYCOBACTERIUM SMEGMATIS*

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Mycobacterium smegmatis, a fast-growing saprophyte, has been used extensively in biochemical and genetic studies because it is a model mycobacterium, which grows rapidly and has no health hazard in the laboratory. In addition, *M. smegmatis* can be transformed effectively by genes for pathogenic antigens and it is a valuable vector for the development of live vaccines. However, little is known on its RNA metabolism. Rne/Rng family proteins are key enzymes in RNA processing and degradation in *Escherichia coli* and other bacteria. To learn more about RNA metabolism in *M. smegmatis*, its Rne/Rng family protein was cloned and expressed and some of its associated proteins were identified. We cloned the *rne* gene into a pET based vector for expression in *E. coli* and an *E. coli* - mycobacterium shuttle vector (pMV262) for expression in *M. smegmatis*. RNase E/G from *M. smegmatis* is a large protein consisting of 1037 amino acids (GenBank accession no. MSMEG_4626). Its calculated molecular mass is 113 kDa, but in SDS-polyacrylamide gel it migrates as a 180 kDa protein, thus resembling its *E. coli* homologue. Following the affinity purification, the Rne/Rng protein preparation was analysed on SDS-polyacrylamide gel and the co-purified proteins were identified by mass spectrometry. Despite the use of protease inhibitors in addition to the full length polypeptide, the preparation also contained its proteolytic forms.
Polynucleotide phosphorylase, enolase and RNA helicase form a large complex with *E. coli* RNase E, referred to as degradosome. This is the prototype of the family of multiprotein complexes involved in the processing and degradation of RNA. The co-purified proteins from *M. smegmatis* were different from those in *E. coli*, as expected after previous mycobacterial studies, though they are not even identical to the associated proteins we found in *M. tuberculosis*. Surprisingly much more proteins were detected in *M. smegmatis*, than in *M. tuberculosis*. Chaperonin GroL was a major member of this complex. Ribosomal proteins were also present. Among others, a negative regulator of genetic competence and a GTP pyrophosphokinase (role in cellular response to starvation) were found.
Our data indicate that, similarly to the enzymes of *E. coli*, mycobacterial Rne/Rng homologues are able to co purify with other proteins. However, the associated proteins are different in the two cases.

SYMPTOMLESS REACTIVATION OF LYMPHOTROPIC HERPESVIRUSES, PAPILLOMAVIRUSES AND TT VIRUS IN HEALTHY PREGNANTS BEFORE PARTURITION IN HUNGARY

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Lymphotropic herpesviruses have been found by many authors in the urine and blood of healthy neonates. The published methodologies of sampling suggested transplacental transmission in addition to the perinatal infection during delivery.

The work presented is the first systematic screening of amniotic fluids taken from healthy pregnants at term and tested for the presence of human herpesvirus types 4 (EBV), 5 (CMV) 6, 7 and 8. The presence of all anogenital papillomavirus genotypes have been examined, too. Amniotic fluids were sampled before artificial rupture of membranes using a closed Vacutainer system. Blood samples were taken from the pregnants simultaneously. With the permission of the Committee for Ethics 106 amniotic fluid samples and maternal blood samples were examined.
Both amniotic fluids and blood samples were tested for the presence of DNA of lymphotropic human herpesviruses. The DNA of human papillomaviruses and TT virus were tested only in the amniotic fluid samples.
The DNA of at least one herpesvirus could be detected in every 4th amniotic fluid sample and in every 8th blood sample. The prevalence of papillomaviruses was 7 of 96 samples. Epstein-Barr virus, human cytomegalovirus and human herpesvirus type 7 were found more frequently in the amniotic fluids than in blood samples (7 to 1).The prevalence of human herpesviruses 6 and 8 was significantly higher in the blood samples than that in the amniotic fluids.
It is well known, that fetal cells can be detected in the maternal circulation. Recently it has been shown, that maternal cells can be transported into fetal tissues, too. Our hypothesis is that the reactivation of latently harboured viruses occurs following materno-fetal transfer of the lymphocytes. The materno-fetal trasport of reactivated viruses cannot be excluded either.
The mean weight of the neonates was not impaired significantly by the presence of viruses passing the placenta at the very end of pregnancy. Possible post partum consequences i.e. possible partial immunotolerance of the neonates to viruses during their later years will be discussed.

ACCUMULATION AND BIOSYNTHESIS OF COMPATIBLE SOLUTES IN THERMOPHILES AND HYPERTHERMOPHILES

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Many organisms that live at very high temperatures have been isolated from shallow marine and abyssal thermal environments, where the geothermally heated water may reach the salinity of the surrounding seawater. These organisms, like all other microorganisms must adjust, within intrinsic limits, to alterations in the water activity of the environment. The majority of microorganisms adjust osmotically by the selective accumulation of small molecular weight organic compounds. Thermophiles and hyperthermophiles accumulate a few compatible solutes that are also common in mesophilic bacteria and Archaea, namely trehalose and glutamate and even the very rare glucosylglycerate (GG). However, the majority of the compatible solutes encountered in hyper/thermophiles are unique to these organisms. These compatible solutes include mannosylglycerate, di-myo-inositol-phosphate and the very rare compatible solutes di-glycerol-phosphate, di-mannosyl-di-myo-inositol-phosphate and mannosylglyceramide.
In recent years we have studied the synthesis of mannosylglycerate (MG) and trehalose in *Thermus thermophilus* (Phylum *Deinococcus*/*Thermus*), *Rubrobacter xylanophilus* (Phylum *Actinobacteria*) and *Persephonella marina* (Order *Aquificales*). The species of the genus *Thermus* have optimum growth temperatures that range between 70 and 75ºC and, with the exception of *Thermus thermophilus*, a maximum growth temperature below 80ºC. The species *Thermus thermophilus* has a maximum growth temperature of about 82 to 83ºC and is capable of growing in media containing 3 to 5% NaCl. The strains of *T. thermophilus* accumulate primarily trehalose and lower levels of mannosylglycerate (MG) during osmotic adjustment.

Recombinant mutants lacking the genes for the synthesis of trehalose, MG or both, result in a profound effect on the ability of organisms to grow in media containing NaCl. The synthesis of MG by *T. thermophilus* proceeds via a two step pathway catalyzed by mannosyl-phosphoglycerate synthase (MpgS) and mannosyl-phosphoglycerate phosphatase (MpgP) from GDP-mannose and 3-phosphoglycerate. These enzymes are very similar to those found in other hyper/thermophilic organisms, however the MpgS and the MpgP from *R. xylanophilus* have little or no identity to the other enzymes. The homologous enzymes of *R. xylanophilus* lead, depending of the substrate, to the synthesis of MG or GG. The thermophilic bacterium *P. marina*, on the other hand, is the only known thermophile to accumulate GG in response to salt stress and possesses genes that are also very different from the ones mentioned above. Moreover, this organism possesses two pathways for the synthesis of GG. The physiological relevance of MG and GG accumulation in these thermophilic bacteria and the evolution of MG and GG biosynthesis in prokaryotes are discussed.

MOLECULAR DIAGNOSTICS AND CLINICAL RELEVANCE OF *CHLAMYDIA TRACHOMATIS* INFECTIONS

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The *Chlamydia trachomatis* (*C.t.*) bacterium is responsible for the most common genital infections in both males and females. The importance of detection has increased because of a new variant of *C.t.* (Ripa T), the asymptomatic infections, and the rising frequency of lymphogranuloma venereum (LGV) cases among men who have sex with men (MSM). A novel mutant *C.t.* strain, discovered in 2006, displays a specific genetic variation (deletion) in the cryptic plasmid such that all current detection systems making use of this plasmid region as a target area fail.

C.t.-relevant methods are needed for the different patient groups. Rapid tests (Clearview chlamydia MF) have been developed for the developing countries (Peeling R). NAATs have been improved for simultaneous detection and genotyping (Xiong L, Molano M): multiplex real-time PCRs (Marshall R), bed-based DNA assay (Lab-on-a-chip (Monaghan PB) and multiplex PCR-based oligonucleotide microarrays (Shi G).

Increases in the incidence of persistent infections have been observed in France and Australia (Massari V, Chen MY). Reinfections and persistent infections are frequent among *C.t.*-positive patients, who should be rescreened (Veldhuijzen I, Peterman T). Screenings of rural and London populations in England revealed a positivity of infection between 2.9-27.4 and 3-12.5%. The prevalence of *C.t.* in metropolitans was 17.7% in Taipei, Taiwan, where 40% of the infections were asymptomatic (M-C Yu), 11.5% in Rio Cuarto, Argentina, and 14.5% an average in Rotterdam, where it was highest among the school population, at 24.5%. Sentinel surveillance has been introduced in Germany, where the *C.t.*-positivity proved to be 6.7% of the have been 46,168 samples. Between the end of December 2005 and January 13, 2006, 107 pregnant women were screened for *C.t.* by Cobas TaqMan real-time PCR method, at the Gynecological Outpatient Clinic and the University Clinic in Szeged. The prevalence of infection was found to be 4.7%. The incidence of *C.t.* infection in our study was significantly associated with younger age, lower education level, unmarried marital status, higher number of lifetime casual sex partners and former STDs. Hidden epidemic (USA) and spatial bridges of spreading (Sweden) of *C.t.* have been revealed. Selective screening is advised by colleagues in The Netherlands. An outbreak started among MSM in Rotterdam in 2003. Retrospective examinations have made possible the detection of rectal LGV cases in Belgium, France, Germany (2004), England, USA (2005), Sweden, Switzerland (2006) and finally

in Australia (2007). The number of antibiotic–resistant *C.t*. strains is increasing. Spontaneous mutation, phenotypic and genotypic resistance have developed against rifampin and rifalasil (Kutlin A). Nucleotide variability has been observed in rpoB in rifampin-sensitive and resistant strains of *C.t.* (Dreses-Werringloer U). Mutations in a 23S RNA gene of *C.t.* were found to be associated with the resistance of macrolides and point mutations in fluoroquinolones-resistant isolates (Misyurina O).

DETECTION OF EPSTEIN-BARR VIRUS IN DISEASES OTHER THAN INFECTIOUS MONONUCLEOSIS, BY QUALITATIVE AND QUANTITATIVE REAL-TIME PCR

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Epstein-Barr virus (EBV) is a ubiquitous virus which is a causative agent of acute infectious mononucleosis (IM), with fever, pharyngitis, lymphocytosis and lymphadenopathy in children (1-6 y), adolescents and young adults worldwide. Chronic IM develops rarely. As the virus is tumorigenic, it is associated etiologically with lymphomas, lymphoproliferative diseases and nasopharyngeal carcinoma.

The aim of the study was to determine viral nucleic acid in atypical cases of EBV infection. For the diagnosis of central nervous system diseases, in cases of cardiomyopathy, and some other diseases of infectious origin, qualitative and quantitative real-time PCR (Artus/Qiagen EBV LC PCR kit, Germany) was performed for diagnosis. Blood (N 39), cerebrospinal fluid (CSF) (N 35), heart biopsy (N 7), pericardial fluid (N 3) and other (N 12) samples were examined between 01 January 2005 and 01 May 2007.

Of the 96 (100.0%) clinical samples, 17 (17.7%) were positive; 7 blood samples, 7 CSF samples, 2 heart biopsy samples and 1 pericardial fluid. Of the 41 males, 11 were positive; of the 55 females 6 were positive. 9 samples were positive from those under 24 y, and 5 samples from those above 50 y.

Some of the CSF-positive samples were of infectious origin, but EBV was detected in epilepsy, sclerosis multiplex and syncope. In 2 cases of dilatative cardiomyopathy and one case of increased pericardial fluid, EBV was detected. In some cases where the symptoms were unsteady, and the seroconversion was already undetectable from serum samples, the suspicious IM diagnosis was confirmed by real-time PCR.

DEVELOPMENT OF A MULTIPLEX RT-PCR TEST FOR THE SIMULTANEOUS DETECTION OF SOME OF THE MAJOR RESPIRATORY VIRAL PATHOGENS OF THE DOG

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Canine respiratory infections still represent a serious problem for small animal practitioners, kennels and pet shelters throughout the world.

The fast and reliable diagnosis can lead to the commencement of a specific treatment that could eventually save even the life of the animal, while it can also significantly reduce the expenses of the symptomatic non-specific treatment. Since the respiratory clinical signs presented by the animals in the incipient stages of the infection can be very similar, the clinician faces a real challenge when he

or she has to decide which one(s) of the existing, but mostly quite expensive tests he or she should ask for. In order to eliminate these time-consuming and expensive diagnostic procedures, a multiplex RT-PCR based test has been developed that can reliably demonstrate the presence of some of the more frequent etiological agents of canine viral respiratory disease, such as canine distemper virus (CDV), parainfluenza virus 2 (CPiV-2), and the novel pathogen of canines, the influenza virus (H3N8), that recently jumped the species barrier from horses to dogs.
The newly designed multiplex test can demonstrate the presence of one or more of these etiological agents in easily obtainable field samples, such as nasal and ocular swabs, significantly reducing the unnecessary stress of the animal, as well as the time interval between the collection of samples and the comprehensive result of the test.

BETA-LACTAM RESISTANCE OF *STREPTOCOCCUS PNEUMONIAE*, DETECTION AND INTERPRETATION

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Surprisingly, no ß–lactamase producing strains or strains with efflux pumps have been observed in pneumococci so far. Intrinsic resistance to the ß–lactam antibiotics is a result of the alterations of the target molecules, the penicillin-binding proteins (PBPs), which catalyse the transpeptidation during the cell wall biosynthesis. The altered PBPs are encoded by so-called mosaic genes that result from the integration of long DNA sequences from other bacteria, often the commensal streptococci.
However, other, less significant resistance mechanisms were also observed recently, like the increased cross-linking of the cell wall by MurM and MurN. In a very recent paper the authors have challenged *S. pneumoniae* to subinhibitory concentrations of penicillin, and as a result, a total of 386 genes were found to be responsive, including *cps* genes or genes causing decreased competence. Such genes may represent potential therapeutic targets for enhancing the activity of penicillin. These observations suggest that beta-lactam resistance of pneumococci might be more complex than we think now.
The penicillin resistance rates in Europe vary widely between countries. The highest levels are reported from Spain and France, whereas the lowest rates are observed in Germany and Austria. The Eastern European countries have no higher resistance than the Western ones. The average resistance is approximately 10-14%, and is probably slightly increasing over the years. The ß–lactam resistance is usually associated with higher antibiotic consumption, but an interesting counter example is Italy, where the consumption is high, but the resistance is rather low (Italian paradox). The penicillin resistance of the Hungarian pneumococci according the nation-wide data is around 5% and seems to show a decreasing tendency, but the non-susceptibility remains stable around 35% over the years.
The determination of resistance in the laboratory can be done either by disc diffusion (using a 1 mg oxacillin disc), or by measuring the MIC by E-test or agar/broth dilution methods. The CLSI guidelines emphasise that in case of disc diffusion, a smaller inhibition zone should not automatically be reported as resistance (which might be the reason of the frequent overestimation of resistance), but measurement of the MIC should be performed. It is very important clinically to distinguish between resistance (MIC > 2 $mg.L^{-1}$) and intermediate resistance (MIC = 0.125 - 1 $mg.L^{-1}$), because infections caused even by the low-level resistant strains could be still treated with higher dose of penicillins. Studies have failed to demonstrate significantly higher mortality of patients infected with penicillin-R rather than penicillin-S pneumococcal strains.
The efficacy of penicillin therapy depends on the site of infection, the MIC of the organism, and the antibiotic concentration achieved, which is determined by the route of administration and dosage.

Therefore, in the recent years, different breakpoints were established for meningitis and non-meningitis cases by the international guidelines. Cephalosporin and carbapenem resistance is linked directly to penicillin resistance, therefore in case of penicillin sensitivity these agents need not to be tested.

TAXONOMICAL DIVERSITY OF MYCOTA IN A PHYTOREMEDIATED CHERNOZEM RELATED TO CADMIUM AND SELENIUM LOADING

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Soil fungal populations, which represent 30-40% of the total biomass, play an important role in the decomposition and other transformation processes in soil. Their level and diversity are considered to be excellent ecological or ecotoxicological markers for biological dynamics of soil. We took soil samples from the Nagyhörcsök Experimental Station HAS RISSAC (loamy calcareous chernozem with 25% clay, developed on loess and containing 3% humus and 3-5% $CaCO_3$ in the ploughed layer) at the middle and at the end of the vegetation period in the years 2004-2006. The soils at these sites were experimentally loaded with huge doses (30, 90 and 270 $mg.kg^{-1}$) of thirteen different microelements in 1991 and have been phytoremediated with usual agronomical methods and cultures for 15 years. To ensure sufficient macronutrient supply for the whole remediation procedure, 100-100 $kg.ha^{-1}$ N, P_2O_5 and K_2O were given yearly. Soil fungi were quantitatively cultured from soil with dilution and microbiological method using modified Pikowskaya's agar and malt-extract agar media. The isolates were identified taxonomically.

Although each experimental site was rich in fungal populations (9.2 - $9.7.10^5$ $CFU.g^{-1}$) detectable differences remained between the control and the treated plots even after a 15 year long phytoremediation procedure. The effect of microelement treatments on the taxonomical diversity of fungal populations was even more evident: 16 species were obtained from the plots treated with cadmium, 11 from the soil treated with selenium, and 28 from untreated control soil. 16 of the total 28 species from the control plot were obtained only from there with a dominance of the species *Acremoniella atra*, *Cladosporium cladosporoides* and *Scytalidium lignicola*. 10 species of the total 16 from the plots treated with cadmium were obtained only from there with a dominance of the species *Acremonium curvulum* and *A. terricola*, and two other isolate types which belong to the genus *Cladosporium*, and *Fusarium solani*, respectively. 3 out of the total 11 species from the plots treated with selenium were cultured only from there with a dominance of the species *Aspergillus ochraceus* and *Chaetomium elatum*. Dependence of the diversity upon seasons also increased in the loaded plots: we revealed 9 and 5 constant species from the soils treated with cadmium and selenium, respectively, while 20 in the control soil.

In summary, the quantitative and qualitative ecotoxicological effect of cadmium and selenium can be investigated with testing soil mycota, similarly as testing nematode fauna [1].

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MOLECULAR ANALYSIS OF VANCOMYCIN RESISTANT *ENTEROCOCCUS FAECALIS* STRAIN ISOLATED IN DEBRECEN

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Colonisation and infection with vancomycin resistant enterococci (VRE) are problems in hospitals worldwide. Despite growing concern about VRE as nosocomial pathogens, especially in the United States, they are rarely isolated in Hungary. We report on the molecular characterization of the first isolation of VRE in Debrecen (Hajdú-Bihar county).

Enterococcus faecalis was isolated from a femoral wound in a patient following mitral prolapse surgery. The *E. faecalis* strain that colonized the wound proved to be highly resistant to vancomycin and teicoplanin (MIC>256 $mg.L^{-1}$ to both ones).

The strain was identified to species level using routine criteria and Rapid ID 32 Strep system. The species was confirmed by PCR for detection of *ddl* gene. Susceptibility to vancomycin, teicoplanin, ampicillin, gentamicin, ciprofloxacin, moxifloxacin, clindamycin and tetracycline was determined using the disk diffusion method and by VITEK 2 system. Vancomycin resistance was also tested on vancomycin screen agar (6 $mg.L^{-1}$) according to CLSI. Resistance to glycopeptides were confirmed by E-test method and by multiplex PCR for detection of *vanA*, *vanB*, *vanC*, *vanD* and *vanE* genes. The PCR test approved the presence of *vanA* gene in the genome. The *vanA* sequence of 620 nucleotides was completely identical to those of published and available in the database. The screening of the rectal swab for VRE carriage was negative for the members of the hospital staff.

This is the first report of femoral wound colonized by *vanA* carrying *E. faecalis* in a patient following cardiac surgery and also the first report of VRE in Hajdú-Bihar county documented by molecular genetic methods.

Further studies are needed in order to examine the source of VRE. To prevent transmission of antimicrobial-resistant pathogens in long-term care and intensive care facilities in which patients have high rates of colonization, infection-control strategies may need to be modified. Potential modifications include enhanced infection-control strategies, such as screening for the staff members for multiresistant pathogens including VRE, initiation of contact-isolation precautions for colonized patients and prudent use of vancomycin.

THE EFFECT OF FUNGAL COMPETITION ON COLONIZATION OF SOYBEAN BY *ASPERGILLUS FLAVUS* AND *TRICHOTHECIUM ROSEUM* AND ON PRODUCTION OF AFLATOXINS

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The relationship between mould biomass and the biosynthesis of aflatoxin B1 (AFB1) and total aflatoxins (AFT) on solid substrates (whole and crushed soybeans) at temperatures from 20 - 40^{o}C and water content in the substrate of 15 - 38 % has been investigated. The experiments have been

carried out with the aflatoxigenous mould *Aspergillus flavus* ATCC 26949 in pure culture and in mixed culture respectively, the latter with the common mould *Trichothecium roseum* ZMPBF 1226. The biomass growth during cultivation was measured by the chitin content, and the concentration of aflatoxins was determined by HPLC method using a Varian instrument with fluorescence detector. It has been established that the biosynthesis of examined aflatoxins and their ratio primarily depend on the temperature of cultivation, rather than on the growth of the mycelium. The biomass of the mixed culture of *A. flavus* and *T. roseum* after 56 days of cultivation reduces the amount of AFB1 by 25 - 60 %, and the amount of AFT by 30 - 75 %. The decrease of investigated toxins concentrations is more pronounced in the substrate with a higher initial water content and a higher temperature of cultivation. The biomass of the pure culture of *A. flavus* after 56 days of cultivation reduces the amount of AFB1 by 15 - 35 % and the amount of AFT by 20 - 45 % at the same parameters of mould growth and water content in the substrate.

IN-VITRO ACTIVITY OF DEHYDROACETIC ACID AND THE NEWLY SYNTHESIZED SCHIFF BASE AGAINST MYCOTOXIGENIC FUNGUS *ASPERGILLUS FLAVUS* ATCC 26949 GROWTH AND AFLATOXIN ACCUMULATION

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The potential for inhibition of aflatoxin accumulation by aflatoxigenic fungus *Aspergillus flavus* ATCC 26949 was investigated using the dehydroacetic acid (DHA) and the newly synthesized Schiff base 3-/2-aminophenylimino (p-toluoyl)/-4-hydroxy-6-(p-tolyl)-2H-pyran-2-one in yeast extract-sucrose (YES) medium at pH 5.5. YES medium was treated with various amounts of DHA and Schiff base after inoculation with *A. flavus*.

Experiments were carried out in a stationary culture at temperatures of 20, 25 and 30ºC during 42 days. Mycelial dry weights were determined gravimetrically and concentration of aflatoxin B1 (AFB1) and total aflatoxins (AFT) were measured by using ELISA method. DHA concentrations of 0.5 μmol . L^{-1} and 1.0 μmol . L^{-1}, respectively, stimulated mould growth and AFB1 and AFT accumulation, but concentrations higher than 10.0 μmol . L^{-1} produced an inhibitory effect. In the presence of low Schiff base concentrations, mould growth was decreased by 75 % and concentrations of AFB1 and AFT by 65 % or completely.

VIRULENCE OF HUMAN PATHOGENIC *YERSINIA* SPECIES

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The genus *Yersinia* comprises three human pathogenic species: *Y. enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis*. The former two are principally considered gastrointestinal pathogens while *Y. pestis* is the causative agent of plague. Interestingly, though eliciting very diverse clinical manifestations *Y. pseudoptuberculosis* and *Y. pestis* are the two closely related species while

Y. enterocolitica is much distantly related to any of the other two taxonomic entities. It has been shown that *Y. pestis* is a recently emerged derivative of *Y. pseudotuberculosis* which has both lost and gained virulence properties during its evolution. Chromosomally encoded and plasmid mediated virulence properties of the three species will be discussed in view of our observations and the relevant literature. Special emphasis will be given to characters encoded by the common virulence plasmid, and to those ones determined by plasmid pPCP1 of *Yersinia pestis*.

REGULATION OF THE EXTRACELLULAR CHITINASE AND PROTEINASES IN AUTOLYSING *ASPERGILLUS NIDULANS* CULTURES

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Carbon starvation can be considered as a kind of stress, when neither the quality nor the quantity of the carbon sources in the medium are satisfactory to support microbial growth. Cells can cope with this stress by utilising weak energy and carbon sources left in the medium and by undergoing both autolysis (an active cell death process followed by a well-organised, bulk degradation of the biopolymers of the dead cells [1], and autophagy. Hydrolases are extensively involved in all these physiological processes, and we demonstrated that in carbon starving *Aspergillus nidulans* cultures the production of extracellular chitinase and proteinases is regulated in quite complex way involving both sporulation-dependent and sporulation-independent signalling pathways.

In the case of the extracellular chitinase (*chiB*), chito-oligomers induced its production, and glucose repressed it *via* CreA transcriptional factor. Chitinase production was induced by the FluG-BrlA asexual sporulation signalling pathway [2], meanwhile glucose inhibited this induction independently of CreA by repressing *brlA*. Yeast extract repressed chitinase production in a *meaB*-dependent manner. Proteinase (*prtA* and *prtB*) production was controlled by the FadA heterotrimeric G-protein signalling pathway, which maintains vegetative growth and represses sporulation [3]. Both the a (FadA) and the bg (SfaD-GpgA) G-protein subunits repressed proteinase production during vegetative growth meanwhile FluG induced proteinase production through the inhibition of FadA signalling. Glucose repressed proteinase production *via* both CreA-dependent and CreA-independent pathways, and the repression of proteinase production by yeast extract was shown to be *meaB*-dependent.

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DEVELOPMENT OF RT-PCR DETECTION ASSAYS FOR AVIAN INFLUENZA AND NEWCASTLE DISEASE VIRUSES

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Avian influenza (AI) and Newcastle disease (ND) are highly contagious and economically important viral diseases of birds. Though type A influenza virus (AIV) belongs to *Orthomyxoviridae* and Newcastle disease virus (NDV) is a member of the *Paramyxoviridae* family, infection by highly pathogenic (HP)AIV or NDV may show similar clinical signs and epidemiology in poultry. The traditional methods to diagnose and confirm HPAI and ND cases are time consuming: the isolation/characterization of the causative virus needs at least 4–7 days to accomplish. Since it has economical and epidemiological significance to identify AIV/NDV as soon as possible, molecular microbiology based methods, like reverse transcription-polymerase chain reaction (RT-PCR) and real-time RT-PCR (RRT-PCR) assays are widely used for the detection of the viruses.

In our laboratory RT-PCR assays have been developed for the rapid and specific detection/discrimination of AIV and NDV. Primers targeting the matrix protein gene (M) of AIV and the fusion protein gene (F) of NDV were evaluated experimentally in simplex and duplex reactions with archive and recent AIV/NDV clinical isolates and vaccine strains. PCR products of the expected size of 144 bp and 316 bp were amplified from AIV/NDV samples, respectively, while no amplification was observed with heterologous avian pathogens. The sensitivity of the method was 10+0.5 50% egg infectious dose (EID50)/0.2 ml for AIV and 10+2.2 EID50/0.2 ml for NDV (on both allantois fluid/spiked fecal samples).

LUX fluorogenic primers targeting the same regions of AIV/NDV genome were designed and tested in RRT-PCR assays. The LUX RRT-PCR exclusively amplified the 145 bp/143 bp products from AIV/NDV samples, respectively, and fluoroscent signal was similarly detected with AIV/NDV samples only, and not with heterologous pathogens. The sensitivity of the method was 10-0.5/+1.5 (AIV); 10+1.2/+2.2 (NDV) EID50/0.2 ml on allantois fluid/spiked fecal specimens, respectively.

Internal amplification controls (IAC) were constructed by using the composite primer technique to eliminate false negative results. The plasmid-borne IAC targets, which can be applied in competitive or non-competitive experimental setup, had no detectable effect on the sensitivity of the original amplification reaction. Plasmid embedded IACs allow simple storage; continuous availability and quality; the determination/calculation of detection limit in target copy numbers; the production of ssDNA and in-vitro RNA transcript IACs from the viral promoters carried on the vector.

The developed (R)RT-PCR assays are rapid, cost-effective tools, which provide powerful and reliable novel means for the early diagnosis of avian influenza and Newcastle disease.

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RAPID INSTRUMENTAL METHODS FOR INVESTIGATION OF MICROBIOLOGICAL CONTAMINATION OF FOODS

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The traditional and standardized methods of microbiological investigations are very time- and labour-consuming, therefore, there is an increasing need for methods which enable investigating numerous samples in a short time. For this purpose, opportunities are developing for application of rapid instrumental methods which are measuring specific physical, physico-chemical and biophysical or biochemical changes related to microbiological contamination of (food) samples. The lecture will review several analytical techniques proved to be or promising to fulfil the above requirements. Already since the second half of the 1980-ies, successful applications of instruments based on

measurement of changes in impedance/conductance or capacitance in relation to growth of microorganisms in appropriate media when inoculated and incubated with microbiologically contaminated samples, due to formation of ionically charged particles during the growth and metabolic activity of microorganisms. An other electrometric method developed recently in Hungary records the decrease of the redox potential of similarly inoculated and incubated samples. The „detection times" estimated by these measurements can be correlated to traditional viable cell counts as references. A well-established procedure, particularly for investigating sanitary conditions of surfaces is the luminometric estimation of the ATP-bioluminescence based on the light emission of the luciferase-luciferin enzymatic reaction of the ATP content of somatic or microbial cells. Recent research shows more-and-more the potential of various spectroscopic (mainly near infrared, Fourier-transform infrared, or spectrofluorimetric) methods, after validation with multivariate statistical calibration, for investigating loss of freshness and/or development of bacterial spoilage of certain foods. Special advantage of some of these rapid methods is their reagent-less and non-destructive character. Similarly, instruments containing selected chemical sensor arrays (electronic noses), supplemented with proper pattern recognition and multivariate statistical techniques, can be „trained" to estimate qualitatively and quantitatively specific complex volatiles formed by microorganisms and taken from the head-spaces of food samples. Various applications of biosensors are also growing for detecting substances of microbial origin, e.g. toxins, or even microbial cells, e.g. pathogens.

DIFFERENCES IN THE ORGANISATION OF THE MITOCHONDRIAL DNA OF *CANDIDA ALBICANS* ISOLATES

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In the study of clinical isolates of *Candida albicans* four mitochondrial RFLP types were revealed by *Hinf*I-digestion of the total DNA. The most frequented type was labelled as number I. This group involves 23 isolates. Eleven isolates belong to Type II, 9 isolates to Type III. The group number IV was represented only by one isolate. The digestion of the purified mtDNA with *Pvu*II revealed very similar RFLP pattern and nearly the same size of the mitochondrial genome in all cases. Other enzymes (ea. *Eco*RV, *Bgl*II) gave distinct result and confirmed the existence of these types. The RFLP pattern of mtDNA Type I coincides with the RFLP pattern of *C. albicans* strain SC5314. The circular mitochondrial genome of this strain consists of 40420 bp as confirmed by DNA sequencing. The nucleotide sequence of fragments and the gene order of the different types were analysed to determine if mtDNA types derived from recombination or mutations.

SPECIFIC DETECTION OF AVIAN INFECTIOUS BRONCHITIS VIRUS SEROTYPE MASSACHUSETTS BY REAL-TIME RT-PCR

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Avian infectious bronchitis virus (IBV), a member of the *Coronaviridae* family, is a highly contagious, economically important pathogen of domestic fowl. The virus, which has a worldwide distribution, is mainly associated with upper-respiratory tract diseases, but some strains can also

replicate in the urogenital tract, causing nephritis and reduced egg production. IBV has a 27.6 kb long, positive-sense single-stranded RNA genome coding for the following structural proteins: surface glycoprotein (spike glycoprotein; S), membrane (M), nucleocapsid (N) and envelope (E) protein. The N-terminal subunit of the S protein, S1, is the most variable part of the viral genome, and it is responsible for the antigenic properties of the virus.

The detection of IBV strains by traditional techniques is labour-intensive, time-consuming, and in several cases the results are uncertain, therefore, PCR-based methods are routinely used for the identification of IBV. Since several serotypes of IBV are recognised in the field that evoke a low level of cross protection, it has great importance regarding diagnostics and vaccine development to have serotype specific virus detecting assays with high accuracy. For this reason, a real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay was developed and optimized for the rapid, specific and efficient detection of IBV serotype Massachusetts using Light Upon eXtension (LUX) fluorogenic primers. Oligonucleotide primers targeting the S1 region were designed using the alignment of nucleotide sequences available in GenBank and at CEVA (M41; H120; Ma5; B48). The specificity of the assay was first demonstrated by testing the primer pair on a panel of samples representing the major serotypes (Massachusetts; Arkansas; Connecticut; 793/B; QX; Italy02) and commonly used vaccine/challenge strains, then in the next step clinical isolates were investigated. Amplification signal and PCR product of the expected size of 74 bp was generated from samples belonging to the Massachusetts serotype, while no amplification/cross-reaction was observed with negative controls and samples of other IBV serotypes. The identity of the PCR amplicon was confirmed by means of DNA sequence analysis and comparative search against GenBank. The sensitivity of the method was determined as approximately 2.5 EID50 (50% egg infectious dose)/ml for virus suspensions (similar to TaqMan assays).

The newly developed real-time RT-PCR method provides rapid and reliable means for the specific detection/identification of IBV serotype Massachusetts and it is a useful tool in challenge studies or quality control procedures during vaccine development. The LUX RT-PCR is suitable for quantitative measurements and enables dissociation curve analysis upon amplification. In contrast to other real-time techniques using hybridization probes, this system is less sensitive to mutations in the target region, which is a great advantage when a hypervariable part of the genome is amplified.

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CORRELATION OF HPV PREVALENCE, CLINICAL AND PATIENT DATA IN INDIVIDUALS WITH ORAL PRECANCEROUS LESIONS

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Human papillomaviruses (HPVs) are able to infect the mucosa of the upper respiratory tract and the oral cavity and may have a role in the development and progression of the head and neck tumours as well as precancerous lesions, such as oral leukoplakia (OL) and oral lichen planus (OLP).

To evaluate the potential role of HPV in the development of oral premalignant lesions, we studied HPV prevalence in exfoliated cells derived from lesions and from healthy mucosa of 27 and 98 patients with OL and OLP, respectively, as compared to a control group consisting of 72 healthy individuals. Detection of HPV DNA was performed by nested PCR using MY/GP consensus primers, the genotype of the virus was determined with restriction analysis of the amplimers. Prevalence data were analyzed with Chi-square test and correlations among age, gender, HPV positivity and clinical appearance of the disease were evaluated with logistic regression. We detected HPV-specific

sequences in 40.5%, 32.7% and 4.2% in OL, OLP patients and the control group, respectively ($p<0.001$ in case of both patient groups compared to the controls). High risk HPV types dominated in both patient groups. Higher frequency of clinical forms with increased risk of malignant transformation (erosive and atrophic lesions) was associated with younger age (<50 years) in OL patients (OR=8.89, p=0.008) as well as with HPV positivity in the OLP group (OR=2,29, p=0,064).
In conclusion, HPV infection was associated with higher-risk OLP, which may indicate a role in the transformation of OLP. Similarly, HPV may have an etiologic role in the development of OL. However further investigations are necessary to shed more light on the issue, as well as to assess whether HPV can be used as a prognostic indicator.
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COMBINED *IN-VITRO* INHIBITORY EFFECT OF SPICES AND RIPENING AGENT APPLIED IN DRY-FERMENTED SAUSAGES

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The originality and the taste of dry-fermented sausages depend mainly on spices and the ripening process applied. Due to the increasing consumers' expectation for food safety, the producers intensively enhanced the hygiene along the meat processing. Thus spices became a marked source of microbial contamination to date. On contrary, there is lot of scientific evidence about the occurrence of in-vitro antimicrobial substances in spice plants that is not considered in the shelf-life calculations. Because those antimicrobials can be susceptible for processing conditions, a thorough study was conducted to explore the relationship among the spices and ripening agents that are used also for microbial growth inhibitors.
Escherichia coli (35033), *Enterococcus faecalis* (ATTC51299), *Staphylococcus aureus* (112002) and a *Bacillus cereus* of sausage origin were applied in various liquid, agar and meat based media for detection of inhibitory effect. Natural form and extracts with different solutions of 6 spices (paprika, white and black pepper, garlic, caraway, rosemary) were tested both with filter paper disc method and mixed in ground meat. Enzyme (lyzozime) amendment was also used for fortification of the ripening agent SRE that contains 40% glucono-delta-lactone as acidifying compound.
As sole factor, natural form of spice plant rosemary, garlic, as well as lyzozyme enzyme proved to be effective against Gram-positive bacteria. The acidification due lactate producing hydrolysis of SRE strongly (with 5 order of magnitude) decreased the count of *B. cereus*, however, the Gram-negative intestinal bacteria survived it. The antimicrobial effect of spices previously ground and salted or dried for sausage processing dropped to 1/10 of natural form. Moderate combination effect was observed when two or more types of additives were applied in ground meat. The consequences of findings to the shelf-life prediction will be discussed.
Acknowledgement: The study was carried out in the framework of RET 09/2005 NKTH project

MUTAROTATION OF D-GALACTOSE ANOMERS PLAYS A SIGNIFICANT ROLE IN THE INDUCTION OF CELLULASES BY LACTOSE IN *TRICHODERMA REESEI*

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The filamentous fungus *Trichoderma reesei* (teleomorph: *Hypocrea jecorina*) is a potent producer of cellulolytic and hemicellulolytic enzymes. While lactose is today the only soluble carbon source on technical scale for this purpose, the mechanism by which it triggers cellulase formation is still not understood. *T. reesei* initiates lactose metabolism by hydrolyzing it first extracellularly, and then taking up and metabolizing the hydrolysis products D-glucose and D-galactose. Recent data showed that while at very low specific growth rates cellulase induction by D-galactose does occur, lactose is still by far a superior inducer of cellulase formation. This is due to the lactose disaccharide itself, since an equimolar mixture of D-glucose and D-galactose yields similar results seen on D-galactose alone. Obviously, despite of hydrolyzing it before taking it up into the cell, *T. reesei* recognizes the presence of hydrolyzed lactose in a different way than the simultaneous presence of D-glucose and D-galactose. What is the difference between hydrolyzed lactose and a mixture of D-glucose and D-galactose? D-galactose released from lactose is the ß-anomer, whereas D-galactose is (because of mutarotation) a mixture of the α- and ß-anomer. Consequently, ß-D-galactose, after entering the cells, will have to be converted to the α-form first in order to become a substrate for galactokinase which is strictly specific for α-D-galactose. To this end, yeasts have a galactomutarotase activity which is part of the Gal10p protein. Interestingly, the *T. reesei* Gal10 protein does not have this domain, and the *T. reesei* genome database does not seem to have a protein with similarity to the Gal10 mutarotase. If there is indeed no such enzyme in *T. reesei*, catabolism of the lactose-derived D-galactose via the Leloir pathway will be considerably delayed because of the time needed for non-enzymatic mutarotation, which at 30°C and pH 6.5 – 7.0 takes several hours.
To test this hypothesis, we have cloned the *Saccharomyces cerevisiae* gene encoding the GAL10 protein into a plasmid after a constitutive promoter (*pyr4*). This construct was transformed into *T. reesei*, yielding a 3-5-copy integration, as confirmed by Southern blotting. According to a Northern blotting analysis, the Gal10 gene was constitutively expressed and subsequently resulted in a high level of mutarotase enzyme activity. Phenotype analysis of the mutarotase gain-of-function *T. reesei* mutants showed that they all transport lactose considerably faster than the re-transformed control strain, but there were no differences in the uptake rate of any other carbon sources tested. Most importantly, both the *cbh1* and the *cbh2* genes encoding cellobiohydrolase I and II proteins, respectively, were expressed at a much lower rate on lactose than in the control strain. These data confirm that mutarotation of D-galactose indeed heavily contributes to the induction of cellulase genes on lactose.

PHENO AND GENOTYPIC ANALYSIS OF SGI1 POSITIVE *SALMONELLA TYPHIMURIUM* ISOLATES IN HUNGARY

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Salmonella bacteria are one of the most frequent food-borne zoonotic pathogens. Its DT104 phage type is often associated with multiple antibiotic resistance, encoded by distinct chromosomal regions. The *Salmonella* Genomic Island 1 – SGI1 (discovered in DT104 *S.* Typhimurium) is a 43kb genomic region encoding pentaresistance (ampicillin, chloramphenicol, streptomycin, sulphonamid,

tetracycline – ACSSuT), described primarily in *S.* Typhimurium but it may also occure in *E. coli*. The phenotypic characteristic of SGI1 in *Salmonella* is thought to be florfenicol resitance.
To check the occurrence of SGI1 in Hungarian *Salmonella* and *E. coli* isolates altogether 140 *Salmonella* (31 *S.* Enteritidis, 20 *S.* Hadar, 30 *S.* Infantis from poultry origin and 59 *S*. Typhimurium from different sources) and 61 bovine *E. coli* belonging to the EHEC, STEC/VTEC and EPEC pathotypes and 17 porcine *E. coli* belonging to the EPEC pathotype were examined for florfenicol resistant phenotype.
We found 18 Hungarian florfenicol resistant *S.* Typhimurium isolates, and 17 of them proved to be positive for tetG tetracycline resistance gene, left junction and right junction of SGI1 in PCR experiments. These strains were freshly isolated from different regions of Hungary and represent different animal species. In order to test their possible genetic relationship these strains were included in further pulsed field gel electrophoresis (PFGE) studies.
PFGE (XbaI) results were analysed by the BioNumerics software package for the phylogenetical analysis performing a band based „Dice" comparison producing UPGMA dendrogram type. Based on our results four distinct groups could be formed concerning the animal origin of the strains but no geographic relationship could be detected.
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PREVALENCE OF HEPATITIS E VIRUS ANTIBODIES IN OCCUPATIONAL GROUPS WITH DIFFERENT EXPOSURE TO SWINE

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A novel, non-A non-B type of infectious hepatitis was described first in 1983 by Balayan et al. The causative virus and the disease is named "Hepatitis E" after its "epidemic", "endemic" and "enterically transmitted" characters. Hepatitis E virus (HEV) is a non-enveloped, positive-sense, single-stranded RNA virus with icosahedral symmetry, belonging to the genus *Hepevirus* in the family Hepeviridae. The infection in humans is associated mainly with an acute, self-limited, icteric hepatitis and with a mortality of 1% (up to 20% in pregnant women). Antibodies to HEV were found in different animal species; however, the HEV infection was asymptomatic. The HEV strains isolated from animals are genetically related to human HEV strains; therefore, the zoonotic transmission is suggested. The aim of this study was to determine the seroprevalence of HEV antibodies in occupational groups with different intensity of exposure to swine, which species is the suspected main reservoir of the virus. 639 sera samples taken from Austrian people of each of the following professions: farmers, swine- and poultry slaughterhouse workers and hunters; and townspeople served as control group. In all sera both HEV IgM and IgG antibodies were determined by recomBlot HEV IgG/IgM® (Mikrogen, Neuried, Germany). The sera were coded, and the investigations were carried out in a blind manner. In addition, in HEV IgM positive sera the attempts were made to detect HEV nucleic acid by RT-PCR assays. Swine farmers and swine slaughterhouse workers are the groups of people who are in everyday contact with swine feces and/or raw meat. In these groups both anti-HEV IgM and IgG were detected in the highest percent of the sera samples (IgM: 25.49 % and 28.42 %, IgG: 36.60 % and 27.37 % respectively). Some of the HEV IgM positive samples, which were also found positive in RT-PCR, were sequenced. The sequences belong to the 3rd genogroup of HEV and show high similarity to HEV sequences of swine origin. By these results we found

connection between the seroprevalence and the profession of the investigated people, which supports the theory of the zoonotic transmission of HEV. The study was carried out in Austria, a country considered non-endemic for HEV infection. Surprisingly, the HEV-positivity even in the townspeople-group (15,71 % IgG, 11,43% IgM) is found much higher than it is reported as general in non-endemic areas (3.3%). These results support the opinion that the "endemic" and "non-endemic" categories should be supervised and new categories should be introduced to describe the epidemiological status of the countries or regions of the world regarding HEV.

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DIVERSITY AND ACTIVITY OF MICROBIAL COMMUNITIES OF POST MINING SUBSTRATES AFFECTED BY *LUMBRICUS RUBELLUS* (OLIGOCHAETA: LUMBRICIDAE)

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Mixing of the substrate is an important part of soil formation, in which process *Lumbricus rubellus* can participate as a dominant pioneer earthworm. A one year laboratory experiment on the earthworm effect has been evaluated. Substrates originated from a post mining site (Sokolov coal mining district, North-West Bohemia, Czech Republic) and were incubated in 400 g aliquots at constant temperature for a year. Subsamples (clay [C]; clay + *A. glutinosa* leaves [CL]; clay + leaves + *L. rubellus* [CLR]; drilosphere [D]) were analyzed after 3, 60, 180, and 395 days. Physico-chemical parameters, respiration and biomass of samples as well as germ counts of bacteria were determined. Microbial communities were studied by chemotaxonomical methods (PLFA, RQ, ergosterol content), DGGE, BIOLOG, and different extracellular enzymatic activity measurements.

During the experiments microbial germ counts, total as well as active biomass (Cmic, PLFAtot), respiration and enzymatic activities increased and deep structural changes of soil microbial communities were observed. The total bacterial CFU counts strongly increased in CLR and D as compared to C samples. PLFA bacterial markers confirmed this increasing trend on cultivable as well as uncultivable level. At the level of bacteria cultivable on plates, an increase of the prevalence of bacterial K-strategists was noted in D. Fungal PLFA markers and ergosterol content indicated promotion of fungal growth in CL and especially in CLR and D. The results of RQ, DGGE and BIOLOG indicated that microbial communities of subsamples were separated on the basis of sampling time. Diversity indices based on the menaquinone profiles of subsamples showed a decrease in the middle of the experimental period and increase to the end. Analysis of total extracted PLFA revealed that mixing of the substrate by *L. rubellus* affected the extractable PLFA. Principal component analysis (PCA) of the PLFA analyses showed a clear separation of the microbial communities among C, CL, CLR and D. The ratio of monounsaturated to saturated PLFA indicated increase of carbon substrate availability in CLR and D. Also spectrum of utilized C substrates increased in treatments with *L. rubellus*, based on BIOLOG investigations.

According to our results soil mixing conducted by *L. rubellus* seems to be important for the accumulation of organic matter in post mining sites.

THE ROLE OF FNRT, AN OXYGEN DEPENDENT REGULATOR, IN THE PHOTOSYNTHETIC PURPLE SULFUR BACTERIUM, *THIOCAPSA ROSEOPERSICINA* BBS

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In facultative anaerobic bacteria the availability of O_2 is one of the most important regulatory signals. In the presence or absence of oxygen different metabolic pathways are switched on or off. The FNR protein is an oxygen responsive transcription regulator functioning as a "switch" between the anaerobic and aerobic metabolic pathways. The FNR contains Fe-S clusters which are oxygen sensitive and the FNR in *E. coli* was shown to regulate the expression of around 110 operons directly or indirectly [1]. *Thiocapsa roseopersicina* BBS is a Gram-negative, purple sulfur photosynthetic bacterium belonging to the Chromatiaceae family in the γ-subdivision of proteobacteria. In addition to the anaerobic photosynthetic growth, the strain is capable to grow aerobically, chemolithotrophically in the dark.

In this work we focused on the role of the FNR analogue, FnrT recently identified in *Thiocapsa roseopersicina. T. roseopersicina* BBS has two sets of membrane-associated [NiFe] hydrogenase genes: the HynSL and HupSL and a third, soluble hydrogenase HoxYH. In our previous report it was demonstrated that the Hyn enzyme was anaerobically induced and the upregulation was mediated by the FnrT [2]. In contrast, the *fnrT* mutation had no effect on the expression of HupSL and HoxYH hydrogenases. Using reporter genes, a slight, negative autoregulation of the FnrT could be noticed. From these observations an interesting question arose: what is the role of an oxygen sensing protein in a preferentially anaerobic bacterium. Since, the FNR had effect on the expression of more that one hundred genes in *E. coli* we assumed the similar global effect of FnrT in *T. roseopersicina*, as well.

To answer these questions an *fnrT* mutant (FNRTM) strain was prepared. Using proteomic approach, genes, metabolic pathways being under the control of FnrT were/are looked for. The protein patterns of the wild type and the mutant strain were compared on 2-D gel electrophoresis. From the silver stained gels several spots with distinct intensities were isolated and the proteins were identified by MALDI-TOF mass spectrometry. In addition, other staining methods are tested to increase the resolution of the protein quantitation, which would allow reliable detection of relatively small differences in the protein level.

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ANTIBIOTIC RESISTANCE OF MAJOR NOSOCOMIAL PATHOGENS IN HUNGARY

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The National Center for Epidemiology has been conducting an antibiotic resistance surveillance system for six years covering more than 60 percent of all tests made in Hungary. Resistance rates for all major nosocomial pathogens and trends in antibiotic susceptibility are reviewed. Among *Staphylococcus aureus* strains isolated from invasive infections the rate for methicillin-resistance has been steadily rising during the last six years and now stands at 25.4 percent. In *Streptococcus pneumoniae* the rate for high level resistance to penicillin has remained low, however, resistance to macrolides is close to 40 percent. The rate for ciprofloxacine and ceftazidime resistance in invasive isolates of *Klebsiella pneumoniae* proved 21 percent and 10 percent respectively in 2006. Vancomycin resistance in enterococci has remained rare in Hungary.

MYCORRHIZAL COLONIZATION RATES AFFECTED BY DROUGHT AND SALINITY IN A MODEL EXPERIMENT

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Our aim in this study was to demonstrate the direct and indirect effect of the plant-mediated physiological actions towards the rhizosphere colonization of the arbuscular mycorrhiza (AM) fungi. Stress-factors, such as the salt and the drought were examined, which are known to be strong environmental stress factors at the salt affected sites.

A pot experiment was carried out with split-roots in the pots. The pots were filled with steam-sterilized calcareous chernozem soil and *Glomus geosporum* inoculum was layered below the white-clover (*Trifolium repens*) seedling. After 8 weeks of growth different treatments were given to the pots: i) control, irrigation with tap water each half of pots, three times a week; ii) salt-treated: irrigation with saline (1% NaCl) water, three times a week; iii) drought-treated: irrigation with tap water, once a week. Four weeks later the mycorrhizal colonization of the plant roots and the electric conductivity of the soil were assessed. Significant differences on the AMF colonization values were found, between the full-salt-stressed "positive" controls and at the half-stressed plants. In those pots the colonization intensity (M%) of the AM fungi was reduced from 76% to 45%. The arbusculum richness of the roots at the same treatment however was especially high (42%), which show that the colonized root segments were heavily arbusculated and functioning in the symbiosis. It is also assumed, that adverse effect of the salinity could be developed only at the low arbusculum richness (A%), where the symbiosis apparently was non-functioning.

The highest values of AMF colonization at drought stress experiment were found at the half-stressed treatment, where the drought stress affected the half-part rhizosphere of the host-plant, which was able to acquire enough water through the other half of the roots. The adverse affect in this experiment is reasonable, and it was predictable, because drought has got a direct mycorrhizal stimulation effect, opposed to the salt stress. To maintain the microsymbiont partner by supplying assimilates, carbohydrates by the host, can be a „profitable investment", which can be generally refunded with macro- and micronutrients. The increased plant acquisition capacity or the limitation of salt uptake are all important conditions of the symbiosis in poor quality salt affected soils According to our findings the enhancement of mycorrhizal colonization in the saline soils can be the beneficial result of a communication process between the macro- and microsymbiont partners.

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THE PD1/PDL COSTIMULATORY PATHWAY HAS A KEY REGULATORY ROLE IN *HISTOPLASMA CAPSULATUM* PATHOGENESIS

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Costimulatory molecules such as members of the CD28/B7 family can either enhance or attenuate T cell responses. The PD1 pathway negatively regulates T cell receptor signalling upon interacting with its two ligands, PDL1 and PDL2 that are expressed on antigen presenting cells (APCs). It is well established that the PD1/PDL system has a crucial role in maintaining self-tolerance. However, pathogens can co-opt this pathway to escape immune responses.

In the present study, we examined the role of the costimulatory PD1/PDL pathway in *Histoplasma capsulatum* (Hc) infection. Hcis an important human pathogenic dimorphic fungus that primarily exists within macrophages *in-vivo*. To characterize populations of macrophages in murine histoplasmosis, peritoneal and alveolar macrophages were isolated and analyzed at different time points after intranasal infection with Hc. qRT-PCR and FACS analysis of these macrophages showed upregulation of PDL1. Splenocytes isolated form Hc infected mice were significant upregulated as well, whereas PDL2 and PD1 levels were unchanged. To investigate the mechanism of PDL1 upregulation in histoplasmosis, we infected IFN-gamma -/- mice and analyzed the macrophages and splenocytes by FACS. Interestingly, there was no upregulation of PDL1 on cells recovered from Hc infected IFN-gamma deficient mice, suggesting that the upregulation of PDL1 is IFN-gamma-mediated. To further study the importance of the PD1/PDL pathway in Hc infection, we used a PD1 deficient mouse model. We have found that PD1-/- mice on the C57BL/6 background were completely resistant to lethal Hc infection. Although they initially developed pulmonary inflammation, they were disease free by day 10, as shown by histological analysis, and the pathogen could no longer be cultured from liver, spleen or kidney. To investigate the therapeutical value of blocking the PD1/PDL pathway, we treated Hc infected mice with a monoclonal antibody to PD1. Seventy percent of the mice treated survived challenge with Hc, whereas all control mice (isotype IgG2b and saline) died ($p<0.001$).

Our results are the first to show the importance of the PD1 pathway in anti-fungal immunity and demonstrate that upregulation of PDL1 by Hc can potently affect the interplay between host and pathogen. Moreover, our studies offer novel potential immunotherapeutic strategies for histoplasmosis.

TARGETED GENE DELETION IN A CLINICAL *CANDIDA PARAPSILOSIS* ISOLATE DEMONSTRATES THE ROLE OF SECRETED LIPASE IN VIRULENCE AND PATHOGENESIS

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Candida parapsilosis is currently the second most common cause of invasive candidiasis. It is particularly associated with disease in premature infants and immunocompromised adults, and as a nosocomial infection in Intensive Care Units. Although it is assumed that secreted lipases play an important role in microbial virulence, the involvement of these enzymes in *C. parapsilosis* virulence has not been precisely defined. We successfully adapted a method for sequential gene disruption in *C. albicans* that is based on the repeated use of the dominant nourseothricin resistance marker (*caSAT1*) and its subsequent deletion by FLP-mediated, site-specific recombination [1] for use in *C. parapsilosis*. We designed a knock out construct to target the lipase locus in the *C. parapsilosis* genome consisting of adjacent genes, *CpLIP1* and *CpLIP2*. Our results showed that this locus had two copies in the genome. The close genomic localization enabled the deletion of the *CpLIP1* and *CpLIP2* genes using one knock-out vector to generate a homozygous "lipase minus" strain. After the gene deletion we reconstructed the *CpLIP2* gene, which restored lipase activity. Lipolytic activity was absent in the null mutants, whereas the wild type, heterozygous and reconstructed mutants showed similar lipase production.

In YPD and minimal YNB medium there were no significant differences in the growth of the wild type and mutant strains. In contrast, in comparison to wild-type *C. parapsilosis* the growth of lipase minus mutants was significantly reduced in both YNB medium with olive oil (3 fold reduction) and YNB supplemented with intralipid parenteral nutrition (1.5 fold reduction, $p=0.02$) suggesting that lipases are important for providing the fungus with carbon and energy sources. Biofilm formation, which is important for candidal virulence, was reduced in the lipase deficient strain that had a 5 fold reduction ($p=0.008$) in XTT activity compared to controls after 48h growth on polystyrene surfaces. The disruptants were significantly less virulent in infections models using inoculation of reconstituted human oral epithelium or murine intraperitoneal challenge. These studies represent the first targeted disruption of a gene in *C. parapsilosis* and show that *C. parapsilosis* secreted lipase is involved in disease pathogenesis.

This efficient system for targeted gene deletion holds great promise for rapidly enhancing our knowledge of the biology and virulence of this increasingly common invasive fungal pathogen.

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EFFECT OF CIDOFOVIR THERAPY ON HPV COPY NUMBER IN A CASE OF JUVENILE RECURRENT RESPIRATORY PAPILLOMATOSIS

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Recurrent respiratory papillomatosis (RRP) is caused by low-risk human papillomaviruses (HPV), especially by human papillomavirus type 6 (HPV6) and 11 (HPV11). In papillomas HPV genome is found in abundance and exists in an episomal form. HPV11 is usually associated with more aggressive, juvenile RRP that often shows extralaryngeal dissemination and is characterized by higher relapse rates, while HPV6 can usually be detected in the less severe adult form of the disease. Papillomas are generally treated surgically, but the recurrent nature of the disease may necessitate periodic surgical treatment and adjuvant chemotherapy may be required. Cidofovir is a nucleoside analogue antiviral drug used in the chemotherapy of RRP. Though reported to be effective, its mechanism of action is not established. While inhibiting the viral DNA polymerases of a number of DNA viruses, in case of HPV lacking viral polymerase it may interfere with E6 and/or E7 proteins of HPVs, preventing the replication of the virus indirectly.

Present study follows up the outcome of cidofovir therapy in case of a 13-year-old male patient suffering from RRP since the age of eighteen months. As surgical and prolonged interferon therapy was unsuccessful, 0.5 $mg.kg^{-1}$ intralesional cidofovir was administered to both visible lesions at weeks 0, 2, 4, 8, 12, 16, 24, 42 and 55. Biopsies were taken from the lesions of the larynx and the soft palate. HPV detection and typing were performed with MY09/MY11 and GP5+/GP6+ nested PCR. The copy numbers of the viral DNA was estimated using real-time PCR with SYBR Green. Derkay's RRP score was used to assess patient status. This course led to clinical improvement (Derkay's scores of 16 before *vs* a maximum of 8 after treatment). Viral copy numbers in laryngeal lesions transiently fell from the pre-treatment 10^5-10^6 copies per µg total DNA to 10^4 during weeks 2 to 12, but rose to and remained at its original levels afterwards. Similar tendency was observed in the soft palate lesions. The fall in copy numbers prove the efficacy of intralesional cidofovir in RRP recurrences. However, permanent suppression of HPV proliferation could not be achieved and the patient's approaching adolescence may also have contributed to the partial clinical success. Moreover, the possibility of the return of the disease to its original aggressive form cannot be excluded due to the relatively short follow-up.
In conclusion, though intralesional cidofovir improves the clinical symptoms, it seems to be unable to permanently suppress HPV proliferation, thus does not reliably eradicate the disease.

RELATIONSHIP BETWEEN IRON TRANSPORT AND XENOBIOTICS BIODEGRADATION IN *SPHINGOMONAS SUBARCTICA* SA1

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Sulfanilic acid is a typical representative of sulfonated aromatic amines widely used and manufactured as an important intermediate in the production of azo dyes, plant protectives and pharmaceuticals. *Sphingomonas subarctica* SA1, a Gram-negative aerobic bacterium capable to grow on sulfanilic acid as sole carbon, nitrogen and sulfur source has been isolated and characterized in our laboratory. In addition to sulfanilic acid our isolate could degrade six other aromatic compounds, like sulfocatechol, protocatechol, para-amino benzoic acid, 3,5-dihydroxy-benzoic acid and oil in soils. Comparison of the protein patterns of cells grown on different substrates revealed that the strain used alternative metabolic pathways for biodegradation of the various compounds. Proteomics approach was applied to identify most of the genes and enzymes catalyzing the sulfanilic acid conversion to oxoadipate entering into the tricarboxylic acid cycle. The genes are organized in two operons.
The first step of sulfanilic acid degradation is not fully understood. The enzyme probably hydroxylating sulfanilic acid was very sensitive to cell disruption indicating that the enzyme catalyzing this reaction was somehow related to the membrane.
Three specifically appearing membrane proteins were sequenced *de novo* by mass spectrometry from cells grown on sulfanilic acid. The aminotransferase is probably one component of the sulfanilic acid converting enzymes catalyzing the deamination of the sulfanilic acid. It is poorly membrane associated. The second protein contained motifs of ATP-binding casettes indicating the energy-dependency of sulfanilic acid uptake. The third protein is a hypothetical TonB-dependent protein, which might play a role in many types of transport including iron uptake. The expression of the TonB-dependent protein is upregulated specifically by xenobiotics/aromatics and iron. Since, two enzymes of the degradation pathway are known to contain iron in their active center, it is plausible to

assume, that the TonB dependent protein is involved in the iron transport to feed the extra iron demand of the enzymes taking part in the biodegradation.
From our data it is assumed that the uptake and conversion of sulfanilic acid is linked to a membrane protein complex and this association can function as a self-defending mechanism for the cell against the cytoplasmic occurrence of the toxic substrates. Furthermore, a potential link between the xenobiotics degradation and iron transport is suggested.
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AMPLIFICATION OF DNA FRAGMENTS FROM NEOLITHIC AND MUMMIFIED REMAINS

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Depending on thermal, chemical and microbial circumstances DNA fragments in archaeological remains are able to survive up to 100 000 years for the longest, although almost complete degradation with in just a few months can also occur during the time. Even DNA of Neanderthal bones can be analysed. The prehistoric data of very early, 7000-5000 years old Neolithic remains can provide crucial information on the origin of modern Europeans.
The two main alteration of DNA molecules with time are fragmentation and base modification.
Following an adequate DNA extraction from human remains, the low copy number authentic DNA templates can be amplified via PCR and analysed further. To assess the authentic DNA template quality, the degree of fragmentation of human mtDNA was estimated using overlapping primer design, with different amplicon sizes ranging from 60 to 600 bp. Samples of markedly different age, as well as the 7000 years old Neolithic remain from Vörs-Máriaasszony sziget, Hungary has been chosen and 150 years old naturally mummified body from the Dominican Crypt of Vác, Hungary. The latter one reported to have authentic pathogen microbial DNA. We are interested in further studies to gain information on the relevance on the presence of these microbial remains.
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ANTIBIOTIC RESISTANT ENTERIC BACTERIA IN BROILER HOUSES

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The emergence of antibiotic resistant microorganisms due to the overuse and misuse of antimicrobials, in- and outside human medicine, is of serious concern all around the world, given the alarming emergence of multiresistant human pathogens. Studies in several countries have demonstrated the association between the use of antimicrobials in food producing animals and

antimicrobial resistance; as fluoroquinolone use in poultry and the emergence of resistant *Salmonella* and *Campylobacter* strains.

Antibiotic use gives rise to a massive resistance reservoir in the normal enteric flora of treated animals, spreading and persisting in their environment. To describe this reservoir, the occurrence and quantity of resistant enterics were measured in the litter of broiler houses. Samples were collected before slaughter, for routine authority examination. Resistant fractions of coliform populations within the samples were measured on antibiotic containing agar plates (Oxytetracycline 8 $mg.L^{-1}$, Chloramphenicol 16 $mg.L^{-1}$, Ciprofloxacin 4 $mg.L^{-1}$) by colony count technique.

Almost all (41 of 42) houses harboured Oxytetracycline resistant enterics, the proportions running to 2,3% as average (90% - 0,01%). Chloramphenicol resistant populations were found in 26 houses, fraction mass average 0,01%; and Ciprofloxacin resistants were present in 18 houses, in small fractions (0,17% - 0,002%, average size 0,003%). Isolated phenicol and Ciprofloxacin resistant strains proved multiresistant, indicating the co-selective effect of Tetracycline, the antibiotic most frequently used for chicken.

Co-selection gives rise to multiresistant strains in the presence of any drug these strains are resistant to; in addition, their enhanced stress tolerance enable them in survival in stressful environments 5-6 times longer than their sensitive counterparts could survive. In conclusion, we suggest to minimize the presence of antibiotic- and disinfectant-residuals in the environment of food producing animals. These residuals maintain selective pressure, raise resistant reservoirs, and select multiresistant strains, altogether increasing the risk of the selection, spread and persistence of multiresistant pathogens.

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ANTI-VCA IGG TITER AND SEROLOGIC MARKERS OF REACTIVATING EPSTEIN-BARR VIRUS INFECTION

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Epstein-Barr-virus (EBV) virus serologic markers were evaluated in four patient groups: primary EBV infection group (N=42), latent infection group with no reactivity to early antigens (EA) of EBV (N=36), latent infection group with anti EBV EA IgG antibodies (N=69) and reactivating EBV infection group determined by the presence of IgA antibodies to any of the tested EBV proteins (N=69). Serologic profiles were determined by immunoblot method. Anti VC-IgG titers were measured by immunofluorescence using B-95-8 cell line as antigen. Anti-VCA-IgM reactivity were detected by ELISA method.

Using immunoblot method, IgM antibodies to EA antigens detected primary infection (98%) more sensitively than anti-VCA IgM (74%). In the other 3 groups, the serologic markers of past EBV infection, anti-EBNA1-IgG and anti-VCAp18-IgG were present in 94 and 98 %, respectively. Nevertheless, lack of developing antibody response to EBNA1 was associated with increased persistence of anti-EA-IgG antibodies (OR: 8,09 CI95:1,4-82,5). In general presence of anti-EA-IgG antibodies was associated with increased anti-VCA-IgG titer ($p<0,01$). Reactivating EBV infection was characterized by the presence of anti-VCA-IgA (94%) and anti-EA-IgA (40%). IgM antibody responses in the latter group were rarely (9%) detected.

FROM PATHOGENICITY TO COMMENSALISM; NEW INSIGHTS INTO THE EVOLUTION OF *ESCHERICHIA COLI*

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Escherichia coli strains belong to one species. They, nevertheless, could exhibit a very diverse set of properties. The majority of *E. coli* strains have the capacity to colonize the intestine of humans and many animals. They are considered as non-pathogenic commensal organisms. Particular variants, however, are able to cause intestinal as well as extraintestinal infections. Over the last few years, the genome architecture of pathogenic as well as commensal *E. coli* has been analyzed. It was shown that pathogenic strains can be distinguished from commensals as the former bacteria harbour "additional" phages, plasmids as well as large genomic blocks, termed "pathogenicity islands". In particular, we have analyzed the genome of strain 536, which is able to cause urinary tract infections in humans. Seven "pathogenicity islands" are part of the *E. coli* 536 genome. On the basis of the genome sequence, we were able to describe the traits encoded by these islands in more detail. Interestingly, island no. VI encodes a new polyketide, which has the capacity to slow down the cell cycle of eukaryotic cells. Interestingly, also commensal *E. coli* strains carry particular islands in their genome, which have been termed "genomic islands". These blocks, which are part the genomes of various strains may also encode for factors, which, in case of pathogenic variants, increase the virulence. In a non-pathogenic background, however, the respective gene products contribute to the fitness of the commensal isolates. This is especially true in the case of iron uptake systems, the already described polyketide or in the case of various metabolic functions. Interestingly, some of the pathogenicity islands have been further developed by the process of reductive evolution. During the presentation, the different genetic mechanisms, leading to pathogenic as well as commensal variants will be described and the processes will be discussed in the light of microbial evolution.

DETECTING VIRUSES IN CULTIVATED AND WILD *AGARICUS* SPECIES BY dsRNA-IMMUNOBLOTTING

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Champignon (white button mushroom) production makes up an important part of the Hungarian vegetable production sector. In the course of mushroom cultivation a large number of pests and infective agents, among them viruses, may endanger yield. Earlier the *La France Isometric Virus* caused significant losses, and lately *Mushroom Virus X* (MVX) has been the major virus giving rise to losses. The unequivocal detection of virus diseases is problematic, because the symptoms are often similar to those caused by errors in cultivation technology. The detection of the MVX-infection presents an extreme difficulty, because up to now it was not possible to identify which of the 23 double-stranded RNA (dsRNA) species, occurring in variable number and size in MVX diseased mushrooms, is causally connected with disease development. This is why the polymerase chain

reaction (PCR) based specific and sensitive methods are still not available for reliable MVX detection.
The aim of our experiments was to introduce a sensitive, simple and reliable method, which will allow us to detect all dsRNAs present in a single step. The dsRNA-immunoblot method used in our experiments is based upon the application of monoclonal antibodies, which specifically recognise dsRNAs, independent of their sequence and nucleotide composition.
We demonstrated that by immunoblotting dsRNAs can be detected directly in unfractionated nucleic acid extracts of champignon, without chromatographic purification on CF11 cellulose. It was found, that even healthy, symptom-free mushroom hybrids collected from different sources may differ in their dsRNA-pattern. In addition, in MVX diseased reference samples as well as in some "suspicious" samples we were able to detect dsRNAs not present in any of the healthy mushrooms. The occurrence of dsRNA species in wild *Agaricus* species was also investigated. We found that dsRNAs, which might be of viral origin, are present in *A. romagnesii*, *A. squamuliferus* and in *A. vaporarius*.

APPLYING SYMBIOTIC FUNGI TO GERMINATE HUNGARIAN NATIVE ORCHIDS

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About 60 orchid species are native in Hungary, all of them are protected plant species, and several are under strict protection. The Hungarian terrestrial orchids have very specific demands with respect to the habitat, and live in symbiosis with fungi. A possible way to conserve the endangered species is the artificial propagation of the orchids, followed by replanting into their natural habitat to increase population density. Co-cultivation with symbiotic fungi during this period may enhance the survival rate after replanting in nature. The aim of our experiments was to establish the optimum conditions for seed germination in the presence of mycorrhizal fungi.
Seeds of 11 orchid species were collected to analyse symbiotic germination and to optimise culture conditions. In contrast to most experiments described in literature we only used mycorrhizal fungi, which have been taxonomically identified by molecular biological methods. The analysed orchid species represent 6 genera (*Anacamptis, Dactylorhiza, Epipactis, Gymnadenia, Ophrys, Orchis*). The seeds were sown on 3 different media, each inoculated with one of the five fungi, which have been isolated earlier from various orchids. The germination percentage was determined.
The presence of mycorrhizal fungi strongly stimulated the germination of *Dactylorhiza incarnata* and *Ophrys sphegodes*, while *Anacamptis pyramidalis* germinated better under asymbiotic conditions. Germination of *Ophrys scolopax* ssp. *scolopax* and *Orchis laxiflora* ssp. *palustris* seeds was dependent on the media. No effect of mycorrhizal fungi on germination was detectable in the case of *Orchis coriophora*. Most fungal lines coexisted with the seedlings, despite the relatively high sugar content of the medium, however, the *Thanatephorus* isolate 5DI1/6A parasitized and finally killed all seedlings except those of *Anacamptis pyramidalis*.
Our results show that the germination stimulating activity of mycorrhizal fungi isolated from orchids is species dependent. Ongoing studies should clarify whether the advantageous influence of the symbiont also prevails during further cultivation.

IDENTIFICATION AND CHARACTERISATION OF A NOVEL NUCLEOBASE-RELATED TRANSPORTER FAMILY IN *ASPERGILLUS NIDULANS*

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Seven homologues (FurA-FurG) of the *Fur4*-like gene family (transporters for uracil/uridine/allantoin/thiamine) of *Saccharomyces cerevisiae* contributing to Fur4, Fui1, Dal4, Thi10/17, respectively were identified in *Aspergillus nidulans* through *in silico* blast analysis of *A. nidulans* genome bank. All but one putative permeases were annotated (AN0660.2, AN4152.2, AN3352.2, AN8416.2, AN9326.2, AN7955.2). The *furD* was found in two separate but overlapping contigs (1.158 and 1.209) and was united into continuous sequence and deposited in NCBI under the accession number EF620426. Each transporter gene was deleted from the genome, and deleted strains were subjected to growth tests to reveal major substrate specificity of the examined transporters. FurA proved to be the exclusive transporter of allantoin. FurD is expressed in germinating conidia and transports uracil. FurE is supposed to be one of the thiamine transporter of *A. nidulans*, while the specificity of FurB, FurC, FurF and FurG still remained hindered. We expected to identify one of the transporters to be a uridine permease and we found none of them is specific for this compound.

PLEUROTUS GREEN MOULD DISEASE: A PCR-BASED TEST FOR THE RAPID DETECTION OF THE CAUSATIVE AGENTS, *TRICHODERMA PLEUROTOPHILUM* AND *TRICHODERMA FULVIDUM*

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Following champignon (*Agaricus bisporus*) and shiitake (*Lentinula edodes*), the oyster mushroom (*Pleurotus ostreatus*) is the third most important commercially grown edible basidiomycete world wide. Since the early 80's severe green mould infections affecting *A. bisporus* have been reported in Europe and North America, and the causative agents were described as *Trichoderma aggressivum* f. sp. *europaeum* and f. sp. *aggressivum*, respectively. In the latest few years case reports have come up about serious green mould epidemics resulting in significant losses in the cultivation of *P. ostreatus* in South Korea, Italy, Hungary and Romania. The fungi responsible for the disease proved to be different from *T. aggressivum* based on their gene sequences (ITS1 and 2, *tef1, chi18-5*), as well as their morphological and physiological properties and therefore have recently been described as the new species *T. fulvidum* sp. nov. and *T. pleurotophilum* sp. nov. Since the green mould disease of *P. ostreatus* is spreading fast world wide, there is an emerging need of a rapid method for the detection of the pathogens in order to be able to control the disease properly. The aim of this work was therefore to develop a PCR based technique for the rapid detection of *T. pleurotophilum* and *T. fulvidum*. PCR primers were designed based on the 4th big intron, the 5th exon and the 5th small intron of *tef1*, which are specific for both *Pleurotus* pathogenic *Trichoderma* species, as well as only

for *T. pleurotophilum*. Besides *T. pleurotophilum* and *T. fulvidum,* we have tested the primers in a multiple PCR with the DNA samples of 28 other *Trichoderma* species and a range of other fungi. Our results demonstrate that *T. pleurotophilum* and *T. fulvidum* can be distinguished unequivocally from each other, as well as from other fungal species by the application of our three-primer set.

Based on our results, the two recently emerged *Pleurotus* pathogenic *Trichoderma* species can be detected rapidly without the need of ITS sequence analysis. This finding may help to recognize and control the green mould disease of *P. ostreatus* in its early phase.

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EARLY GROWTH RETARDATION OF MYCORRHIZA-INOCULATED TOMATO, BUT BETTER YIELD FINALLY IN A SOIL FREE SYSTEM

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The microbial inoculation techniques are promising tools in the sustainable agriculture. Among them, the microsymbiont bacteria and fungi are potential beneficial partners in the "struggle for life" strategy of several hosts [1]. One of the key points of this technology is how to avoid the competition of autochthonous populations and how to regulate the proper nutrient availability for the beneficial symbiosis formation [2]. An automated soilless "hydroponic" system was developed to study the effect of arbuscular mycorrhizal fungi (AMF) on the growth of several vegetable crops, among them the tomato (*Lycopersicum aesculentum* cv. red cloud). Inocula of an "aggressive" environmentally well-adapted mycorrhizal (AM) fungus (*Glomus intraradices* L.) as spores and hyphae in clay-beads were layered below the tomato seedlings (of two-weak-old) in 2 % (V/V), grown in peat-coco shell-perlite (V:V:V=1:1:1) mixtures. An automated water/nutrient supply was used, where a commercial half-strength N/P/K mixture was diluted in the water according to the suppliers suggestions. Yield of tomato was assessed regularly for quantity and quality. Statistical analysis ANOVA was used for the proper evaluation.

An early growth retardation of the tomato was found due to the initial symbiosis formation by the aggressive mycorrhiza fungi. After 1 months of growth a 20% less of tomato yield was found at the mycorrhiza inoculated treatments. At the 2nd and 3rd sampling months the interrelation between the macro- and microsymbiont partners has resulted in a better yield, by 15- and 25%, respectively. Beside the total amount of the tomato biomass there was a quality improvement recorded, as e.g. the size of the tomato fruits. It is assumed, that under soil free situation a greater nutrient-supply is necessary at the beginning of the symbiosis formation for the host plant. Beside the infectivity of the fungi, the efficiency and functioning might be a changeable character, which is directly regulated by the macro- and microsymbiont partners, as a function of the particular environmental conditions. The soil free system proved to be an appropriate method for the potential upscaling of the AM fungi and also in a new growth-technology among the greenhouse conditions.

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MONITORING OF SOIL BIOREMEDIATION PROCESSES USING LIPIDS AS BIOMARKERS

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Membrane lipids can be used as biomarkers for the analysis of microbial population changes as well as for the physiological status of micro-organisms. The investigation of changes in lipid composition is of common use for the assessment of physiological conditions in pure cultures. However, as lipid composition does not show drastic diversity among living organisms the use of lipids as biomarkers in mixed cultures and environmental samples has certain limitations. Therefore, special marker phospholipid fatty acids as well as modern statistical analysis of the results are necessary to receive certain information about the qualitative and quantitative changes of e.g. a soil microflora due to a contamination with organic compounds and its bioremediation.

The use of lipids as biomarkers in monitoring bioremediation is shown at the Hradčany site, a former Russian air force base in the Czech Republic that operated until 1990. In total an area of 32 ha soil and groundwater were contaminated with kerosene and BTEX compounds in an amount of 7,150 tons. Since about 6 years this highly contaminated site is treated with the so-called air sparging method to clean-up the contamination by aerobic biodegradation.

PLFA analysis could reveal a dramatic increase in bacterial biomass as well as a community shift to a Gram-negative microflora during the air-sparging treatment over about 5 years. The results, including a principal component analysis (PCA) of the obtained fatty acid profiles, showed that the air sparging leads to substantial differences in microbial communities depending on the contamination levels and length of treatment, respectively. Obviously, the length of air sparging treatment controlling the BTEX concentration in soils causes temporal changes of bacterial community and adaptations of its respective members. These results were verified either by classical culture methods as well as by a detailed molecular biological analysis of clone libraries.

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NOD1, IL-8 AND TLR4 GENETIC POLYMORPHISMS IN *HELICOBACTER PYLORI*-POSITIVE PATIENTS WITH DUODENAL ULCER OR GASTRITIS

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The intracellular receptor NOD1 is involved in the recognition of *Helicobacter pylori* (HP), which results in considerable interleukin-8 (IL-8) production in gastric epithelial cells. The aim was to investigate the association between Single Nucleotide Polymorphisms (SNPs) of Nod1, IL-8 or TLR4 genes and the development of gastritis or duodenal ulcer (DU) in HP-infected patients.

221 HP-positive patients with dyspeptic symptoms were examined by gastroduodenoscopy. HP-positivity was detected by 13C-UBT and histopathology. DU was found in 85 and gastritis in 136 patients. 75 HP-positive persons without gastric or duodenal disease served as controls.
The G796A polymorphism of CARD4/ Nod1 gene was determined by RFLP, while the T-251A polymorphism of IL-8 gene was analysed by ARMS method. TLR4 polymorphisms (A12874G and C13174T) were allocated by melting point analysis with a real-time PCR method. Statistical analysis was performed by using the Fisher exact test or χ^2 test as appropriate.
AA homozygote mutant variants of NOD1 were detected in 17 of 85 HP-positive patients with DU (20%) vs. 10 of 136 HP-positive patients with gastritis (7.4%), the difference being significant (p=0.010, OR: 3.150, 95% CI=1.367 - 7.26). Conversely, the prevalence of GG genotype was significantly lower in the group of patients with DU (44.7%) than in the gastritis group (62.5%) (p=0.012, OR: 2.061, 95% CI=1.188 - 3.561). The IL-8 heterozygote mutant variant at position -251 was detected with a significantly higher frequency among both ulcer patients (57.6%) and patients with gastritis (55.9 %) than among the HP-positive control persons (40%). However, no significant correlation in the frequency of the *TLR4* gene polymorphisms could be revealed between these two groups. The genotype frequency of AG heterozygotes concerning gene polymorphism A12874G was 13.4% in the patients with gastritis vs. 8.5% in DU patients. Similarly there was 13.4% frequency of CT heterozygotes concerning the C13174T gene polymorphism in the patients with gastritis vs. 8.5% in DU patients.
Genetic polymorphism of IL-8 might be a predisposing factor in DU and also in gastritis, but Nod1 polymorphism is rather important only in the pathomechanism of ulcer formation. The TLR4 polymorphisms do not increase the risk of disease manifestation in HP-positive patients. These observations draw attention to the importance of host genetic factors in the pathomechanism ofHP-induced diseases.

MODERN CHALLENGES FOR THE APPLICATION OF LACTIC ACID BACTERIA FOR THE BENEFIT OF MANKIND

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Microbial interactions in the human environment form part of our daily life and our cultural history. Lactic acid bacteria (LAB) are amongst the beneficial microorganisms. Traditional fermentation processes such as sauerkraut fermentation in industrialised countries and numerous other processes in developing countries offer valuable models to study the protection of the human GIT through microbial interactions. Preservation mechanisms may include competition, and antimicrobial metabolites such as lactic acid, acetic acid, alcohol, and antimicrobial peptides such as bacteriocins. The underlying mechanisms of these beneficial effects are subjects of increasingly intensive research in our time. In addition to preservation, additional benefits from LAB fermentations may include improvement/enrichment of the diet, biological enrichment by release or production of proteins, essential amino acids, essential fatty acids, and vitamins, detoxification during fermentation and degradation of antinutritive factors, improvement of the digestibility, resulting in reduction of preparation time and energy required, and favourable (functional) effects on the GIT by probiotic activities. Particular focus is placed on the selection and use of LAB as “multifunctional” strains, addressing both the food environment (for implementation as starter and protective cultures) and the human GIT (as functional or probiotic cultures). In this context, microbiologists, food scientists, nutritionists and biomedical researchers are challenged towards (a) the development of improved

selection techniques for functional strains, (b) defining "functional properties" and identification of "biomarkers" or desired "end-points" for functionality and application, and thereby leading to (c) the development and selection of "multifunctional" strains with an array of desirable features, both related to health benefits, food bio-preservation and/or food fermentation. Strains with multiple beneficial features are known but not well studied. In addition to a number of defined "probiotic" properties, selected strains may exert additional positive effects in food, e.g. by anti-oxidative activity, and by producing antimicrobial substances other than lactic acid or bacteriocins, and also by the formation of technically valuable compounds such as exopolysaccharides and flavour substances. Novel applications of lactobacilli as delivery vehicles for therapeutic molecules are under investigation; whether they will find application within the food context may remain an open question on the medium term. An interesting development is the envisaged use of non-pathogenic LAB for oral delivery of vaccine and therapeutic products. In addition, a few peptides of LAB strains have been found to exert anti-viral activities. The bacteriocin operons of a number of strains have been sequenced and expressed in bacterial hosts. Two bacteriocins, produced by *Lactobacillus plantarum* and *Pediococcus damnosus*, have been expressed in *Saccharomyces cerevisiae*. Production of a number of bacteriocins has been increased by selection of medium components and changes in growth conditions.

COMPARATIVE BACTERIOLOGICAL INVESTIGATIONS ON THE DRINKING WATER NETWORK OF BUDAPEST

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Microbial communities of potable water can change significantly in the collecting and distributing pipelines from the wells to the consumers. Usually pathogens and corrosive microbes are investigated by routine microbiological methods, so the real microbial diversity of water is only partially known in such systems. In a recent study diversity and changes of bacterial community of a discrete section of drinking water collecting and distribution system of Budapest was investigated.

Ten water samples were collected (three from riverbank filtration wells, three from collecting tubes in the Szentendrei Island, and four chlorinated drinking water samples from the distribution system) and cultured on oligotrophic agar plates (R2A, PYE, M27, Ravan media) and used for parallel molecular investigations (10 L water was filtered). Community DNA was isolated from the filters (MoBio Ultra Clean™ Water DNA Kit), and after partial 16S rDNA amplification T-RFLP analysis was performed. Bacterial community of samples was compared and Shannon's diversity index of each sample was calculated from size and relative quantity of terminal fragments. According to the first results of cultivation, plate count values were very low on all applied media. Unchlorinated (wells and raw water collecting system) and chlorinated (pumping stations of distribution system) part of the network had 10^{-1} - 1 CFU.ml^{-1} and 1 - 10^{2} CFU.ml^{-1} plate count, respectively. Highest plate counts were detected on R2A and PYE plates, the least colonies were detected on M27 media.

Diversity indexes varied from 1.7 to 3.6 values, highest diversity was observed in wells and collecting tubes, chlorinated samples were characterised by the lowest diversity. Chlorinated and unchlorinated samples separated based on their T-RFLP profile. Although water of "Újpalota" pumping station was chlorinated, its T-RFLP profile and diversity index were similar to unchlorinated samples. It may indicate that microbial enrichment occurs in the distal part of drinking water distribution system.

MIGRATION AND SYPHILIS IN CENTRAL EUROPE

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STIs showed an unusually high incidence in the Central and Eastern European Region in the years prior to and after the Millennium. Great interest was raised, and rightly so, by the HIV and syphilis epidemic that developed in the Central- Eastern European Region. Its coincidence with the far-reaching political and economic changes that took place at that time suggested a link between the two events. Hungary, where these infections had low incidence before the period mentioned, also experienced an increase in STI incidence. Migration – as a particular of horizontal mobility – subserves the speread of STDs. After the political changes of 1989, migration has increased significantly in estern and central Europe. The abrupt increase in the incidence of syphilis is clearly shown for the two counties accommodating refugee camps. The reduction of the number of camp-dwelling refugees was followed by a slow decrease in the number of infections occurring in the involved region.

STUDIES ON THE DEVELOPMENT OF SPECIFIC BACTERIAL SPOILAGE BIOTA IN CHILLED MINCED PORK MEAT

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The purpose of this study was to detect and identify the dominant species and strains of bacteria in minced pork meat during refrigerated storage under tightly packed, i.e. somewhat reduced aerobic conditions for 8 days at 4ºC. It has been proven that growth of microbiota is altered under refrigerated circumstances so that a few or often a single microbial species became dominant. Depending on the availability of oxygen these species usually belong to the genera *Pseudomonas, Brochothrix, Lactobacillus*. Under aerobic atmosphere the main spoiling species belong to the genus *Pseudomonas* that are able to compete successfully with other spoiling bacteria.

During our studies we determined the total number of aerobic bacteria on general nutrient agar (TGE) and performed Gram staining, oxydase and catalase probes. Further characterization of the isolates was made by inoculation into *Pseudomonas* selective culture media such as Cetrimide agar and GSP agar (Merck). The bacterial growth curves were estimated by curve-fitting of the colony count by DMfit programme package of the ComBase softwares, using Baranyi's dynamic growth model.

We identified the presumed *Pseudomonas* isolates with the application of API20NE system. According to these results the majority of the isolates belonged to *Pseudomonas putida* while some of them were identified as *Pseudomonas fluorescens*. As expected the *Pseudomonas fluorescens* isolates showed fluorescence under UV light. We used RAPD-PCR technique for molecular typing of the isolates aiming to cluster them into similarity groups according to their DNA sequence.

A PHYLOGENETIC STUDY ON DIFFERENT *PHOMA* SPECIES

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Phytopathogenic, opportunistic parasite and saprophyte species of *Phoma* genus have a world-wide occurrence. Identification for *Phoma* species by morphological characteristics has been almost the only method available for fungal taxonomists so far. However molecular identification methods are useful tools for a confirmative or distinctive complement.

In this study we have found suitable molecular markers which can be used as phylogenetic markers in the molecular based classification in the *Phoma* genus. With the present work we introduce the obtained DNA sequences from ITS regions and translation elongation factor (*tef1*) coding genes to resolve phylogenetic relationships among several *Phoma* species, since it has been shown that usage of multigene datasets can improve the resolution of molecular phylogenetic analyses.

The total genomic DNA from each of the isolates were extracted and a fraction of the *tef1* gene and the ITS regions were amplified. The phylogenetic analysis was conducted with PAUP*4.0b. Phylogenetic relationships of *Phoma* strains were inferred by the parsimony analysis of *tef1* and ITS sequences respectively. Topological robustness in parsimony analysis was estimated using 1000 bootstrap replicates.

Different *Phoma* sequences were compared to the closely related *Ascochyta* sequences. In some cases, the strains in the constructed phylogenetic tree, however does not fit to the traditional *Phoma* sections based on morphological characters. But we could manage suitable distinction of *Phoma* strains from *Ascochyta* ones comparing their *tef1* and ITS sequences by parsimony analysis.

We have proved that *tef1* and ITS could be useful phylogenetic markers to resolve phylogenetic relationships on the species level within *Phoma* genus. However more investigations are necessary to clarify whether the *tef1* gene sequence and/or ITS regions as phylogenetic molecular markers are well-suited for the classification of *Phoma* species.

ASPECTS OF MYCOPARASITISM OF *BOTRYTIS* SPP. BY *TRICHODERMA REESEI*

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A study was made to investigate the antagonistic efficiency and mycoparasitic activity of *Trichoderma reesei* against *Botrytis* spp., *B. cinerea, B. allii* and *B. fabae*.

To evaluate the efficacy of *Trichoderma reesei,* we studied the antagonistic effect of *T. reesei* on mycelial growth of *Botrytis* spp. *in-vitro*. A reduction of mycelial growth diameter was clearly observed against *B. cinerea*. However, no effect was found against *B. allii* and *B. fabae.* Hyphal and sclerotial mycparasitism of *T. reesei* against *Botrytis* spp. was microscopically investigated. Penetration, coiling and stunted hyphae were observed as signs of hyphal parasitism. The three afore mentioned signs of parasitism were observed against hyphae of *B. cinerea*. Stunted hyphae and coiling were the only signs of parasitism against *B. fabae* and *B. allii*, respectively. On the other hand, sclerotia of *B. cinerea* were parasitized but sclerotia of *B. fabae* and *B. allii* were not.

RNA POLYMERASE MODULATION AND TRANSCRIPTION ACTIVITY

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Stringent response is activated following nutritional deprivation or growth arrest in *E. coli* cells via the increase in the level of (p)ppGpp, a modulator of RNA polymerase. The elevated (p)ppGpp levels along with DksA, an RNA polymerase accessory protein, lead to reduction in transcription of some genes such as stable RNAs genes and to increase in transcription of some genes including amino acid biosynthetic and virulence genes. Mutations in subunits of RNA polymerase produce stringent RNA polymerase mutants such as rpo* mutations which suppress the UV sensitivity of *E. coli* cells lacking RuvABC protein. These mutations mimic the effect of elevated (p)ppGpp on gene expression. It was found that rpo*35 causes reduction in accumulation of RNA polymerase arrays. Moreover, it improves viability and survival of relA spoT dksA ruvA mutant. It is possible that generation of rpo* mutations in pathogenic *E. coli* cells lacking RelA may increase the transcription of virulence genes independent of (p)ppGpp and DksA.

COMPARATIVE STUDY OF THE METABOLIC FINGERPRINT OF *HISTOPHILUS SOMNI* STRAINS ISOLATED FROM FARM ANIMALS

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

Semen samples were taken in 7 different sheep flocks, vaginal swabs from cattle and lung samples from dead calves in 4 and 12 cattle stocks of Hungary, respectively. We involved *H. somni* type strain ATCC 43625 into the examination. Using adequate culturing methods, 92 bacterial strains were isolated, and identified as *H. somni* on the bases of morphological, cultural and biochemical characteristics. The comparative study of the metabolic fingerprint of 44 different *H. somni* strains was carried out, using the Biolog Microstation ID System (Biolog, Ca). The system analyses the ability of the utilization of 95 single carbon sources simultaneously thus allows the detection of slight differences among the strains.

Out of the 44 strains, 20 originated from testicles and semen samples of rams, 14 from calf lung samples and 9 strains from vaginal samples of cattle. There were 33 carbon sources that can be utilized by at least one strain. α-D-glucose, N-acetyl-glucosamine and dextrin can be utilized by more than the 90% of the examined strains. There was only 1 carbon source (α-D-glucose) that can be utilized by 100% of the strains isolated from calf lungs. All the strains originated from sheep samples could utilize 3 carbon sources (dextrine, α-D-glucose, turanose). All strains from cattle vaginal

samples were able to utilize 7 carbon sources (dextrin, N-acetyl-glucosamine, D-cellobiose, D-fructose, α-D-glucose, D-mannitol, D,L-lactic acid).
The relationship among the strains according to the carbon source utilizationwas also evaluated.

PROBIOTIC YOGURTS FERMENTED BY *BIFIDOBACTERIUM BREVE* AND *LACTOBACILLUS PARACASEI*, BASED ON LACTOSE-HYDROLYSED MILK, SUPPLEMENTED WITH SOYA-MILK

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The role of probiotic starter culture in dairy fermentation is to assist in the next characters: i. the preservation of the milk by the generation of lactic acid and antimicrobial agents, bacteriocins; ii. the production of flavour compounds (e.g. acetaldehyde in yogurt; iii. the production of other metabolites (e.g. extracellular polysaccharides) that will provide a product with organoleptic properties desired by consumer; iv. the enhancement of nutritional value, as in the synthesis of vitamins of the B-group; v. the provision of special or prophylactic properties due to the presence (at the time of consumption) of several million viable bacterial cells of starter origin. Special supplementation with prebiotic compounds may be useful both to consumers, and probiotic cells. For example, the soya-milk is able to stimulate the probiotic starters and it is very important for healthy life-style.
The antibiotic resistance of probiotic bacteria were controlled with Resistest disk method. The fermentations were run in 2 duplicate, at 37 C, until complete coagulation. *Bifidobacterium breve* (3%) and *Lactobacillus paracasei* (3%) were used for inoculation. Before the inoculation 2/3 part lactose-hydrolysed milk was supplemented 1/3 part soya-milk. During the fermentation the titritable acidity was measured. The viable cell count of *Bifidobacterium breve* and *Lactobacillus paracasei* was determined by microscopic (Breed) method. After 4-5 hours fermentation and cooling the organoleptic properties were evaluated by Kramer method, by 10 panelists. An "electric tongue" was used to compare the yogurts, fermented for lactose-sensitive persons.
Bifidobacterium breve had higher antibiotic-sensitivity, than *Lactobacillus paracasei*. The fermentation was faster, the acidity was higher (included acetic acid) in case of the samples, which were supplemented with soya milk. The taste of soya-milk was a bit unusual for the panelists.

SEQUENCING AND COMPARATIVE ANALYSIS OF A GOOSE AND A TURKEY ADENOVIRUS

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From the genus *Aviadenovirus*, the genomes of only two members are fully characterized. Both of them, fowl adenovirus (FAdV) 1 and 9, originate from chicken. From waterfowl, no adenovirus sequence is available yet in the GenBank, although we have previously recognized the distinctness of a goose and a Muscovy duck adenovirus from FAdVs by the analysis of partial hexon gene

sequences. Similarly, there is no sequence released from turkey aviadenoviruses. The only sequenced turkey AdV genome is that of the highly pathogenic turkey hemorrhagic enteritis virus (TAdV-3), a member of an other genus, *Siadenovirus*. To learn more about the phylogenetic position and genome organization of waterfowl and non-pathogenic turkey AdVs, we decided to sequence the entire genome of two Hungarian isolates, a goose and a turkey AdV strain.
Different methods were used for the two genomes. The DNA of the turkey adenovirus was randomly cloned and sequenced after cleavage with several restriction endonucleases (*Bam*HI, *Pst*I, *Sac*I). The ends of the genome were disentangled from the terminal protein by alkali treatment; the DNA was digested by *Bam*HI and cloned into *Bam*HI and *Eco*RV cut plasmid. For the goose adenovirus genome, the more expensive, but much quicker shotgun sequencing was applied. After non-specific shearing of the purified viral DNA, the molecule population corresponding to size range of 600 – 1,500 bp was extracted from agarose gel and cloned as blunt-ended fragments. The massive amount of sequences, obtained from 750 clones, was processed (sorted, aligned and joined) by the Pregap and Gap4 programs of the Staden package. Missing parts were amplified by PCR. The ends of this genome were sequenced directly on purified genomic DNA by a custom made primer specific to the leftmost end of the contiguous genome sequence. Phylogenetic calculations, based on deduced amino acid sequence alignments (MultAlin), were made by distance matrix analysis (Phylip program package, visualization by TreeView).
Physical map of the turkey adenovirus DNA were determined for *Bam*HI enzyme. It seems that the whole genome has been successfully cloned, and the nucleotide sequence of approximately half of the genome is determined. Missing parts are being subcloned for further sequencing or genome walking is performed by custom designed primers. Several structural genes have been fully sequenced; the largest contiguous sequence consists of 5,042 bp. The genome of the goose adenovirus is ca. 43,450 bp long with a G+C-content of 44.63%. In the middle part, the homologues of 16 conserved genes, found in every adenovirus studied to date, were identified. Bioinformatic analysis suggests the presence of the U-exon, and the existence of only one fiber gene as opposed to two fiber genes in FAdV-1. The hypothesis on the co-evolution of adenoviruses with their vertebrate hosts seems to be further supported. On the phylogenetic tree, the goose (as well as the Muscovy duck) adenovirus formed a separate branch and lineage, distinct from that of fowl adenoviruses. The turkey adenovirus clustered together with FAdVs.
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SELECTION OF ANTAGONOSTIC YEAST FROM APPLE SURFACE MICROBIOTA

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The vegetables and fruits are usually colonized by complex microbial populations. In this study apple was chosen as a model fruit to examine the composition of this microbial population. We find that there are many kinds of yeast strains and filamentous fungi on apples throughout the vegetative period. For taxonomic identification the yeast isolates were tested for growth under different conditions. We identified several groups, which may correspond to several species. Bacteria were not investigated.
It is well established, that many kinds of filamentous fungi can induce post harvest rotting, which can be a serious problem during storage. To find a biological agent for their inhibition, we selected yeast strains with antifungal activities from the surface microbiota. *Metscnikowia* strains were found to be

most active. Their activity was tested against *Botrytis* and *Penicillium* in several ways: on agar medium, in liquid medium and on apples.

INFLUENCE OF FERMENTATION CONDITIONS ON VIABILITY AND ETHANOL PRODUCTION OF *KLUYVEROMYCES LACTIS* AND *K. MARXIANUS*

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Kluyveromyces lactis and *K. marxianus* belong to those unique yeast species that are able to ferment lactose into ethanol; therefore they could be candidates for bioethanol production from lactose containing wastes and by-products.

Our aim was to investigate the viability and ethanol production of these two yeast species in a batch fermentation system. Experiments were carried out by using a shaken fermenter (Monitshaker, BIA Ltd, Ljubljana), which allows online measurement of dissolved oxygen and pH. Fermentations were performed under strong oxygen limitation in culture media containing glucose and lactose in different concentrations.

During lag phase both species used up the dissolved oxygen rapidly from the culture media but its concentration increased gradually after they started growth. This indicated that their metabolism switched from respiration into fermentation at the beginning of growth. Concentration of dissolved oxygen remained constant during the stationary phase of growth. Gradual decrease of pH was observed on both carbon-sources to pH 5 under non-buffered conditions. Viability of *K. lactis* was not affected considerably by the combination of the increasing ethanol content (up to 2 wv%) and decreasing pH (up to pH 5) but *K. marxianus* was very sensitive for the combination of these factors. At the beginning of the exponential phase of growth the ratio of viable cells decreased to 40% if glucose was added as carbon-source and to 10% if lactose was the utilizable carbon-source. Ratio of viable cells however increased gradually during further growth up to 50%, which indicated its adaptation to the inhibitory conditions.

In separate experiments the inhibitory effects and interaction of ethanol and pH were determined. As the main conclusion *K. lactis* seems more suitable for ethanol production from lactose than *K. marxianus* because the former species can tolerate stress factors more than the second one.

DETECTION OF VIRUSES IN HUNGARIAN SURFACE WATERS

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It is generally impossible to determine the etiological agent of most water-related disease outbreaks. Most of these infections are probably due to water-borne viruses, such as Hepatitis A virus, Calicivirus (norovirus) or enterovirus, which are shed into sewage by the infected human population. Detection of potentially pathogenic viral presence in water is challenging as viral concentrations are generally very low and hitherto techniques for routine virological analysis of water are not readily available. There is no previous data on the prevalence of viruses in Hungarian surface waters.

Methods for the concentration and detection of human enteric viruses from surface waters, developed in the EU funded project Virobathe (www.virobathe.org) were implemented during the present study. Large volume (10 L) water samples were concentrated to 10 mL by glass-wool filtration and organic flocculation. Viral nucleic acids was extracted from the concentrates and analyzed for the presence of Hepatitis A virus, human adenovirus, noroviruses and enteroviruses by (RT)-PCR.
The performance of the method was verified using tapwater and surface water samples seeded with known strains of enterovirus and adenovirus. The findings indicated that 2 Enterovirus particles could be detected using 5µl samples by qPCR in seeded samples. This sensitivity corresponds to 5,000 genomes in the surface water concentrate. Test surface water samples were collected from the Danube and Tisza rivers and Danube dead-branches. Concentration and nucleic acid extraction were successful. All samples were also tested for enterovirus, adenovirus and norovirus by ELISA. One sample was positive for Caliciviruses by ELISA and for adenoviruses using rapid line test. These samples were however negative by PCR. Negative results are potentially due to chemical inactivation of the viruses or the presence of PCR inhibitors in the concentrate.

MODELLING SEPTATION IN FISSION YEAST

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Nowadays challenges for microbiology are studies on application of microbes in industry, environment and food sciences. In these researches the examinations of the individual microorganisms are coming to the front. The mapping of the cell cycle and the cellular behaviour regulating complex protein network is the targets of new scientific approaches.
The common test organisms are bacteria (e.g. *Escherichia coli*) and yeasts (e.g. *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*). Our group worked out mathematical models for yeast cell cycle regulation.
The three most important events of the cell cycle are DNA replication, mitosis and cell division (cytokinesis). One of the main goals of our research is to understand these processes by mathematical models. My talk will show our new model of the regulatory network of cell division in *S. pombe* cells.
The rod-shaped fission yeasts divide by forming a septum in the middle of the cell. The regulating protein network of cell division is called septation initiation network (SIN). We worked out a simple mathematical model of SIN, and tested it by computational simulations. The SIN behaves like an adaptive system (in the same way as bacterial chemotaxis). The upstream regulator of SIN is the Cdc2/Cdc13 kinase, which is the main regulatory protein of mitosis as well. The network can not be activated at low and at high Cdc2/Cdc13 activity; but SIN has an activity peak when Cdc2/Cdc13 drops from high to low level. This drop of Cdc2/Cdc13 kinase occurs only after mitosis. This connection between SIN and the mitotic kinase guarantees that septum formation is initiated after mitosis, only once per cell cycle.
We incorporated this module of SIN into our earlier mathematical model, describing DNA replication and mitosis in fission yeast cells. We thus developed a new model which explains all three major cell cycle transitions. We demonstrated by computational simulations that our model works perfectly not only for wild type, but different uncontrolled septation mutant cells as well.

INDUCTION OF EXTRACELLULAR B-GALACTOSIDASE (BGA1) FORMATION BY D-GALACTOSE IN *HYPOCREA JECORINA* IS MEDIATED BY GALACTITOL

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The ability of *Hypocrea jecorina* (*Trichoderma reesei*) to grow on lactose strongly depends on the formation of an extracellular GH family 35 β-galactosidase, encoded by the *bga1* gene. Previous studies, using batch or transfer cultures of pregrown cells, had shown that this *bga1* is induced by lactose and d-galactose, but to a lesser extent by galactitol. To test whether the induction level is influenced by the different growth rates attainable on these carbon sources, we compared *bga1* expression in carbon limited chemostat cultivations at defined dilution (= specific growth) rates. Data show that *bga1* expression by lactose, d-galactose and galactitol positively correlates with the dilution rate, and that galactitol and d-galactose induce the highest activities of β-galactosidase at comparable growth rates. To know more about the actual inducer for β-galactosidase formation we compared its expression in *H. jecorina* strains impaired in the first steps of the two d-galactose degrading pathways. Induction by d-galactose and galactitol was still found in strains deleted in the galactokinase encoding gene *gal1*, which is responsible for the first step of the Leloir pathway of d-galactose catabolism. However, in a strain deleted in the aldose/d-xylose reductase gene *xyl1*, which performs the reduction of d-galactose to galactitol in a recently identified second pathway, induction by d-galactose, but not by galactitol, is impaired. On the other hand, induction by d-galactose and galactitol is not affected in an l-arabinitol 4-dehydrogenase (*lad1*) deleted strain which is impaired in the subsequent step of galactitol degradation. These results indicate that galactitol is the actual inducer of Bga1 formation during growth on d-galactose in *H. jecorina*.

DIVERSITY OF THERMOTOLERANT CAMPYLOBACTERS IN CHICKEN-DERIVED ABATTOIR AND COMMERCIAL EGG SAMPLES IN A HUNGARIAN COUNTY

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We tracked thermotolerant *Campylobacter* species in broiler stocks from farm to fork. We also studied *Campylobacter* carriage in commercial eggs of two consecutive layer stocks. Present report is

concerned with the diversity found in abattoir environmental and broiler carcass samples, commercial eggshells and meat from retail shops.
We followed up five broiler stocks consecutively kept in the same henhouse between April and December 2006, another stock of the same breeder kept at a different location in November-December 2006, and a stock of a different owner in December 2006 - January 2007. The stocks of the same breeder were slaughtered and processed at the same abattoir and sold in the same retail shops, while the different owner used another abattoir and the meat was sold to different shops.
During processing of each stock samples from the abattoir environment as well as five neck skins and five breast filet samples from carcasses were collected. Commercial eggs were collected fortnightly, altogether 1090 pools were examined. Samples were cultured according to the official guidelines. Campylobacters were identified by species-specific PCR.
Practically all meat samples were contaminated. We isolated 14, 15, 33, 30 and 45 Campylobacters from 1090 egg, 149 environmental, 35 neck skin, 35 breast filet and 55 retail meat samples, respectively. Out of these we typed 70 and 26 *C. jejuni* (CJ) as well as 20 and 14 *C. coli* (CC) isolates using ERIC-PCR and flagellin gene RFLP, respectively. Both methods discriminated the two species, fla-typing and ERIC-PCR revealed two and one CC as well as five and three CJ clusters. Clusters revealed by fla-typing generally corresponded to samples derived from stocks of different locations, and isolates connected to consecutive stocks were frequently grouped into the same clusters. Egg-derived isolates were separated from meat-derived isolates by fla-typing but not by ERIC-PCR, while grouped into several different clusters with both methods.
We observed carryover of strains from the meat of one stock to that of another both in case of CC and CJ. This may derive from survival and carryover of strains between the consecutive stocks in the henhouse or from their survival in the abattoir environment. Then the contamination was transmitted to retail meat in case of all stocks. Shop-specific patterns were not observed, suggesting that retail shops do not seem to be a significant reservoir of thermotolerant Campylobacters.
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THE IMPORTANCE OF MULTIRESISTANT *PSEUDOMONAS AERUGINOSA* ON BIOREMEDIATED SITES

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The widespread antibiotic resistance of the opportunistic pathogen *Pseudomonas aeruginosa* and the appearance of multiresistance in clinical surroundings were detected for decades. At the same time the occurrence of increased antibiotic resistance in the environment has been described only in a few cases. In our research the possibility of increased antibiotic resistance was considered with the examination of *P. aeruginosa* strains isolated from hydrocarbon contaminated soil and groundwater. From 2003 to 2006 29 isolates of *P. aeruginosa* were collected from hydrocarbon contaminated sites. The isolation and identification of *P. aeruginosa* were executed in several steps including phenotypic, biochemical and genetic methods recommended by the relevant Hungarian Standard (MSZ 21470/77:1988), API 20NE method (bioMérieux) and ETA method (exhibition of Exotoxin A gene sequence using PCR technology).
The antibiotic resistance profiles of the environmental isolates were compared with nosocomial *P. aeruginosa* strains that were isolated from human infections (included the generally used reference ATCC 27853, three isolates with increased resistance and one multiresistant nosocomial strain). The

comparative antibiotic resistance research between environmental and clinical strains of *P. aeruginosa* was executed with disc diffusion susceptibility testing based on the internationally accepted methodology and evaluated according to the NCCLS. In our investigation 31 antimicrobial products were applied (from 9 different classes) that are in common use in clinical practice.
In our results 22 of the 29 strains (75%) had higher rate of resistance than the standard level of ATCC 27853 comparative clinical strain and reached the increased category. Two environment originated strains of *P. aeruginosa* were multiresistant. It was established that the proposed classes of antibiotics (e.g. Aminoglycosides) for treatment of *P. aeruginosa* are not effective in every time – the increase of the antibiotic resistance in the environment is verified. Moreover in several cases the proposed antibiotics (e.g. Piperacillin) are completely inefficient for treatment of the environment originated strains. On the basis of the results the antibiotic resistance and multiresistance has great importance – not only in nosocomial surroundings but in the nature as well. This fact entails the necessity of the reinterpreted approach of bioremediation procedures. Namely unidentified inocula with excellent biodegradation capability often contain opportunistic pathogen microorganisms – e.g. *P. aeruginosa*. Moreover during *in-situ* biostimulation (soil treatment for the stimulation of soil biota during remediation e.g. soil aeration) pathogen microbes – possibly with increased antibiotic resistance – can proliferate in uncontrollable way and antibiotic resistance mechanisms can be spread immediately among species of natural microbiota.
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EPIDEMIOLOGICAL IMPORTANCE OF SPORADIC HUMAN CALICIVIRUSES

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Among the small round-structured viruses (SRSVs), such as rota-, adeno- and astroviruses, the human caliciviruses (HuCVs) are the most frequent etiological agents of acute, non-bacterial gastroenteritis worldwide. The sources of infection are stool-contaminated water, food and aerosol. A very rapid spread can occur within closed communities, because 10-100 virus particles are sufficient for infection. Our knowledge on sporadic HuCV infections is very deficient. According, one goal of our study was to detect HuCVs from gastroenteritic faeces samples.
With traditional methods, the HuCVs cannot be cultivated; the diagnostic methods are the antigen-ELISA and the traditional PCR. Between January 1, 2003 and March 31, 2007, 2098 stool samples were analysed. For the antigen detection, we used the Norovirus I/II antigen ELISA (DacoCytomation) and the Norovirus 1 and 2 antigen ELISA (IDEIATM Norovirus) kits. For the viral RNA detection, we used the traditional RT-PCR and the real-time RT-PCR methods. In this time interval, we analysed the prevalence of rota-, adeno- and astroviruses.
Of 2098 samples, 320 (15.25%) proved to be positive for human caliciviruses. We determined the prevalence of the human caliciviruses between the different age groups and sexes with biostatistical methods. We found 320 HuCV-containing samples (15.25%). During the sample preparation, ELISA was used 873 times, RT-PCR 142 times and real-time RT-PCR in 1095 cases. We applied different methods to detect HuCVs in 93 cases. We attempted to find connections between the incidence of positive cases and the outcome of the Hungarian pandemics between 2003 and 2007. We compared the nationwide data and our own data.
We found correlations between the occurrence of HuCVs and Hungarian pandemics. There were several pandemics in 2007 in the different departments at the University of Szeged, and on each occasion we detected sporadic cases before the pandemics. We used antigen-ELISA kits for the

routine HuCV diagnostics, but for phylogenetic assays and for the determination of the different phyla we have to use molecular genetic methods. After 4 years of study, we can say that in Szeged human caliciviruses are responsible for gastroenteritis in most cases, in second place we find rotaviruses (9.7%), then adenoviruses (4.1%) and human astroviruses (3.9%).

CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* STRAINS COLLECTED FROM PATIENTS WITH ATOPIC DERMATITIS IN HUNGARY

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Atopic dermatitis (AD) is a pruritic, chronic, recurrent, inflammatory disease that is often triggered by bacterial, fungal or in some rare cases, viral infection. A number of studies have shown that the skin of patients with atopic dermatitis is colonized by *Staphylococcus aureus*, and their superantigens (SAgs) may represent a substantial immunological trigger factor for this disease. *S. aureus* strains (168) isolated from the throat (53), nose (57) and skin (58) of 150 children with AD, were screened for their carriage of genes encoding SAgs (the classical *entA-E*, *tst* and the newly described *entG-J*), exfoliative toxins (et), biofilm forming capacity (ica), and methicillin resistance (*mecA*). Furthermore biofilm formation was investigated *in-vitro* as *S. aureus* is known to form biofilms on injured skin using fibrin and glycocalyx. Of these strains from atopic patients, 139 strains (82.7%) carry at least one of the SAgs genes. The genes *entG* and *entI* were amplified from both atopic (65.5% and 53.6%, respectively) and healthy individuals (76.7% and 70%, respectively). About half (47%) of the screened strains from AD patients carried the classical SAgs genes and the carriage of these toxins genes was highest amongst the skin isolates (58.6%). *EntE*, et and *mecA* were not identified in any of the strains. Genes associated with biofilm formation were detected in 73.2% of the AD strains, but this was similar to those strains isolated from healthy subjects. There were fewer strains possessing the Ica phenotype on Congo Red Agar than there were strains harbouring the ica gene. Our results suggest that the classical SAgs may be responsible for the exacerbation of AD. The high rate (42.1%) of *S. aureus* carriage in the nasopharynx amongst AD patients suggests the possibility of self-inoculation from both the throat and nose.

CULTIVATION BASED BACTERIAL DIVERSITY OF THE SEDIMENT OF LAKE BALATON

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Numerous studies were published about material- and energy-turnover of the benthic region of Lake Balaton but little information is available about the microbial communities of the sediment in the biggest lake of Hungary and Middle Europe. The aim of this study was to reveal the seasonal variations of bacterial diversity with culture-based investigations by choosing sampling sites with different trophic state.

Samples were taken from the upper 3-5 cm horizon of the sediment from the mesotrophic Tihany-basin [B1] and eutrophic Keszthely-basin [B2] in May and August, 2005. 215 strains were isolated using four different media. After cell morphological and biochemical investigations, hierarchical cluster analysis was carried out on the basis of 30 phenotypic test results. 69 representative strains were chosen for ARDRA from clusters (represent more than 85% similarity), and strains with different genotypic fingerprint were identified with 16S rDNA sequence comparisons.
On the basis of Principal Component Analysis with Centered Euclidean biplot method, the two sampling sites clearly separated from each other in both season. More Gram-positive organisms were cultivated from the eutrophic basin, but for example the presence of fermentative bacteria was characteristic rather in the mesotrophic region. The strains were fairly inactive in most of the biochemical tests, although in the summer period the biopolymers were utilised in greater extent. Forty percent of the strains showed the highest sequence similarity with species of genus *Bacillus* (e.g. *B. koguryoae, B. megaterium, B. pumilus, B. subtilis*), which may play key role in the mineralization of larger organic compounds. Gram-positive bacteria were found also from the genera *Cellulomonas, Kocuria* [B2], *Microbacterium* [B2], *Rhodococcus* [B1] and *Sporosarcina*. Other strains were identified as members of the genera *Ensifer* [B2], *Sphingomonas* (Alpha-), *Acidovorax* [B2] (Beta-), *Aeromonas, Arthrobacter*, *Pseudomonas*, *Pseudoxanthomonas, Rheinheimera* and *Shewanella* [B1] (Gamma-Proteobacteria). Species of *Aeromonas* were found mainly in Tihany-basin, while *Pseudomonas* in Kesztely-basin. Both taxa may be important in the decomposition of smaller organic compounds. One of the strains identified as *Flavobacterium succinians* from Tihany-basin belonged to Bacteroidetes.

ENDOTOXIN ANALYSIS BY ELECTROPHORESIS AND MICROCHIP TECHNOLOGY

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Bacterial endotoxins (lipopolysaccharides, LPSs) are highly active components of the outer membrane of Gram-negative bacteria and are released during growth, division and lysis. They have been recognized as the most potent stimulants of mammalian immune systems, causing a wide spectrum of pirogenic and toxic reactions.
LPS consists of a lipid region, termed lipid-A (which is a phosphorylated glycolipid) covalently attached to a polysaccharide region (which consists of a longer O-side chain and a shorter core section). The molecular mass of an endotoxin monomer is between 2-20 kDa. In physiological solutions, the amphipathic LPS molecules aggregate and form detergent like vesicles (MW around 1 million Da), which exhibit negative net charge due to their phosphate groups.
Though several methods have been used for endotoxin analysis (e.g. SDS-PAGE), much progress is still needed to separate and identify the many subclasses of LPS from individual strains. The lack of strongly UV-active groups or chromophores in the LPS molecule and its strong tendency to aggregate in aqueous solution makes the detection of the underivatized substances difficult.
We have developed two different and novel methods for endotoxin analysis: 1) capillary electrophoresis to monitor endotoxin-hemoglobin complexes by UV-light, and 2) micro-chip electrophoresis with laser induced fluorescence (LIF) detection of LPS samples containing SDS.
Since native human hemoglobin (Hb) forms complexes with LPS, we utilized this complex formation to detect LPS in capillary electrophoresis (BioFocus 3000, Bio-Rad Laboratories, Hercules, CA). LPSs (1mg.ml^{-1}) and hemoglobin (1mg.ml^{-1}) were mixed, incubated (37.5^{o}C) and analysed in coated

fused-silica capillaries. The mobility of hemoglobin changed upon a complex formation with endotoxins. The electrophoretic pattern obtained after incubation with endotoxins extracted from *Escherichia coli*, *Salmonella minnesota*, *Shigella sonnei* strains were different.
Microchip electrophoretic analysis of different LPSs was carried out in a commercial instrument (Agilent Bioanalyzer 2100, Waldbronn, Germany). Exploiting the advantages of the micro-device (shorter analysis time, simultaneous analysis of large sample numbers, etc.) we worked out conditions for the application of a microfluidic chip for protein assays to the analysis of lipopolysaccharides. From a series of experiments we can conclude that i) 4 % (w/v) SDS concentration in the sample is the best for LPS disaggregation, ii) the electrophoretic profile depends on the structure of LPS and the results are comparable with the SDS-PAGE patterns of endotoxins.

NUCLEOTIDE SEQUENCE ANALYSIS OF PEANUT STUNT VIRUS RP ISOLATE, PROVE THE ROLE OF RECOMBINATION IN CUCUMOVIRUS EVOLUTION

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Peanut stunt virus (PSV) is an economically important pathogen of legumes worldwide. PSV is the member of the genus *Cucumovirus* in the family Bromoviridae. PSV like other cucumoviruses, has a tripartite genome of positive-strand RNAs. The four complete nucleotide (nt) sequences available in the GeneBank are classified into three distinct subgroups (I, II, III) based on RNA sequence comparison. The nt sequence identities between strains in the same subgroup are greater than 90%, whereas those between strains in different subgroups are in the range of 70% to 80%.
PSV-Rp was isolated and identified from black locust (*Robinia pseudoacacia*) in Gödöllő, Hungary. PSV-Rp caused mild mosaic symptoms in black locust. The isolate was propagated on tobacco and the virion was purified. cDNA from the viral RNAs was synthesized according to the protocol of 5' Rapid Amplification of cDNA Ends (RACE) kit, than the viral RNA was amplified by PCR. The PCR products were cloned and cDNA inserts representing PSV-Rp RNAs 1, 2 and 3 respectively were selected for automatic fluorescent nt sequencing. Sequences were analyzed using EBI Emboss pairwise alignment, Clustal X and Treeview programs. Since only four complete nt sequences are available in the GeneBank and neither of them is originated from black locust, we were curious to supply data in this respect.
The full length nt sequence of RNA 1, 2 and 3 of PSV-Rp were determined to be 3325, 2942 and 2208 nt, respectively, coding five ORFs typical for cucumoviruses. This PSV-Rp nt sequence is the first full sequence data reported in Europe, and the first one originated from black locust. Nucleotide sequence identities among PSV-Rp and the earlier described PSV RNAs were analysed. The percent nt identities for RNAs 1 were between 79,2 % and 84,6 %, for RNAs 2 were between 74,1 % and 82,3 %, and for RNAs 3 were between 80,9 % and 83,9 %. Based on these identities, we propose to establish a fourth subgroup of PSV.
Phylogenetic analyses of RNAs and ORFs showed that PSV-Rp RNA 1, 2 and 3 are most closely related to subgroup II strain. Interestingly, phylogenetic analyses of the CP gene coding by RNA 3 displayed closer relationship to subgroup III strains. Possibility of an intraspecific recombination was studied by Topali v2 program. PDM analysis detected 2 recombination points in the RNA3 genome.

Data indicated that PSV-Rp 1-1200 nt and 1886-2234 nt are related to subgroup II, while nucleotide sequence between 1201 and 1885 nt (including CP gene) related to subgroup III. Recombination data proved an intraspecific ancient recombination during PSV evolution among subgroup II and III. This is the first report of a well characterized recombination event in a *Cucumovirus* genus isolated from natural ecosystem.

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CONTRIBUTION OF *SPI-4* GENES TO THE VIRULENCE OF *SALMONELLA ENTERICA*

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Salmonella pathogenicity island-4 (SPI-4) is a 27 kb region that carries six genes designated *siiABCDEF*. SiiC, SiiD, and SiiF form a type I secretion apparatus for the secretion of SiiE, a huge (~600 kDa) non-fimbrial adhesion contributing to colonization and intestinal inflammation. Here we show that loss of SPI-4 attenuates oral virulence of *Salmonella enterica* serovars Typhimurium and Enteritidis in mice. 50% lethal doses (LD50) were elevated in both serovars upon the loss of SPI-4. Moreover, ΔSPI-4 mutants were outcompeted in systemic organs by their wild-type strains in a co-challenge model. Contribution of SPI-4 to virulence appeared less pronounced in the *S.* Enteritidis strain, which is justified by lower levels of the secreted protein SiiE in this strain in comparison to *S.* Typhimurium. Competition assays with isogenic mutants lacking individual genes of the island showed that all six genes were required for full virulence of *S.* Typhimurium. Δ*siiA* and Δ*siiB* mutants were, nevertheless, able to secrete SiiE to culture supernatants. The amount of secreted SiiE was, however, reduced in these two mutants compared to the wild-type strain. Furthermore, the impact of different structural and regulatory LPS mutations on the expression of SiiE is shown and discussed.

MONITORING THE EFFECT OF TEMPERATURE INCREASE ON THE BIOGAS PRODUCING MICROBIAL COMMUNITY BY MOLECULAR METHODS

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Anaerobic digestion is a widely applied method for treating organic wastes, due to the high efficiency in organic matter degradation producing biogas, which can be further used as an energy source.
This process is mainly performed at two temperature ranges, namely mesophilic (35°C) and thermophilic (55°C). Although the microbial aspects of both ranges have been studied by many authors, the effect of the gradual change of mesophilic conditions to thermophilic on the bacterial community has never been observed. However, this method is common to pose the thermophilic operation. Thus, our aim was to examine the biological aspects of heating up the mesophilic reactors, and to correlate them with the changes of the physical and chemical parameters.

Our survey passed by means of two pilot-scale continuous stirred-tank reactors (CSTR) of South-Pest Wastewater Treatment Plant (SPWWTP). To gain information about the changes of community structure DNA and RNA based methods were used. After the amplification of a 16S rDNA (or cDNA) fragment, a molecular fingerprint method was applied called Terminal Restriction Fragment Length Polymorphism (T-RFLP). Clone libraries were made to identify the peaks of T-RFLP chromatograms. To estimate the ratio of the bacterial biomass existing at different temperatures Real Time PCR was used. Biogas production and composition, total VFA concentration, alkalinity, pH and redoxi potential were measured in the accredited laboratory of SPWWTP.
According to our results a thermotolerant mesophilic community emerged, its activity was sufficient up to 47°C. The microbial community of this temperature range were dominated by different clones belonging to *Chloroflexi* subphylum I and *Nostocoida limicola*, moreover up to 43°C a *Planctomycetes* clone was established. With respect to domain *Archaea* an acetoklastic methanogen, *Methanosaeta concilii* appeared predominant. At 51°C, the activity of this species, together with the biogas production reduced, while an eight fold increment was detected at the level of total VFA. Achieving 55°C a new eubacterial and archaeal community evolved. The dominant archaeon was a *Methanosarcina* sp., while the domain Bacteria was represented by a *Coprothermobacter* sp., an uncultured *Thermotogales* related clone and a species belonging to *Chloroflexi* subphylum I. In spite of the results concerning the mesophilic range, stable thermophilic methanogenesis was found in a narrow temperature interval, from 55°C to 59°C. At 63°C the acetoklastic methanogens (*Methanosarcina* sp*., Methanosaeta* sp.) were overpopulated by hydrogenotrophs (*Methanoculleus* sp*., Methanothermobacter thermoautotrophicus*), the acetic acid concentration irreversibly increased, whereas the biogas production and methane content dropped.

ASSOCIATION OF BETA-DEFENSIN-1 SINGLE NUCLEOTIDE POLYMORPHISMS WITH *HELICOBACTER PYLORI* INDUCED GASTRITIS AND WITH CROHN'S DISEASE

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Defensins are antimicrobial peptides with a broad spectrum of activity against Gram-positive and Gram-negative bacteria, fungi, yeast and enveloped viruses. Defensin deficiency might be responsible for impaired mucosal barrier function against different bacteria. The aim of our study was to investigate single nucleotide polymorphisms (SNPs) of the β-defensin-1 gene (*DEFB1*) in Crohn's disease and in *Helicobacter pylori* induced gastritis.
Three SNPs of the *DEFB1* gene *DEFB1* G-20A, *DEFB1* C-44G and *DEFB1* G-52A were genotyped either by Custom TaqMan SNP Genotyping Assays, or by restriction fragment length polymorphisms (RFLP), in 190 patients with Crohn's disease, 150 patients with gastritis and 95 controls.
Strong association between the G-20A and C-44G SNPs and the colonic and ileocolonic localization of the disease, respectively, but no association was detected as concerns the ileal localization. A significantly higher frequency of the GA genotype of G-20A was observed among patiens with colonic localization (60%) as compared with the healthy controls (38%), with OR 2,4. The GG genotype of C-44G SNP, which can be regarded as a protective genotype, was much less frequent (3%) among the patients than among the controls (11%), OR 0,29. Occurrence of DEFB1 G-20A and G-52A SNPs together were significantly higher (42%) in patients with H. pylori induced gastritis

then in controls (28%). These results indicate that genetic variations in *DEFB1* gene encoding constitutive human β-defensin-1 may associated to risk for *H. pylori* induced gastritis and for Crohn's disease.

PREVALENCE OF CYTOTOXIN GENES IN AUSTRIAN (AT), HUNGARIAN (HU) AND MACEDONIAN (MK) METHICILLIN-RESISTANT (MRSA) AND SENSITIVE (MSSA) *STAPHYLOCOCCUS AUREUS* STRAINS

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The purpose of this study was to detect the presence of cytotoxin genes in *Staphylococcus aureus* strains isolated from inpatient infections, and compare according to country origin and methicillin resistance. The phenotypical identification of the strains was done by classical microbiological methods. The genetical confirmation of the strains was done by detecting genes for thermostabile endonuclease (*nucA*) and 23S rRNA. According to the presence of the gene encoding methicillin resistance (*mecA*) 48 MRSA and 128 MSSA from Austria, 110 MRSA and 94 MSSA from Hungary, 73 MRSA and 29 MSSA strains from Macedonia were examined. The genes responsible for cytotoxins were detected by polymerase chain reaction. The pulsed-field gel electrophoresis of the strains was also done, the evaluation of results is in progress.

The Panton-Valentine leukocidin genes (*lukS-PV, lukF-PV*) were only found in 2.3% of AT MSSA, in 4.3% of HU MSSA and in 1.4% of MK MRSA strains. The alpha- and delta- haemolysin genes (*hla*, *hld*) were detected in all HU and MK strains. The *hla* gene was carried in 94% and 86% by AT MRSA and MSSA strains. The AT MRSA and MSSA strains harboured the *hld* gene in 96% and 98%. The *hlb* gene encoding beta-haemolysin was detected in 33% and 52% of HU MRSA and MSSA, in 68% and 76% of MK MRSA and MSSA strains, and in 58% and 40% in AT MRSA and MSSA strains, respectively. The gamma-haemolysin gene (*hlg*) carriage was 96% and 93% in MK MRSA and MSSA, 88% and 68% in HU MRSA and MSSA, 69% and 74% in AT MRSA and MSSA strains. The haemolysin gamma variant gene (*hlgv*) was found in 100% in the MK strains, 100% and 84% in HU MRSA and MSSA, 100% and 56% in AT MRSA and MSSA strains. Comparing all the MRSA with MSSA strains independently from country origin the presence of *hlg* and *hlgv* genes was significant for MRSA strains. The presence of 5-gene combination pattern composed of *hla, hlb, hlg, hlgv, hld* genes was significant for both the MK MSSA and MRSA strains. For the AT MSSA strains the 3-gene combination comprised of *hla, hlg, hld* genes was significant. The HU MRSA strains were characterised significantly with the 4-gene combination pattern included *hla, hlg, hlgv, hld* genes.

Significant differences could be detected in the presence of cytotoxin genes between MRSA and MSSA strains according to country origin. Characteristic country specific gene combination patterns were also found in both cases of MSSA and MRSA strains. MRSA and MSSA strains do have geographical features in the aspect of cytotoxin genes.

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PCR-BASED DETECTION OF HUNGARIAN *FUSARIUM GRAMINEARUM* ISOLATES

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Species-specific PCR assay was used for the identification of Hungarian *Fusarium graminearum* isolates in pure mycelial culture. *Fg16F/Fg16R* primer pair of the three known species-specific primers appeared to be most appropriate one to identify *F. graminearum* from pure cultures. The PCR assay described in this study can be used for routine detection and identification of *F. graminearum.*

Two methods were used for comparative determination of the amplicon size of *F. graminearum* strains. One of them was a traditional agarose gel electrophoresis, other was a chip electrophoresis (DNA 500 LabChip Kit, Agilent 2100 Bioanalyzer, Agilent Technologies, USA).

Our results have shown that the chip electrophoresis is an easy-to-use, time-efficient substitute for conventional agarose gel electrophoresis; moreover it provides a more precise size determination of amplicons. Amplicon size ranging from 415 bp to 421 bp in tested isolates may be associated with genetic diversity in the Hungarian population of *F. graminearum*.

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THERAPEUTIC OPTIONS FOR INFECTIONS CAUSED BY ESBL-PRODUCING ENTEROBACTERIACEAE

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The spectrum of human interaction with ESBL-producing enterobacteria ranges from mere colonisation to severe, life-threatening infection. These infections are particularly problematic because choices for antimicrobial therapy, both empirical and definitive, may be severely restricted given that these organisms are often resistant to multiple antibiotics and antibiotic classes. Inadequate initial antibiotic treatment is an independent risk factor for mortality in non-urinary infections caused by ESBL-producing Enterobacteriaceae.

No clinical trial exists which proves that one particular antibiotic is better than any other one for treatment of ESBL-producers. *In-vitro* susceptibility data show that carbapenems are the most active drug class against ESBL-producing organisms. Observational studies of serious infections with ESBL-producers have consistently shown that carbapenem (imipenem and meropenem) use is associated with a superior outcome than occurs with other antibiotic classes. Ertapenem shares the good *in-vitro* activity of the other carbapenems, and has been successfully used in a variety of infections (e.g. diabetic foot, intra-abdominal and complicated urinary tract infections).

There is a strong association between quinolone resistance and ESBL production, therefore the quinolones should not be first-line therapy for infections with organisms suspected to produce ESBLs. Quinolones would be reserved to treat urinary tract infections caused by ESBL-producers that have documented quinolone susceptibility. Nitrofurantion and fosfomycin may also be alternatives for uncomplicated urinary tract infections. Aminoglycosides are not recommended as monotherapy for serious infections, and no clinical data exist favouring routine use of combination therapy for infections with ESBL-producing organisms. Tigecycline, a recently approved tetracycline derivative, shows excellent *in-vitro* activity against ESBL-producers but published clinical data are

lacking. Colistin and polymyxin B may be the antibiotics of last resort against panresistant ESBL-producing enterobacteria. Treatment of colonised patients is usually not recommended.
In a nosocomial outbreak situation polymyxin B, neomycin plus nalidixic acid, and colistin plus tobramycin have been successfully used for decolonisation of the gastrointestinal tract.

THE MICROBIAL CONTAMINATION OF DRIED PAPRIKA BERRIES

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Paprika is the dried, ground pods of *Capsicum annum* L. Its quality is mainly determined by its seasoning power and natural colour. Paprika is the most distinctive element of Hungarian cuisine, and the average consumption in Hungary is 3 times higher than in the rest of Europe. Foreign products are often used to improve the colour of the Hungarian red spice paprika powders. The relatively high microbial contamination of the paprika powder (total microbial count, involving high spore count and mould contamination) and the potential to contain mycotoxins underlines the importance of the investigation of the microbial status of the production chain from the field to the table.
The microbiological investigations involve the whole chain of production from the field to the final product. Here the results of microbial contamination of dried paprika berries stored in one of the paprika producer factory are presented.
Sixty dried paprika berry samples of different origin (56 samples were grown at different places in 2004-2006 in Hungary; 1 sample originated from South-Africa, 1 sample from Peru and 2 samples from Brazil), were investigated after grinding for their microbiological quality (mesophilic aerobic total count, mould and yeast, coliforms and *Escherichia coli, Salmonella* sp. and *Listeria* sp.). The water activity of the powders was also measured. To study the overall mould contamination, the ergosterol content of the samples was also measured by HPLC method and compared to the plate count results.
The mesophilic aerobic total counts of the samples were between 10^3 and 10^8 CFU.g^{-1}, the fungal and yeast counts also varied greatly between <10 and 10^5 CFU.g^{-1}. The coliform counts were 10^1 - 10^5 CFU.g^{-1} and the *E. coli* counts were <10 - 10^3 CFU.g^{-1}. The samples could not be grouped by their microbial status. Paprika sample from Brazil had higher mould contamination and higher water activity than the Hungarian samples. The water activity values of the paprika powder samples were low enough to inhibit the growth of the moulds (a_w 0.233 – 0.561). There was no correlation between the mould counts and the ergosterol content, indicating that the overall contaminations (including field and storage contamination and fungal growth) of the samples were different.
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ANALYSIS OF HISTONE H3 AND H4 ACETYLATION AND HISTONE H3-K4 METHYLATION AT THE LATENT EBV PROMOTER LMP-1

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Epstein-Barr virus (EBV) is a ubiquitous human gammaherpesvirus that is associated with numerous malignancies. EBV latent membrane protein 1 (LMP-1) is essential for the EBV-induced immortalization of B lymphocytes *in-vitro*. To understand the host cell dependent expression of the *LMP-1* gene, it is important to analyse the regulatory mechanisms of the *LMP-1* promoter (LMP-1p). Because histone acetylation and histone H3-K4 methylation regulates gene expression through opening the chromatin structure for the transcription, we examined the acetylation state of histones and the level of histone H3-K4 methylation on well characterised cell lines of type I, II and type III latency carrying strictly latent EBV genomes with the method of chromatin immunoprecipitation (ChIP) assay combined with real-time PCR. These ChIP results showed that the active LMP-1p contained more acetylated and H3-K4 methylated histones than the inactive ones.

ANTIFUNGAL ACTIVITY OF LEVOMENTHOL, 2-PHENYLETHANOL, THYMOL AND HYDROQUINONE

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Many biological effects have been documented for simply aromatic (phenolics) and cyclic alcohols of natural origin. The antimicrobial effects of these low-molecular-weight plant metabolites could be promising agents against superficial mycoses of man and animals when used alone or in combination with synthetic antimycotics. For this purpose, the aim of this study was to investigate *in-vitro* antifungal activity of levomenthol, 2-phenylethanol, thymol and hydroquinone against clinical isolates of *Candida albicans*, *Microsporum gypseum* and *Trichophyton mentagrophytes* (N=5 per species). Broth microdilution method was performed according to CLSI (ex NCCLS) M38-P guidelines for determination of minimal inhibitory concentrations (MIC). Yeasts and dermatophytes were sensitive to all investigated alcohols with MIC values below or equal 5.625 $mg.mL^{-1}$. The lowest MIC values demonstrated aromatic alcohols, thymol and hydroquinone against *M. gypseum* (MIC 0.061 $mg.mL^{-1}$, MIC 0.049 $mg.mL^{-1}$, respectively) and *C. albicans* (MIC 1.719 $mg.mL^{-1}$, MIC 1.563 $mg.mL^{-1}$, respectively) strains. Hydroquinone and 2-phenylethanol showed the lowest MIC values against *T. mentagrophytes* strains with MIC values 0.488 $mg.mL^{-1}$ and 0.821 $mg.mL^{-1}$. When chemical structure was compared with antifungal activity, aromatic alcohols with one or two hydroxyl groups, such as thymol and hydroquinone showed the strongest activity against *C. albicans* strains. Dermatophytes sensitivity was species-depended. The strongest activity was observed using thymol, hydroquinine and levomenthol against *M. gypseum*, and thymol, hydroquinone and 2-phenylethanol against *T. mentagrophytes* strains. In conclusion, the causative agents of superficial mycoses are more sensitive to thymol and hydroquinone. They showed significantly lower MIC values than aromatic 2-phenylethanol and cyclic levomenthol. Double hydroxyl group in molecule of these investigated natural alcohols enhances antifungal activity.

CHARACTERIZATION OF CCW7P CELL WALL PROTEIN OF FILM-FORMING *SACCHAROMYCES CEREVISIAE* WINE YEAST STRAINS AT GENE AND PROTEIN LEVEL

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Aging of the Hungarian botrytized wine *Tokaji szamorodni* results aromatic compounds produced as by-products of the oxidative metabolism of yeasts that are forming a biofilm at the surface of wines. Film-forming strains show a planktonic growth during the main fermentation phase but the cells rise to the surface afterward and form a multilayered biofilm on it. The cells metabolize mainly ethanol and glycerol during maturation and produce characteristic aromatic compounds like acetaldehyde. However, the mechanism of attachment and the role of cell surface molecules in film formation are not clarified at all.

Our aim was to determine the possible role of certain cell wall proteins in film formation, therefore we looked for differences in the cell wall proteins of sedimenting and film-forming *Saccharomyces cerevisiae* strains.

We found different protein patterns among the alkali extracted cell wall proteins of laboratory and film-forming *Saccharomyces cerevisiae* strains. In the case of film-forming strains a smaller ccw7 protein was observed and isolated. For further investigation we designed a specific primer pair for the amplification of the Ccw7p encoding gene *HSP150*. PCR amplification of the gene confirmed the size difference between the ccw7 proteins of the laboratory and film-forming strains. Almost all the film-forming isolates generated a single PCR product of the same size but two of them contained additional amplicons, a shorter or a longer one. Sequence analysis of the amplified common PCR products of film-forming yeasts revealed two deletions in the internal repetitive region of the gene. In the case of TD04 strain that has an additional longer PCR amplicon we induced meiotic segregation aiming to check whether there is any correlation between the film forming ability and the *CCW7* alleles. The alleles segregated during meiosis, however, both types of the segregants maintained the film forming ability. *CCW7* amplicons of sedimenting wine yeast strains belonged to six PCR profiles due to the combination of five amplicons of different lengths. By the use of RFLP analysis of the *CCW7* amplicons we found that majority of film forming yeasts formed a separate cluster of 100% similarity, while the sedimenting wine yeast strains showed an extended length polymorphism.

TREATMENT OF FIRE BLIGHT BY BACTERIOPHAGES

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Fire blight, caused by *Erwinia amylovora*, is a serious disease of various plants, including the agriculturally important fruits like apples. This destructive bacterial disease kills blossoms, shoots, limbs and sometimes entire trees. Control of diseased wood means removal pruning, but till now there is no cure or prevention of fireblight is known. Aim of this project was to develop an effective new method against *E. amylovora* causing fire blight of apple plants. The new virus-based product contains several both genetically and morphologically characterized phage strains. Altogether we have isolated 72 phage plaques. Purification of the plaques was followed by morphological characterisation by electron microscopy. Genetic characterisation of the bacteriophage strains was carried out by RFLP and PCR. 26 distinct phage strains could be distinguished. Aim of the genetic

characterisation was also to test our strains on the presence of antibiotic resistance genes in the viral genomes and by this to rule out the occurrence of possible horizontal transfer of antibiotic genes. Our phage products were tested in laboratory against 6 strains of *E. amylovora* isolated from different firebligt cases. Beside laboratory testing we also treated fire blight infected apple-plantation by our phage product. Phage-resistance experiments were also carried out during the project to get a hint about the frequency of spontaneous resistance among *E. amylovora* strains against phages.

CHANGES IN THE SEROPREVALENCE OF BOVINE VIRAL DIARRHOEA VIRUS (BVDV) IN HUNGARY

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Bovine viral diarrhoea virus (BVDV) is a highly important pathogen of cattle herds. Besides direct losses caused by diarrhoea (reflected in the name of the disease), fetopathies, immunosuppression, and mucosal disease (MD) caused by cytopathic strains (cpBVDV), the non cytopathic variants of the virus (ncpBVDV) is able to cause persistent infection (PI). To minimize the economic losses, vacciantion by live, non virulent or inactivated vaccines is used. Many authors indicated however, that live vaccines, mostly produced from the less virulent ncpBVDV strains, can spread in the herds, hence recently only inactivated vaccines are used. Besides that, to reduce the economic impact of the infection, eradication programmes were launched in many countries of Europe. To join this campaign may be essential in the future.

In Hungary the last surveys were made several years ago, when the attenuated vaccine was widely used, hence investigations providing new data on the prevalence of the infection and serve as a basis for later monitoring were needed to estimate the present status. In our project 762 samples were collected from 30 farms. At least 15 animals per farm were tested using commercial ELISA kits (IDEXX Herdcheck), ages varied between 6 months and 6 years. Surprisingly the ratio of seropositive samples was much lower than it was previously estimated, and we found many naturally BVDV-free farms in the country. These farms could serve as sources of BVDV free animals when and if an eradication process will start.

Investigating the samples, we found that 329 samples were seropositive (43,2%) and 29 gave dubious results in the tests (3,8%), while 404 sera were negative (53.0%). From the 30 farms sending samples for investigation in case of 10 (30%) we could not detect seropositivity among the samples. This is in inconsistence with previous results that stated that the prevalence of BVDV is above 80% in Hungary. One explanation of the drop may be the ban on using live, attenuated vaccines, which ceased the spreading of the vaccine strain in the herds. This theory is supported by the decreasing number of MD cases. The prevalence of ncpBVDV strains hence occurrence of PI calves dropped, therefore superinfection of animals by the cpBVD type became rarer. An other reason can be the reduction of the cattle stock number in Hungary, which reduces the chances of the spreading of the wild BVDV strains.

ASPERGILLUS TAMARII KERATITIS

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Aspergillus strains are among the most common organisms causing fungal keratitis in the case of rural agricultural workers in South-India. Certain *Aspergillus* species, mainly *A. flavus*, *A. terreus*, *A. fumigatus* and *A. niger* have long been regarded as important pathogens in eye infections, especially keratitis. Most of the *Aspergillus* strains isolated from keratomycosis are being identified and reported at the genus level only. Their molecular identification at the species level would be of great importance, as the pathogenic potential may vary between different species of the genus.

A 32-year-old female was presented to the AravindEyeHospital, Coimbatore, India with complaints of pain, redness and defective vision in the left eye after an ocular injury by an iron piece. Anterior segment examination of the left eye showed lid oedema and conjunctival congestion. The cornea showed a central ulcer with an anterior mid stromal infiltrate with feathery edges and surrounding oedema. Topical antifungal therapy was started with 5% natamycin suspension and 2% econazole drops along with 1% homatropine. When reviewed after 3 days, the anterior chamber showed a hypopyon. The patient was admitted as inpatient and advised to continue the same medications along with 200 mg oral ketoconazole and 0.2% subconjunctival fluconazole. During the next three weeks, the infiltrate reduced gradually and the anterior segment inflammations subsided. The patient was advised best glasses and to report for review after 6 months.

On the basis of the macroscopic features, micromorphology and the results of ITS-, β-tubulin- and calmodulin sequence analysis, the strain was identified as *A. tamarii,* a member of *Aspergillus* section *Flavi*. The detected antifungal susceptibility values for itraconazole, ketokonazole, voriconazole, amphotericin B and natamycin were within the value ranges determined previously for a large number of *Aspergillus* strains isolated from corneal ulcers. The low MIC of ketoconazole correlated with the clinical outcome of the therapy.

A. tamarii is widely used in the food industry for the production of soy sauce, and in the fermentation industry for the production of various enzymes. Although *A. tamarii* is able to produce several toxic secondary metabolites including cyclopiazonic acid and fumigaclavines, it has rarely been encountered as a human pathogen. To our knowledge, the presented case of fungal keratitis is the first report on an ocular infection caused by *A. tamarii,* and the third known case world-wide with the involvement of this unusual opportunistic human pathogen.

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CLONING AND EXPRESSION OF CATECHOL 1,2 DIOXYGENASES FROM *RHODOCOCCUS GLOBERULUS* AK36 AND *R. RHODOCHROUS* AK40 STRAINS

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From the results of the parallel genome-projects on three *Rhodococcus* species it is obvious that these microbes encode huge number of different dioxygenases. These enzymes catalyze the incorporation of both atoms of molecular oxygen into aromatic substrates. Dioxygenases are classified into two groups on the base of their mode of action. Intradiol enzymes use a non-hem Fe (III) to cleave the aromatic ring between two hydroxyl groups (ortho-cleavage), whereas extradiol enzymes use a non-hem Fe (II) to cleave the aromatic ring between a hydroxylated carbon and an adjacent non-hydroxylated carbon (meta-cleavage). Enzymes that belong to the intradiol family are the catechol 1,2-dioxygenases (1,2-CTD) which play key roles in the degradation of aromatic pollutants by soil inhabiting rhodococci. The aim of this work was the molecular characterization of aromatic decomposer *Rhodococcus* strains originating from the Agruniver Holding Strain Collection. In particular we focused on their dioxygenase enzymes.

Based on data from the finished genome projects and the published sequences we constructed six primer pairs (A-F) to clone catechol 1,2 isoenzymes from our *Rhodococcus* strain collection. PCR reactions revealed that the strains have fairly polymorphic cathecol 1,2-dioxygenase profiles. We chose *R. globerulus* AK36 for cloning ctdA and *R. rhodochrous* for cloning *ctdD* genes, respectively, using the Invitrogen directional TOPO cloning kit. Sequence comparisons of the encoded proteins CtdA and CtdD revealed 92% and 95% homology values, respectively, for known catechol 1,2 dioxygenases, By PFAM structure analysis we could identify the catechol dioxygenase specific catalytic domain (alpha/beta C-terminal domain) and the connected beta N-terminal domain as well.

To characterize the gene products His-tagged fusion constructs were prepared and the recombinant proteins were expressed in *E. coli* BL21 DE3 strain. The expressed enzymes were purified by NiNTA affinity chromatography. Both enzymes showed significant activity against catechol substrate measured by the standard spectrophotometric method. The purified proteins were separated into two bands in native PAGE gel with apparent molecular masses of 130 and 33 kDa. The analysis of the high-molecular-weight form by denaturing SDS-PAGE resulted in the appearance of the minor, 33 kDa isoform. These data suggest that catechol 1,2-dioxygenase CtdA and CtdD are tetramers of identical subunits.

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GENOMIC INSIGHTS INTO THE ENZYME FACTORY *HYPOCREA JECORINA* (*TRICHODERMA REESEI*)

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Mutant strains of the ascomycete *Hypocrea jecorina* (anamorph: *Trichoderma reesei*) are used as industrial producers of cellulases, and are promising hosts for the production of heterologous proteins as well. Expression of the respective genes adaptive, i.e. induced by cellulose and some other products related to plant cell-wall degradation. Transduction of the signal from cellulose to cellulase expression is not known. The availability of the genome sequence of *H. jecorina* has now enabled an improved and in-depth investigation of the genetics and biochemistry of this process. In my talk, I will explain how the genome organization of *H. jecorina* has given rise to its potent cellulase production, and present recent data from my lab on the involvement of G-alpha proteins in the cellulase induction process. Finally, I will pinpoint an 80-kb (28 gene) deletion in the

hypercellulolytic strain "*T. reesei*" RUT C-30 and discuss its physiological implications for this strain.

THE HISTORY OF THE ERADICATION PROGRAM AGAINST SYLVATIC RABIES IN HUNGARY

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Rabies is a zoonotic viral disease, which causes acute encephalitis in domestic and wild mammalians. It is transmitted through close contact with saliva from infected animals (i.e. bites, scratches, lick on injured skin and mucous membranes). Once symptoms of the disease develop, rabies is fatal to both humans and animals. Rabies virus belongs to the genus Lyssavirus having non-segmented, negative-stranded RNA genome. The disease has three forms: urbane-, sylvatic- and bat-rabies. In Hungary, only the urbane rabies was present at the beginning of the 20th century. Rabies is subjected to an obligatory notification in Hungary since 1928. By the end of the thirties as a result of the implementation of the strict rules for dog keeping (keeping record on dogs) and the obligatory immunisation of dogs in each year, Hungary was the first country all over the world that became free of urbane rabies. After the II. World War the country for a short period lost its rabies free status. The sylvatic rabies was introduced into Hungary from the north in 1954 and until 1966 occurred only sporadically cases to the east of the Danube. In 1967 the disease spread also to Transdanubia and the whole country had become infected by the end of 1971. At the beginning the protection against sylvatic rabies was carried out through measures to decrease the number of foxes (extermination in burrows with phosgene), but it lead to poor results. The Hungary started experimentally the oral immunisation of foxes in 1992 and this program has been completing every year two times. Between autumn of 1992 and spring of 1996 the vaccination was trialled first at an area of 5.000 km^2 thereafter of 6.000 km^2, near the western border of Hungary. Between autumns of 1996 and 2000 the western part of the country (Transdanubia) was covered by baits. As a result by the end of 2000 the rabies almost disappeared from Transdanubia (only 5 cases). Between spring of 2001 and autumn of 2003 the territory between the river "Danube" and the river "Tisza" had been involved in the immunization campaigns, while in Transdanubia only emergency ring vaccinations were carried out, where positive cases were detected. Since 2004 the bait distribution has been extended over the whole country. The efficacy of the oral immunization of foxes can be demonstrated by considerable decrease of rabies incidence in the country. Between 1986 and 1995, in average 1104 cases were found in Hungary. In 2006 only 3 cases were found in the whole country.

THE BIOLOGICAL FEATURES OF A CLASSICAL SWINE FEVER VIRUS WITH WILD BOAR ORIGIN: OUTCOMES OF A TEST IN DOMESTIC PIGS

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Classical swine fever (CSF) is a fatal disease of pigs and wild boar (*Sus scrofa*). This latest play a significant role as a virus reservoir in the epidemiology of the disease. The aim of our scientific collaboration is to develop a marker vaccine against CSF for oral immunisation of wild boars. The vaccine candidate strain was subjected to animal experiment in order to be examined its biological features, like virulence, dissemination and kinetics of immune response compared to other virulent CSFV strains.

In this study the characterisation of CSFV strain isolated from wild boar recently (11722-WIL) was aimed. A group of 28 piglets was infected oro-nasally with $2x10^{5.0}$ TCID50 CSFV. The average weight of the animals was ca. 15 kg. Body temperature was daily observed, the clinical symptoms were scored. According to a previously established time-table blood samples were collected from the animals and two animals were exterminated on each given day. Pathological examinations were carried out on carcasses and organ specimens were collected.

The clinical manifestations of the disease and fever have been emerged from the 6. post-infection day (DPI), while from the 5. DPI leucopoenia was detected. The most frequently observed clinical symptoms were the follows: bleeding in lymph nodes, punctual haemorrhages in kidney and lobular pneumonia. The most typical histopathological lesions were detected from 2. DPI.

The viral antigens of CSFV were detectable by immunehistochemistry in tonsils from 1. DPI, while in submandibular lymph node and spleen from 2. DPI. The viral antigens were present in the kidney only from 17. DPI. Virus-specific antibodies were found by virus neutralisation probe and ELISA only in one animal after 17. DPI. According to the examinations the principal replication site is the tonsil. Beside tonsil the virus spread to submandibular and mesenteric lymph nodes, thymus, lungs, spleen and ileum earliest. The whole blood, sera, heart, muscles and kidney did not proved to be reliable to detect the virus. The 11722-WIL strain showed significant virulence in piglets.

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METHYLATION CASSETTE ASSAY TESTING FOR INACTIVATION OF HUMAN IL-10 PROXIMAL PROMOTER

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Locally elevated IL-10 production is commonly detected in HPV associated malignant and premalignant lesions of the uterine cervix. Although IL-10 is produced also in the human squamous epithelium, there is a lack of IL-10 production in human cell lines of epithelial origin at level of either transcription or translation. Previously, we could show by using bisulfite sequencing that the proximal part of human IL-10 promoter has a methylated profile in epithelial cell lines. In contrast, in IL-10 producing lymphoid cells this promoter region appeared unmethylated. In this work we tested the effect of CpG methylation on promoter function using cassette methylation of reporter constructs.

We performed the methylation cassette assay using reporter constructs containing 1082 bp and 617 bp fragments of human IL-10 proximal promoter. Transcriptional activity was monitored by measuring luciferase activity of transiently transfected HeLa cells.

The activities of IL-10 promoter-reporter constructs transiently transfected in HeLa cells were repressed by *in-vitro* cassette methylation. In the case of both reporter constructs luciferase activity was reduced 2 to 3 fold compared to the unmethylated constructs. Epithelial cells ensure all necessary

transcription factors for IL-10 promoter activation. Nevertheless, promoter CpG methylation might account for the consistent silencing of IL-10 expression in these cell lines.

MECHANISM OF ACTION OF THE ANTIFUNGAL PROTEIN, PAF PRODUCED BY *PENICILLIUM CHRYSOGENUM*

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The antifungal protein PAF is a small, basic, cysteine-rich protein secreted by the filamentous fungus *Penicillium chrysogenum*. PAF inhibits the growth of various important plant- and zoopathogenic filamentous fungi, e.g. *Botrytis cinerea*, *Fusarium oxysporum*, *Trichoderma koningii*, *Aspergillus fumigatus*, *Absidia* sp., *Rhizopus* sp. PAF induces morphological changes of the sensitive fungi: crippled, hyperbranched hyphae appear after PAF treatment [1]. PAF most likely interacts with specific receptor(s) in *A. nidulans*, which is accompanied by the active internalization of the protein and induces a heterotrimeric G-protein signalling pathway. In the presence of PAF K+ efflux takes place in sensitive fungi, such as A. nidulans. This phenomenon evokes the immediate hyperpolarisation of the plasma membrane of *A. nidulans* hyphae, which might be account for the morphological changes of the hyphal tips [2]. Exposure of *A. nidulans* to PAF results in an increased level of reactive oxygen species (ROS). ROS damages the mitochondria and induces mitoptosis, which was determined by transmission electron microscopy. The disintegration of mitochondria changes the basic physiological functions in the cell and induces programmed cell death. In the presence of PAF *A. nidulans* shows apoptosis-like phenotype. The increased exposure of phosphatydyl serine on the surface of PAF-treated protoplasts and the appearance of DNA strand breaks, are proved by Annexin V staining and TUNEL assay, respectively. Furthermore ultrastructural analysis of *A. nidulans* hyphae after PAF treatment reveals several cell injury: loss of cell wall granulity, shrinkage of the plasma membrane, microvesicle formation between the cell wall and the cell membrane named apoptotic blebbing and appearance of large vacuoles [3].
In virtue of these effects the antifungal protein PAF is a promising tool for further characterisation of apoptotic-like events in filamentous fungi.

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IN-VITRO MODELLING OF THE PREBIOTIC EFFECTS OF INULIN APPLIED AS BISCUIT ADDITIVE

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Inulins belong to a group of natural fructose-containing oligosaccharides, known as fructans. They are mainly comprised of 2 to 150 fructose units typically having a terminal glucose. While inulin cannot be digested in the human the upper gastrointestinal tract it reaches the colon and selectively stimulates the proliferation of beneficial colonic bacteria like *Bifidobacterium* ssp., *Lactobacillus* ssp. Several examinations justified this prebiotic effect, but we have less information about the changes in it as a consequence of the modified structured due to the high heat treatment.

The aim of our work was to assess the alterations in the utilization of inulin that were previously exposed to heat treatment of backing. Four intestinal bacteria (*Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Escherichia coli*, and *Enterococcus faecalis*) were tested for growth rate *in-vitro*. They were cultured in TPY medium amended with heat treated (140 - 190ºC) inulin as carbon source. Photometrical measurements were performed after 24 hours of 37ºC anaerobic cultivation. Contrary to *E. coli* and *E. faecalis*, *B. bifidum* preferred the non treated inulin much better than fructose and glucose used as control. However, the heat treatment of inulin significantly increased its utilization by all the test bacteria. The fastest proliferation was observed in 190ºC treatment of inulin. *B. bifidum* proliferated better at all heat treatments than the other bacteria.
The influence of heat treatment on the competition between intestinal bacteria and the facultative pathogen *Pseudomonas aeruginosa* was also studied in an equal proportion mixture of all the five microorganisms. The colony counts assessed on selective media after 48 hours of incubation showed that *P. aeruginosa* was almost outcompeted in medium containing heat treated inulin, whereas the non treated one did not result in reduction of *P. aeruginosa* population size.
Acknowledgement: The research was carried out in the framework of RET 09/2005 NKTH project.

TRENDS IN THE PREVALENCE RATE OF ESBL AND ACQUIRED METALLO-B-LACTAMASE PRODUCERS AMONG GRAM-NEGATIVE CLINICAL ISOLATES IN HUNGARY

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SHV-5 and SHV-2a genes were found to be dominant among extended-spectrum b-lactamase-producing Enterobacteriaceae strains in Hungary between 1998 and August 2003. In the majority of the isolates the *bla*SHV genes were on transferable plasmids of 94kb. During 2003 countrywide spread of a CTX-M-15-producing *Klebsiella pneumoniae* clone in Hungary was detected. The *bla* CTX-M-15 gene was located on a 137KB self-transmissible plasmid. In 2005 the number of infections caused by CTX-M-producing *K. pneumoniae* rose sharply in Hungary. In addition, a shift was demonstrated in the occurrence of CTX-M-producing *K. pneumoniae* clones. Three multidrug resistant clones were detected in 38 healthcare facilities causing large outbreaks and individual nosocomial infections. The first metallo-β-lactamase (MBL) producing *P. aeruginosa* clinical isolates in Hungary were characterised in 2003. Following this a monitoring system was set up that identified VIM metallo-β-lactamase-producing serotype O11 or O12 *Pseudomonas aeruginosa* isolates infecting or colonizing 17 patients from five hospitals between January and November 2005 in Hungary. Macrorestriction analysis revealed that VIM-4-producing *P. aeruginosa* isolates with a close clonal relatedness were recovered from hospitals in three different towns in North-West Hungary. Our studies indicate that serotype O11 and O12 multiresistant clones of *P. aeruginosa* play an important role in the dissemination of *bla*VIM through clonal spread but other mechanisms, such as horizontal transfer are also involved.

SENSITIVITY OF DIFFERENT ZYGOMYCETES TO THE SESTERTERPENE, OPHIOBOLIN A

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Ophiobolin A and its analogues are a group of sesterterpene type phytotoxins produced by the members of the genus *Bipolaris*. Several members of the class Zygomycetes are important as postharvest pathogens of agricultural products; *Rhizopus*, *Mucor* and *Gilbertella* species are among the most frequently isolated causative agents of fungal rots. Other representatives of this group are known to be opportunistic pathogens of humans and animals. These fungi have a substantial intrinsic resistance to most of the widely used antifungal drugs. In this study, effects of the ophiobolin A and B on selected Zygomycetes species were investigated.

A total of 17 fungal isolates representing 6 different genera (*Micromucor, Mortierella, Mucor, Rhizomucor, Rhizopus* and *Gilbertella*) were tested. The *in-vitro* antifungal activity of ophiobolins was determined with 96-well microtiter plate bioassay by measuring the absorbance of fungal cultures at 620 nm. For calculation of the inhibition rates, absorbance of the untreated control cultures was referred to 100% of growth, in each case. Each experiment was repeated three times.

The antifungal effect of the two ophiobolin compound was found to be diverse. Ophiobolin A showed higher antifungal activity against the tested isolates than ophiobolin B. *Mortierella wolfii* and *Micromucor ramanniana* proved to be the less sensitive to ophiobolins; ophiobolin A inhibited the germination of spores in the cases of both species at a concentration of 50 μg/ml. Ophiobolin A was very active against the other tested strains: it completely blocked the spore germination at concentrations of 3.2 - 12.5 $\mu g.ml^{-1}$, respectively.

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DERMAVIR PATCH VACCINE FOR THE TREATMENT OF HIV/AIDS

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Present antiretroviral treatment involves the combination of drugs targeting various stages of the virus life cycle, which enables suppression of viral replication. These drugs must be administered continuously to minimize the risk of the development of clinical AIDS or death. New medicines that capable to eliminate some of the HIV-infected cells might provide additional clinical benefit even if the interruption of antiretroviral drugs is instituted.

In HIV infected individuals we investigate a novel medicine, called DermaVir Patch that can expand the HIV-specific T cells to destroy HIV infected cells by cytotoxic killing. The active pharmaceutical in DermaVir is a plasmid DNA expressing most of the HIV genes. The DNA is formulated to a mannosylated nanoparticle and topically applied under a Patch using an experimental vaccine administration device. The expression of the DermaVir DNA in dendritic cells in the draining lymph node supports the induction of HIV-specific memory T cells with broad-specificity.

Preclinical studies demonstrated the safety and antiviral activity of DermaVir Patch treatment. In naïve macaques DermaVir induced potent virus-specific memory T cell responses. These animals controlled viremia after challenge with pathogenic viruses. In chronically infected macaques DermaVir provided immunologic, virologic and clinical benefit. In HAART treated macaques Patch treatment augmented virus-specific T cell responses and controlled viral load rebound during drug treatment interruptions. DermaVir Patch used as mono-therapy, improved survival of infected macaques. GLP toxicity studies demonstrated transient, mild local erythema associated with repeated topical vaccinations.

In the GIHU004 study HIV infected individuals were treated with different doses of DermaVir (0.1 – 0.8 mg DNA). The Patch treatment was well tolerated, there were no significant adverse events associated with the treatment. Repeated DermaVir Patch treatment studies are presently under way in the USA (ACTG5176) and Sweden (DermHivImm) including antiretroviral drug-treated individuals.
In conclusion: 1) Preclinical macaque studies demonstrated the feasibility of the expansion of virus-specific memory T cell pool during chronic infection. 2) Viral load control was associated with the expansion of memory T cells. 3) Preclinical and clinical use of DermaVir Patch suggest the safety and tolerability of this vaccine in HIV infected individuals. 4) Repeated DermaVir Patch treatment improved antiviral activity in the absence of additional toxicities. These results encourage further investigation of DermaVir Patch for the treatment of chronic HIV infection.

SOIL ALGAE AS POTENTIAL HYDROGEN PRODUCERS

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Nowadays due to the global warming and the predictable lack of fossil energy more and more agricultural product is used as source of clean, environmentally friendly and renewable energy. Algae including soil algae can be used as a new source of high level energy products like biodiesel or biogas that can be added to the agricultural energy products. Some algae can produce hydrogen under specific conditions via biophotolysis. The hydrogen production method is quite different in the Chlorophyta (eg. *Chlamydomonas* sp.) and the Cyanobacteria (eg. *Anabaena* sp.) cells as they use different enzymes for this process.
The hydrogen production depends on the biological capabilities like the size of antenne pigments, enzyme activity, oxygen tolerance of the hydrogenase and growth rate of the algae. External conditions like the sunlight intensity, temperature, composition of the media and the aeration also influence the hydrogen production. Due to the special needs of the hydrogen producer algae special photobioreactors are needed in which the photosynthesis and the process of the hydrogen production can be separated. The selection of strains is based on their productivity. The biomass production is measured by cell counting and turbidimetry. The hydrogen production is measured *in-vivo* and *in-vitro* with gas chromatography. *In-vitro* measurements show higher hydrogen productivity in case of a wild type *C. reinhardtii* strain (CC-125). There are sharp differences in the algae productivity depending on type of media. It can be established that the Sueoka medium [1] is one of the most effective media for green algae especially for *Chlamydomonas* cultures.
Considering the above mentioned potentials and limitations 7 *Chlamydomonas* strains are investigated in laboratory experiments to measure and optimize the hydrogen production potential. The present paper summarises the first preliminary result of these experiments.
1. Sueoka et al. 1960. *Proc. Nat. Acad. Sci. USA,* 46, 83.

A COMPARATIVE STUDY ON THE ANTIBIOTIC RESISTANCE OF MRSA AND MSSA STRAINS ISOLATED IN HUNGARY, AUSTRIA AND MACEDONIA

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The aim of the study was to compare the quantitative susceptibility of methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains of *Staphylococcus aureus* to antistaphylococcal agents. Antimicrobial sensitivity of 123 MSSA and 158 MRSA strains isolated in Hungary, 115 MSSA and 40 MRSA strains isolated in Austria, 35 MSSA and 72 MRSA strains isolated in Macedonia were tested. Identification of *S. aureus* strains was performed by classical and molecular methods (presence of catalase, clumping factor, *nucA* and 23S rDNA genes). The *mecA* gene was detected by polymerase chain reaction (PCR). Minimum inhibitory concentrations (MICs) of antibiotics were determined by broth microdilution method according to NCCLS/CLSI recommendations. PFGE analysis of the strains is in progress. All tested strains were sensitive to vancomycin. The majority of Hungarian and Austrian MRSA strains were sensitive to amikacin, while 70.8% of Macedonian strains were resistant. Resistance of Austrian and Macedonian MRSA strains to gentamicin exceeded 90%, Hungarian MRSA strains were gentamicin resistant in 73.7%. To clindamycin 2.5% of the Hungarian MRSA strains and 8.3% of the Macedonian MRSA strains proved to be sensitive. To clarithromycin, ciprofloxacin, levofloxacin and moxifloxacin more than 90% of MRSA strains were resistant, except for moxifloxacin, to which the percentage of the resistant strains from Macedonia was slightly lower (88.6%). All tested MRSA strains were multidrug resistant. The most frequent resistance phenotype of Hungarian and Austrian strains was the resistance to gentamicin, clindamycin, clarythromycin and to fluoroquinolones. The most common phenotype of Macedonian strains was the resistance to these antibiotics and amikacin in addition. We can state that MSSA strains were mainly sensitive to aminoglycosides, however the susceptibility rates for amikacin proved to slightly lower among Austrian and Hungarian strains. The resistance to clindamycin and clarithromycin was about 25-30% among the Austrian and Hungarian MSSA strains, while the Macedonian strains were resistant only in 9%. MSSA strains were sensitive to all 3 tested fluoroquinolones (75-95%), Hungarian strains were more sensitive than the others. Moxifloxacin is still the most effective fluoroquinolone.

Resistance rates and degrees of MRSA strains to a variety of antimicrobials were significantly higher than those of the MSSA strains. Therapeutic options differ according to countries. In MSSA infections all antistaphylococcal drugs except for penicillin and clindamycin can be used, while in MRSA infections for empiric therapy only vancomycin and teicoplanin is recommended. The susceptibility rates and resistance patterns could correlate with the antibiotic usage in the examined countries.

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A CASE OF KERATITIS DUE TO *NEOCOSMOSPORA VASINFECTA* IN COIMBATORE, SOUTH INDIA

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We report the first case of an ocular infection caused by *Neocosmospora vasinfecta* in an immunocompetent patient in South India. The patient did not recall any trauma or injury to the eye, but might have had trivial injury during the course of his duties as a farmer. Corneal scrapings revealed fungal filaments on direct microscopy, and the initial isolate of the fungus was identified as a *Fusarium* species. Further mycological and molecular examination resulted in the reidentification of the fungus as *N. vasinfecta,* a Hypocrealean fungus not hitherto reported from ocular infections. Given the rarity of *N. vasinfecta* as a human pathogen, we did not consider this fungus as an etiological factor at first, therefore antifungal drugs with documented activity against *Fusarium* were administered. Natamycin (5% suspension) and 2% econazole half hourly, combined with 1% homatropine (three times daily) proved to be unsuccessful. Despite further 52 days of intensive therapy including systemic ketoconazole (200 mg twice a day), a full thickness therapeutic keratoplasty had to be performed. Post-operative treatment with amphotrericin B (0.15 $mg.ml^{-1}$) and 1% clotrimazole drops half hourly combined with 2% cyclosporine twice a day resulted in a complete cure. The presented data demonstrate that the correct identification of the aetiological agent is of great importance in order to administer the correct treatment in cases of corneal ulcers that can be caused by various fungi.

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ASPERGILLUS KERATITIS: EPIDEMIOLOGICAL FEATURES, MOLECULAR IDENTIFICATION, ANTIFUNGAL SUSCEPTIBILITY AND CLINICAL OUTCOME AT THE ARAVIND EYE HOSPITAL IN SOUTH INDIA

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The objectives of this study were to analyze the epidemiological features of *Aspergillus* keratitis in South India, to perform morphology-based and molecular identification of *Aspergillus* strains isolated from keratomycosis patients, to determine the antifungal susceptibilities of the isolates and to study their genetic diversity.

26 *Aspergillus* strains isolated from keratomycosis in the Aravind Eye Hospital between August 2005 and February 2006 were involved in this study. Epidemiological data were recorded for the patients. Morphological examination was performed by microscopy, culture characteristics were studied on malt extract agar. Molecular identification was carried out by sequence analysis of the ITS region. Antifungal susceptibilities were determined by the E-test method modified for moulds.

The 26 patients included 17 males and 9 females. Rural, semiurban and urban populations were represented among the patients with 23, 50 and 27%, respectively. Farmer was the most frequent occupation among the male patients, while most of the female patients were housewives. Corneal trauma was reported as the potential predisposing condition of the infection for 57.7% of the patients, the traumatizing agents were dust or iron particles, insects or oil and mustard seeds. Among the further possible predisposing conditions, systemic diseases like diabetes mellitus and hypertension proved to be frequent. The antifungal drugs applied for the therapy of patients included natamycin, itraconazole, ketoconazole, econazole, clotrimazole and amphotericin B. Therapeutic keratoplasty

was performed in the case of 9 patients. Most of the isolates proved to belong to the species *A. flavus*, however, other species of the genus including *A. terreus*, *A. fumigatus* and A. *tamarii* were also represented in the sample. The initial identification of the isolates based on conidial- and colony morphology could be confirmed by ITS sequence analysis. All of the examined strains proved to be resistant to fluconazole with MIC values above 256 μg/ml. MIC-ranges of the other examined antifungal drugs were in the following ranges ($\mu g.ml^{-1}$): 0.064 - 4 for amphotericin B, 0.25 - 1 for ketoconazole, 0.064 - 32 for itraconazole and 0.064 - 1 for voriconazole.

Our data provide important information on the current incidence of *Aspergillus* species in corneal ulcers in South-India, as well as useful ideas for the selection of the appropriate antifungal agents.

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ENVIRONMENTAL IMPACT OF SOIL POLLUTION WITH TOXIC ELEMENTS

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Toxic elements from the Pb/Zn mine at Gyöngyösoroszi (North-East-Hungary) cause pollution and potential risk for the ecosystem. The aim of this work was to determine the contamination level at two sites planted with *Salix* sp. and *Zea mays* L. along Toka valley, to evaluate soil quality by biological and biochemical parameters and to assess potential risk for human health.

Total As, Cd, Cu, Pb, Zn, Hg contents in polluted soils reached values up to 384, 28.8, 493, 2827, 4417, 4.46 $mg.kg^{-1}$, respectively. These values were 11 - 67 times higher than the local background levels. All measured parameters therefore indicated soil pollution: plant emergence %, plant and microbial biomass decreased while water extractable organic carbon, acid phosphatase activity and maize root infection by AM fungi and toxic metal content of the tested plants increased significantly. The results showed a high soil pollution level and consequently a great potential risk for human health and indicate the necessity for separate procedure (remediation) of the observed site.

THE MULTIFUNCTIONAL LATENCY ASSOCIATED NUCLEAR ANTIGEN AND VIRAL INTERLEUKIN 6 OF

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Human herpesvirus 8/Kaposi's sarcoma associated herpesvirus (HHV-8/KSHV) is a large double stranded DNA virus that has been implicated in Kaposi's sarcoma and several lymphoproliferative disorders. Similar to other herpesviruses, KSHV establishes a latent state that persists for the life of the host. During latency, little or no virus is present, and the viral genome is maintained as a multi-copy circular episome. Few viral genes are expressed during latency, and these are involved in maintenance of the episome and modulation of the host cell environment, making it permissive for latency. One of the viral proteins expressed during latency is the latency associated nuclear antigen 1 (LANA1). LANA1 is a sequence-specific DNA binding protein, expressed in all latently infected

cells, and has been shown to be critical in establishment and maintenance of latency. Furthermore, LANA1 may also play a role in oncogenic transformation of lymphocytes. Viral interleukin-6 (vIL-6) is a homolog of cellular IL-6 that is encoded by the KSHV genome. vIL-6 binds to the IL-6 signal transducer gp130 without the cooperation of the IL-6 high affinity receptor to induce STAT3 DNA binding and cell proliferation. vIL-6 is believed to be important in the pathogenesis of KSHV-induced diseases and our recent study shows that vIL-6 is a transforming protein.
The goal of this review to discuss these multiple functions of LANA1 and vIL-6.

VESICULAR STOMATITIS VIRUS INDUCES APOPTOSIS ASSOCIATED WITH DECREASED BCL-2 AND INCREASED P18 BAX LEVELS IN THE WONG-KILBOURNE DERIVATIVE OF THE CHANG CONJUNCTIVAL CELL LINE

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Virotherapy represents a novel therapeutic modality for the treatment of malignant tumors. *Vesicular stomatitis virus* (VSV), a member of the *Vesiculovirus* genus of the *Rhabdoviridae* family, has been shown to exert antitumor effects in several tumor types.
Since the potential oncolytic activity of VSV has not yet been evaluated in epithelial tumors of the conjunctiva, we set out to investigate the susceptibility of the immortalized Wong-Kilbourne derivative of the Chang conjunctival (WK) cell line to VSV, and analyzed the role of apoptosis in the VSV-mediated induction of cell death. Furthermore, in order to gain an insight into the underlying molecular mechanisms implicated in the apoptogenic properties of this virus, we also determined the effects of VSV infection on the levels of the Bcl-2 and Bax proteins.
WK cells were infected with VSV at various multiplicities and maintained for different periods of time. VSV-infected cells were analyzed by inverted microscopy for the development of a cytopathic effect (CPE), and virus replication was measured by indirect immunofluorescence assay, Western blot analysis and plaque titration. Our results demonstrated that the WK cell line was highly permissive to VSV replication and was highly susceptible to the CPE of this virus. The results of ELISA for detection of the enrichment of nucleosomes in the cytoplasm of apoptotic cells revealed that VSV infection elicits the apoptotic death of WK cells. Western blot analyses showed that the mock-infected cells exhibited endogenous expression of Bcl-2 and p21 Bax. VSV infection caused a significant decrease in the expression level of Bcl-2. Moreover, in parallel with a slight decrease in the level of p21 Bax, p18 Bax accumulated in VSV-infected WK cells. Together, these data demonstrate that VSV is a powerful inducer of apoptosis in immortalized WK cells. The VSV-mediated alterations in the expressions of Bcl-2 and Bax may play important roles in the apoptotic responses of infected cells and may also sensitize to other apoptotic stimuli. Our findings suggest that this virus may possess oncolytic activity in epithelial tumors of the conjunctiva.
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THE HERPES SIMPLEX VIRUS-INDUCED DEMISE OF KERATINOCYTES IS ASSOCIATED WITH A DYSREGULATED PATTERN OF P63 EXPRESSION

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The cell demise elicited by *Herpes simplex virus* type 1 (HSV-1) contributes to the development of organ-specific dysfunctions observed during infections. HSV-1 can trigger several forms of cell death, including necrosis, apoptosis and anoikis. The cytopathic effect (CPE) of HSV-1 is cell-type specific and can be classified into three groups. HSV-1 triggers apoptotic demise in myeloid and lymphoid tissues. Epithelial cells, in which HSV-1 causes lytic infection, die primarily by way of necrosis, and apoptosis plays only a minor role. Finally, in persistently infected tissues in the central nervous system, the inhibition of apoptosis predominates and maintains cell survival. Since the underlying mechanisms involved in the cytopathogenicity of HSV-1 have not yet been fully defined, we set out to investigate the susceptibilities of the HaCaT keratinocyte cell line and primary keratinocytes to HSV-1, and analyzed the role of apoptosis in the HSV-1-mediated induction of cell death. We also determined the effects of HSV-1 infection on the expression levels of p63, p53 and Bax proteins. The keratinocytes were infected with HSV-1 at various multiplicities and maintained for different periods of time. HSV-1-infected cells were analyzed by inverted microscopy for the development of a CPE, and virus replication was measured by indirect immunofluorescence assay, Western blot analysis and plaque titration. Our results demonstrated that the HaCaT cell line and the primary keratinocytes were permissive to HSV-1 replication and were highly susceptible to the CPE of this virus. The results of ELISA for detection of the enrichment of nucleosomes in the cytoplasm of apoptotic cells revealed that HSV-1 infection elicits low levels of apoptotic death in keratinocytes. Western blot analyses showed that the mock-infected HaCaT cells displayed the endogenous expression of DNp63a, mutant p53 (p53mt) and p21 Bax. The levels of DNp63a and p53mt were decreased; p21 Bax remained unaffected, while the expressions of the p26 Bax and p63 isoforms migrating near 51, 53, 57 and 62 kDa were highly increased in the HSV-1-infected HaCaT cells. The mock-infected primary keratinocytes displayed the endogenous expression of DNp63a, wild-type p53 (p53wt) and p21 Bax. The level of DNp63a was decreased; p21 Bax and p53wt remained unaffected, while the expressions of the p26 Bax and p63 isoforms migrating near 51, 53, 57 and 62 kDa were highly increased in the HSV-1-infected primary keratinocytes. Together, these data demonstrate that HSV-1 infection alters the stoichiometric ratios of the p63 and Bax isoforms. The dysregulated pattern of p63 expression observed in HSV-1-infected keratinocytes may represent a mechanism by which HSV-1 perturbs the functions of keratinocytes and leads to their demise.

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TRANSCRIPTIONAL REGULATION IN *SCHIZOSACCHAROMYCES POMBE* CELLS

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Trancriptional regulation of the polymerase II dependent genes requires components of the Mediator complex. This complex acts as both a positive and a negative regulator. Its subunit composition in *S. cerevisiae* has been studied in detail [1,2]. Most subunits can also be found in higher organisms, and they are evolutionally conserved. They have probably similar functions, but the exact role of the subunits is not clear. Our aim was to get more information about two subunits (sep10/Med31 and sep15/Med8) of the *S. pombe* Mediator complex.

The genome-wide gene expression profiling of sep10 and sep15 mutants revealed, that large, partially overlapping sets of genes of diverse biological functions are dependent on the two subunits. These genes are scattered over the entire genome. Sep10 and sep15 proteins are also involved in the transcription of transposons and genes functioning in sexual differentiation and energy household. Six cell separation genes, which are regulated by the Sep1-Ace2 transcriptional regulators, are also among the target genes. In both mutants, the repressed genes show significant overlaps with genes repressed in mutants defective in *rpb4+*. We also report the cloning and sequencing of the ts allele *sep15-598*. It contains a point mutation at the 3' end of the intron that impairs the efficiency of splicing. The *sep15-598* mutation is synthetically lethal with *sep10::ura4+*.

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FREQUENT INTRAPATIENT RECOMBINATION BETWEEN HIV-1 R5 AND X4 ENVELOPES: IMPLICATIONS FOR CORECEPTOR SWITCH

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Emergence of HIV-1 populations that switch or broaden coreceptor usage from CCR5 to CXCR4 is intimately coupled to CD4+ cell depletion and disease progression towards AIDS. To better understand the molecular mechanisms involved in the coreceptor switch, we determined the nucleotide sequence of 253 V1-V3 env clones from 27 sequential HIV-1 subtype B isolates from 4 patients with virus populations that switch coreceptor usage. Coreceptor usage of clones from dualtropic R5X4 isolates was characterized experimentally. Sequence analysis revealed that 9% of the clones from CXCR4-using isolates had originated by recombination events between R5 and X4 viruses. The majority (73%) of the recombinants used CXCR4. Furthermore, coreceptor usage of the recombinants was determined by a small region of the envelope, including V3. This is the first report demonstrating that intrapatient recombinations between viruses with distinct coreceptor usage occur frequently. It has been proposed that X4 viruses are more easily suppressed by the immune system than R5 viruses. We hypothesize that recombination between circulating R5 viruses and X4 viruses can result in chimeric viruses with the potential to both evade the immune system and infect CXCR4 expressing cells. The broadening in cell tropism of the viral population to include CXCR4 expressing cells would gradually impair the immune system and eventually allow the X4 population to expand. In conclusion, intrapatient recombinations between viruses with distinct coreceptor usage may contribute to the emergence of X4 viruses in later stages of infection.

MICROBIAL CONTAMINATION OF CELL CULTURES: A 2 YEARS STUDY

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Cell line contamination is a major drawback of main cell banks of the world and it has cost of losing important biological products or valuable research. The causative „agents" are different chemicals, invertebrates, bacteria, fungi, parasites, viral species and even other cell lines. In this retrospective study, cell lines from various species such as human, fish, insect, animals either offered or accessed through usual official accession in CGBRI were studied during 2 years (2002-2004) to detect their microbial contaminations and the causative organisms. Samples were taken for sterility test upon cell lines receipt and upon each cell line sub-culture. Samples were examined for bacterial (including mycoplasmas) and fungal contamination using conventional microbiological techniques. The study excluded parasites, viruses and other contaminating agents. This study revealed 39% of specimens were contaminated. The major contaminating agents were mycoplasmas (19%) followed by mixed infection (8%), fungi (8%) and bacteria (4%). Among various bacterial species (except mycoplasmas) *Bacillus* sp., *Enterococcus* sp. and *Staphylococcus* sp. are main agents and among various fungi *Aspergillus* sp. followed by *Penicillium* sp., *Sepedonium* sp. and *Botrytis* sp. were main fungal causative agents of CGBRI cell line contamination. Our study also delineates each cell line contamination rate and its causative agents. This is the first report of cell culture contamination from cell banks of Middle-East countries like Iran.

BACTERIAL COMMUNITIES OF THERMAL-KARSTIC SPRINGS OF SOUTH TRANSDANUBIUM

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Microbial communities from South-western Hungarian thermal-karstic springs located near Harkány were investigated by cultivation independent methods. Microscopic investigations of the pellet from the hydrogen-sulfide containing spring confirmed the presence of filamentous bacteria. The filaments were *Thiothrix*-like with variable length, contained septa between individual cells and deposited sulphur granules. A 16S rDNA based clone library was also constructed and Thiothrix specific primers were used to amplify the DNA extracted from the pellet. More than 140 clones were grouped by ARDRA and 21 representatives with different electrophoretic patterns were sequenced. The identified species were *Thiothrix fructosivorans*, *Thiothrix unzii*, *Thiohalomonas denitrificans*, *Methylomonas rubra*, *Leptothrix cholodnii*, *Dechloromonas* sp. Other clones showed high sequence similarities to hitherto uncultured *Thiobacillus* and *Nitrosospira* species. The detected mainly chemolitotrophic sulphur-oxidizing bacteria may play important role in the sulphuric cycle of the thermal baths of South Transdanubium.

COMPARISON OF PATHOGENIC PROPERTIES OF FIVE MURID HERPESVIRUS 4 (MUHV 4) ISOLATES POSSESSING DNA DELETIONS AS RELATED TO THE PROTOTYPE MOUSE HERPESVIRUS (MHV-68) GENOME

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The lymphotropic MuHV 4 infection in mice serves as animal model for studying human gammaherpesvirus infections. The prototype strain MHV-68 along with isolates MHV-60, MHV-72, MHV-76 and MHV-Sum belong to the MuHV 4 species, a member of *Rhadinovirus* genus of the subfamily Gammaherpesvirinae. The left 5´-end of the MHV-68 genome encodes several virus-specific genes (*M1*, *M2*, *M3* and *M4*) and is the site of transcription of 8 small tRNA molecules. Deletions of the corresponding ORFs allowed to follow the role of above mentioned genes for virus replication *in-vitro* as well as for their pathogenesis *in-vivo*. The growth in cell cultures of recombinant virus mutants, which had artificial deletions within the 5´-end region, was not hampered. All mutants in question were able to infect Balb/c mice, but had varying impact on virus load at acute infection, on the establishment of latency and/or on the rate of reactivation. The most interesting results in this respect are coming from strain MHV-76, which has a natural 9.53 kbp deletion at the 5´-end of the MHV-68 genome [1]. During a 27-month follow up of Balb/c mice, which were infected with MHV-76, neither tumors nor leukemia-like syndrome were observed. This is in contrast with oncogenic strains such as MHV-68 and/or MHV-72; during latency when established with the latter, lymphoproliferative disorders, lymphomas and even sarcomas developed at a frequency of 7 to 22%. The MHV-76 strain was eliminated from lungs already on day 10 p.i., but still spread to many organs (spleen, liver, kidney, thymus, bone marrow and brain). Viremia was accompanied with mild elevation of leukocyte counts (only a few atypical mononuclear cells appeared in blood smears) and with no significant splenomegaly. The titer of virus neutralizing antibodies was low, although the MHV antigen as well as the infectious virus were detected at chronic intervals (ranging from 2 to 27 months) in all tested organs with the exception of lungs. Surprisingly, MHV-76 was found in the liver, brain and intestinal wall; in the latter it was especially abundant at 27 months p.i. We hypothesize that the M4 gene, preserved in all oncogenic MHV strains, might be related (directly or indirectly) to their transforming properties.

1. Macrae et al. 2001. J. Virol. 75, 5315.

A STUDY ON CHROMOSOMAL INTERACTIONS AND SOMATIC PAIRING IN DIPLOID *SCHIZOSACCHAROMYCES POMBE* CELLS

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Several cytological and genetic studies investigate the positioning and interaction of chromosomes in different cell types. Homologous chromosome pairing in meiotic prophase has been demonstrated and examined in a number of model organisms, but the mechanism by which homologous chromosomes recognize each other is still largely unknown. Outside the meiotic program the

association of homologous chromosomes is investigated in premeiotic and somatic cells. Pairing in premeiotic cells has been detected in plants and both in budding and fission yeast. It was proposed to facilitate the process of homologous juxtaposition. Observations on the behaviour of chromosomes in vegetative cells show a diverse picture. In Dipteran insects somatic pairing is a normal part of nuclear organization, while in other organisms it is related to trans-sensing effects, and/or it is restricted to specific loci/sequences or cell types.

We set up a system to investigate the overall chromosomal interactions in a diploid nucleus and to detect somatic pairing in fission yeast if it exists. To obtain stable vegetative cultures strains of identical mating-types were united by protoplast fusion, and chromosomal interactions were examined with the application of the Cre/loxP system. In this system Cre recombinase promotes site-specific recombination between two 34bp long *lox*P sequences of bacteriophage P1. The probability of recombination over time is a reflection of the local concentration of two sites. Therefore, the relative frequencies of recombination between *loxP* sites integrated at different positions in the genome reflects relative spatial disposition within the nucleus. In our system one *loxP* site follows the strong *adh1* promoter, and the other one is in front of a promoterless *ura4* gene. Recombination between the two constructs results in turning on the expression of the *ura4* gene, thus it can be determined genetically.

Recombination frequencies were determined between *loxP* sites located at allelic positions of homologous chromosomes, nonallelic positions of homologous chromosomes, on the same physical chromosome (intrachromosomal recombination) and between loci on nonhomologous chromosomes. The main tendencies of chromosomal interactions can be summarized as follows. 1. Allelic interaction frequencies are higher than interaction frequencies for non-allelic loci on homologs and exceed the majority of nonhomolog interactions as well. This suggests a tendency for chromosomes to be colocalized along their length. 2. Significant pairing of homologous chromosomes was measured at telomere proximal loci indicating active homology recognition in the region. 3. At the centromere proximal loci the robust clustering of centromeres (Rabl orientation) overrides homology differentiation. 4. The slight preference for allelic interactions at the interstitial chromosomal regions may be a consequence of the flexibility of long chromosomes and the dynamic nature of the nucleus. Finally, the factors whose interplay defines the probability of chromosomal interactions and nuclear architecture will be discussed.

YEAST CELL WALL PROTEINS AND THEIR BIOTECHNOLOGICAL POTENTIAL

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Fungal cell walls attracts attention for their use as an ideal potential target for antimycotics, but also as a cellular structure by which the cell corresponds with its surrounding. Budding yeast wall has a bilayered structure. The inner layer consists mainly of a network of linear ß-1,3-glucan, and the outer layer is composed of an unusually high number of mannoproteins. Yeasts have evolved three different ways of attaching proteins to the polysaccharide network. Some proteins are bound to ß-1,3-glucan noncovalently (Scw – soluble cell wall proteins; extracted by hot SDS), while others are attached covalently (Ccw – covalently linked cell wall proteins; extracted by glucanases) either through glycosylphosphatidyl inositol (GPI) anchor and ß-1,6-glucan, or directly to ß-1,3-glucan by an alkali labile ester linkage between the γ-carboxyl groups of glutamic acid and hydroxyl groups of

glucoses (Pir – proteins with internal repeats; extracted by mild alkalis). The exact physiological roles for most cell wall proteins are still unknown.
Molecular mechanisms for incorporation of different groups of wall proteins, as well as the potential use of these mechanisms for cell surface engineering will be reviewed. Also, the potential of cell wall proteins for identification and characterisation of wine yeasts will be discussed.

A RAPID METHOD TO DETERMINE THE GROWTH AND MORPHOLOGICAL SENSITIVITY OF *TRICHODERMA* SPP. COLONIES TO METALS IN POLLUTED AGAR MEDIUM

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An automated image analysis procedure was developed for simultaneous quantification of changes in extension of colony, area, as well as edge morphology caused by heavy metals. This fully described technique, which allows rapid, accurate and inexpensive survey of high number of environmental factors at a large collection of filamentous microorganisms, was used for assessment of growth and morphological sensitivity of 13 *Trichoderma* spp. to ten pollutant metals (Cd, Co, Cu, Hg, Mn, Mo, Ni, Pb, Se, Zn) at seven concentrations. The lowest adverse effect concentration was calculated for colony area and edge morphology for each microbe x metal combinations. Generally, changes in morphological development could be detected at significantly higher concentrations than in mycelial growth. Although all of metals retarded the growth of test fungi, great differences were found among them in the tendency of morphological changes. Mo, Ni and Se increased the fractal dimension of colony edge of thirteen, eleven, and four strains, respectively. Very precise differentiation of sensitivity of fungal strains could be examined with the elaborated technique, which may be very useful in various ecological, physiological or genetical studies dealing description, strain selection or improvement.

"GENTLY ROUGH": OPTIMAL DOWN-REGULATION OF LPS IN RFAH MUTANTS FOR LIVE-ATTENUATED ENTEROBACTERIAL VACCINE CANDIDATES

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Enteric bacteria, such as *Escherichia coli*, *Salmonella enterica* and *Shigella* spp. consist of a very large number of serovariants due to the extreme heterogeneity of their surface antigens, particularly LPS. In order to raise cross-protective immunity among heterologous serovariants a tempting strategy would be to use mutants with defects in LPS synthesis (i.e. rough mutants). On the other hand, LPS is an important virulence factor contributing to several steps of the infectious process. Consequently, rough mutants tend to be over attenuated and are usually considered to be inappropriate as live vaccines. Ideal live-attenuated vaccines find a balance between being attenuated (safe) and retaining immunogenicity (efficacy). We have tested several structural LPS mutants of *S. enterica* sv. Typhimurium and have shown that loss of the O-antigens itself (Δ*waaL*) did not elicit sufficient attenuation, whereas truncation at the depth of the inner core (Δ*waaG*) rendered the mutant over

attenuated, i.e. unable to induce an efficient immune response. RfaH is a transcriptional anti-terminator in enterobacteria required for the transcription of long operons including those encoding LPS synthesis. Mutants lacking RfaH were described before to exhibit deep rough-phenotype. However, in the current study we provide evidence that the truncation of LPS molecules in *rfaH* mutants is partial, only, i.e. different lengths of LPS chains are expressed. The majority of LPS molecules are truncated at the level of core oligosaccharides, indeed. A small part of LPS molecules, however, contain an intact core, some of which are capped by O-antigens. This was confirmed by the existing, although decreased potential of *S.* Typhimurium *rfaH* mutants to be transduced by the O-antigen-specific phage P22. Furthermore, an *rfaH* mutant of *Shigella flexneri,* was still agglutinated by O-specific serum and parts of the O-antigen ladder could still be detected by silver staining on the SDS-PAGE gels suggesting that the amount of the O-antigens retained in mutants of in this species could be higher than in *S.* Typhimurium. This LPS structure - designated here as "gently rough" phenotype - sufficiently attenuates virulence while retaining immunogenicity of *rfaH* mutants. LD50 value of the *S.* Typhimuriummutant increased $>10^4$-fold in the murine typhoid model, while that of *S. flexneri* increased >10-fold in the mouse lung model. Vaccination with *rfaH* mutants induced protective immunity against the homologous serotypes in both species. Furthermore, in case of *Salmonella* cross-protective immunity was detected, likely to be mediated by conserved outer membrane proteins shared by various representatives of *Enterobacteriaceae*.

EVOLUTIONARY INSTABILITY OF FRUITING BODY TYPES AMONG COPRINOID HOMOBASIDIOMYCETES

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The family *Psathyrellaceae (=Coprinaceae p. p.*) represents a heterogenous group of agaric fungi including several important taxa that are widely used in genetic and breeding studies. Recent phylogenetic studies and consequent taxonomic and nomenclatural changes caused considerable debate in the scientific community on the instant applicability of molecular results to classificational questions. The evolution of the group, which in our opinion could fill the gap between phylogenetic studies and classification, is, however, still very poorly understood.

In this study we attempt to reconstruct the evolution of coprinoid fungi based on molecular and morphological traits. We used the nuclear encoded ribosomal large subunit gene (nrLSU) to infer phylogenetic relationships of the group. Then we mapped morphological characters onto the phylogeny using the stochastic mapping algorithm of Heulsenbeck & al. [1] to reconstruct the ancestral character states of critical nodes of the phylogeny.

Our results strongly suggest that changes from non-deliquescent (*Psathyrella*-like) to deliquescent (*Coprinus*-like) fruit body types occurred at least 3 times during evolution of the group. As deliquescent fruiting bodies of these taxa need much less time for spore dispersal, we suggest that such changes provided these taxa with the opportunity to colonize new substrates, such as herbivore dung, places or seasons with very short wet periods (e.g. morning moisture). We have also examined other morphological changes accompanying alterations in fruit body types. We found that bimorphic basidia, voluminous hymenial cystidia, and the emergence of highly structured *velum universale* are peculiarities of deliquescent fruiting bodies. Their evolutionary role and the possible classification of coprinoid taxa in view of the new results are also discussed and compared with other examples from the *Agaricomycetes*.

1. Heulsenbeck et al. 2003. *Syst. Biol.* 52, 131.

FUNGAL DIVERSITY OF HUNGARIAN SANDY REGIONS

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The authors present the main results of a systematic inventory of fungi on the Great Hungarian Plain. Results of intensive recording of mostly *Basidiomycetes* during the past 10 years and previously published records are combined to estimate the current knowledge about fungi in the region and are discussed in terms of conservation on a regional and international scale.

Presently, roughly 1300 fungal species are known from the region with certainty (i.e. represented by herbarium material). During the last 10 years, more than 180 species have been reported as new for Hungary, which reflects the special position of sandy regions within the Hungarian mycobiota. The typical species composition was found to be a mixture of taxa from western European sand-dunes and of eastern steppe-like vegetation, which is unique to Europe. Due to the unique environmental conditions the proportion of rare species is unexpectedly high, this makes this region mycologically very valuable on both Hungarian and European scale. Furthermore, several new taxa have been described and remain to be described in the near future, many of them known only from these Hungarian sand regions. The authors attempt to compile a list of taxa that could be candidates for inclusion in the Red List of Hungarian macrofungi or legal protection. Some examples of new and rare species are discussed in detail.

Perspectives of future research on badly represented taxa and habitats are also concerned, and the need for long-term inventories of fungi – being fundamental to most conservational, taxonomic and ecological research fields – is stressed.

SIGNIFICANCE OF HIV DRUG RESISTANCE IN THE MANAGEMENT OF AIDS

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Hungary belongs to low HIV endemic area of Europe. Between 1986-2006 altogether 511 AIDS cases had been registered. More than 70% of them acquired infection by homo/bisexual transmission. During the rapid replication of HIV, high levels of genetic mutations occur, resulting in emergence of drug resistant HIV variants. In a country as Hungary with very favourable HIV/AIDS epidemic situation, HIV genotyping before therapy should be performed almost in every case. Mutations occur in the region of the HIV genome for the enzymes targeted by the current anti-HIV drugs i.e. the reverse transcriptase (RT) gene and the protease gene. We determined the mutations in the HIV-1 pol gene associated with resistance to antiretroviral drugs in primary HIV infected individuals who did not receive antiretroviral treatment. Integrated provirus DNAs were purified from patients' lymphocytes. Nested PCR was used for amplifying HIV pol sequences. Drug resistance genotyping of HIV RT was done by in situ DNA hybridization using a Line Probe Assay (Inno-LiPA).

Viral variants harbouring resistance mutations such as: M41, T69R, K70R, M184V, T215Y and others in the pol gene were detected in 15% of the subjects. M184V and T215Y was found most frequently indicating resistance against RT inhibitors *zidovudine* (AZT), *stavudine* (D4T), *lamivudine* (3TC) and in less frequently *emtricitabine* (FTC). HIV mutants resistant to NRT inhibitors were revealed in 10 % of those infected before and in 20% in patients infected after the year 2000. Multiple drug resistant viruses (2-3 drug classes) were present in 3.5% of those studied, mainly in

recently infected patients. Amino acid substitutions in RT were only found in those infected before year 2000 as T69D (4.5%), T215S/D (6%) and T215A/V (3%). These findings indicate the evolution of drug resistance showing a correlation with the time of introduction of combination therapy in our country.
Surveillance of antiretroviral resistance is a main objective of our anti-HIV program. This study identified antiretroviral resistant mutations in HIV-1 RT gene in HIV infected therapy-naïve patients. This confirms the transmission of drug-resistant HIV revealed by genotype testing during primary infection and raises serious clinical and public health consequences. Development of resistance leads to viruses escaping the control of drug combination therapy and cause disease progression. Resistant mutations observed with preliminary drug resistance appear to be far more stable, presumably because there are no competing drug-sensitive strains. Drug resistance testing (HIV genotyping) at the time of diagnosis should be the standard of care.

MICROBICIDAL ACTIVITY OF NEW DISINFECTANTS - EXPERIENCES OF TESTING FOR FOOD INDUSTRY USE

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The Food-microbiology Department of National Institute of Food Safety and Nutrition serially tests new chemical disinfectants, used in food, industrial, domestic and institutional areas. Since 2002 we use EN standard test methods: EN 1276:1997 (for bactericidal activity), EN 1650:1997 (for fungicidal activity).
In the practice only few customers and manufacturers know exactly the efficiency of their disinfectants, and take the other influential factors into consideration. Often they couldn't give even the exact parameters of product use (concentration, contact time, temperature).
Microbiological testing determines the ideal parameters for application of disinfectants. This is necessary for appropriate use to prevent the emergence of disinfectant resistant microorganisms, and to minimize chemical amounts released to the environment. Tendencies experienced in Hungary (in the last 5 years) and the results from testing new disinfectants will be demonstrated on the poster.

A NOVEL MULTIPLEX TAXON-SPECIFIC SINGLE NUCLEOTIDE PRIMER EXTENSION ASSAY FOR THE DETECTION OF MICROORGANISMS

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A new molecular approach based on single nucleotide primer extension (SNuPE), a method used in genetic typing and mutation analysis, was adapted for environmental samples in order to allow the parallel detection of DNA sequences of different target microorganisms. In the primer extension reaction, a DNA polymerase is used to label a specific primer hybridized to the target sequence by incorporating a single fluorescently labelled dideoxynucleotide. The specificity and sensitivity of the

primer extension assay was assessed by single and multiplex reactions using defined template mixtures of 16S rRNA gene PCR products obtained from pure bacterial cultures. The one mismatch discrimination potential of SNuPE was investigated by means of altering a specific primer to obtain different mismatch annealing. The priming proved to be very specific and the cyclic fluorescent labelling of the hybridized primers via extension resulted in a significant increase to the detection sensitivity of the polymerase chain reaction. For a first application on environmental samples, a SNuPE assay in combination with taxon-specific PCR for the detection and typing of *Dehalococcoides* populations was developed. Only one sequence type belonging to the Pinellas phylogenetic subgroup was detected in a Bitterfeld/Wolfen region aquifer containing chlorinated ethenes as main contaminants. The three primers hybridization assay provided a fast and easy to implement triple proof verification of taxon-specific PCR allowing a rapid additional taxonomic affiliation. In addition, reverse transcribed 16S rRNA was successfully used as a template to prove the applicability of SNuPE in a PCR-independent manner. This study demonstrates the great potential of SNuPE for parallel detection and typing of different nucleic acid sequences from environmental samples.

NOVEL APPROACH USING SUBSTRATE-MEDIATED RADIOLABELLING OF RNA TO LINK METABOLIC FUNCTION WITH STRUCTURE OF MICROBIAL COMMUNITIES

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A novel concept was developed applying radio-isotope-labelled substrate incorporation into the biomass. The resulting radiolabelled RNA is used both as an indicator of activity and as a template for gaining structural and functional information about a substrate-utilizing microbial community. Sequences of PCR products obtained from a microbial community are separated via cloning or using molecular fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE). Nucleic acids from predominant clones or the whole DGGE patterns are transferred to a nylon membrane and hybridized with the radiolabelled sample RNA. Scanning of the hybridized blots for radioactivity indicates the members involved in the utilization of the substrate. This novel "random walk" approach using radio isotope probing was evaluated in a model community experiment. A bacterial culture was incubated with ^{14}C-acetate and labelled RNA was obtained. The radiolabelled RNA was hybridized to dot-blots of ssDNA from various bacteria to investigate the specificity of the reaction and to prove the principle of the concept.

INVESTIGATION OF *SALMONELLA* CONTAMINATION AND PRELIMINARY CHARACTERIZATION OF THE STRAINS ISOLATED FROM BROILER CHICKEN FLOCKS, CARCASSES AND MEAT PRODUCTS IN THREE EAST-HUNGARIAN FARMS

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Salmonella contamination of broiler chicken flocks of three farms located in Hajdú-Bihar-county, as well as contamination of the carcass and the raw meat products originating from them was investigated by isolation and partial characterization of the strains.

During the study period (April 2006 - January 2007) chicken faecal samples were collected according to standard methods in the 5 successive flocks set in a fowl-house in the farm A. In the farms B and C one flock in one fowl-house was sampled. Prior to the settings, samples were taken from the water, feed and the environment of the fowl-houses. During processing of all investigated flocks, environmental and carcass samples were taken in the processing plant. Raw meat bought in retail shops supplied by the abattoir and *Salmonella* strains isolated from human diseases in the same period and region were also examined. Isolation and serotyping of the strains were done according to standard methods; characterization was done by phage- and antibiotic resistance typing; genetic relationship was assessed by PFGE.

The environment of the fowl-house and the 5 flocks of the farm A were negative for *Salmonella*. However, 35 *S.* Infantis strains were isolated from 50 samples taken in the abattoir and the retail shops during processing of the flocks of the farm A. The environmental samples of the farm B were *Salmonella* negative, but the flock was contaminated: from the faecal samples mostly the *S.* Infantis was identified which serotype also predominated among the strains isolated from the carcass and raw meat samples. From the fowl-house of the farm C *S.* Infantis was cultured from the environmental samples taken prior to the setting. The flock was positive for *S.* Infantis during the whole rearing period, and this serotype was dominantly identified among the carcass and meat samples. The vast majority (123/126) of the identified *S.* Infantis strains (including all animal and human strains) belonged to the phage types 217 and 213, all but one were characterised by the nalidixic acid-streptomycin-sulphonamide-tetracycline R-type and showed 88,6 % genetic similarity, irrespectively of their origin.

This study showed that the same multidrug resistant *S.* Infantis clone was spread in the food-chain, form 2 out of the 3 examined broiler farms to the broiler carcasses, the raw meat and finally the human population of the region that was earlier detected as the dominant clone characteristic of the broiler and the human population of the country.

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MOULD AS A PROBLEM OF THE PANEL-BUILDING RECONSTRUCTION – 1. ARCHITECTURAL AND MYCOLOGICAL ASPECTS

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Many mistakes were done during the realization of the loosely adapted plans of blockflat buildind in the 70ies. Our official serial studies of the situation, with adequate, sophisticated methods revealed both conceptual and practical errors equally to hygienic and social requirements not correctly considered. Even invaded concrete elements were also set in, while the running of the finished buildings by the tenants have also produced many faults. Anyway all the errors together are in close contact to the water - vapor - heat economy of the buildings, especially the living quarters within

them. Mould films develop when enough water is given (either from rain, or by condensations from indoor air oversaturated with vapor) on the inside surfaces of the boarding walls (condensation on cold surfaces) together with nutrients. To clean up, our department started to study with sophisticated international methods (*in-situ* microscopy, culture from mold colonies on wall and air sampling).
It has been proven (in serial studies of hundreds of cases from 1985) that the contribution of the following processes seem to be essential: vapor over production and weak ventillation in the small cubature (also in kitchen and bathroom, with tightly built in windows and doors), Low heat insulation by the panel (broken concrete elements, damaged plastic foam as middle layer, higher density of the block, at originally weak outside heat insulation, apex phenomenon. The molud invasion, however, is dangerous to the building, but is also agent of troublesome human health events: the bronchial asthma, especially in children.
To eliminate molds from the sick building is a great challenge. Some disinfectants can't conquer the thick and melanin incrusted cell-wall, etc.

GENITAL HERPES – AN UPDATE

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Herpes simplex virus (HSV) 1 and 2 infections constitute a major, global public health problem. During the last decades there have been major changes in the epidemiology, clinical significance, diagnostic procedures and treatment regimen of HSV infections.
Due to improvements of living and hygiene conditions, national gross domestic products, a decrease in childhood HSV-1 seroconversion occurs. More individuals acquire genital HSV-2 infections without pre-existing cross-reacting HSV-1 antibodies that predisposes to severe clinical outcome. Practice of oral-genital sex due to frequent asymptomatic salivary shedding results in primary genital HSV-1 infections. This might account for more than 50% of genital herpes cases in some geographic regions! By and large, HSV-2 seroprevalence is decreasing. The increasing number of female patients with genital HSV-1 and male patients acquiring genital HSV-1 infection at younger age is the major characteristics of recent epidemiological trends. Both HSV-1 and HSV-2 act as major cofactors of HIV-1 transmission and transactivation consequently accelerate AIDS spread and progression.
Over the past 15 years, HSV-1 and HSV-2 specific diagnostic tests (e.g. purified or synthetic glycoproteins to detect species specific antibodies by immunofluorescence or ELISA, or specific traditional and real-time PCRs) became commercially available. In our Hungarian cohort study, both immunofluorescence and PCR verified 5.9% of HSV-1 infection as etiology of recurrent (>4 episodes/year) infections.
Acyclovir (e.g. Telviran) is widely available and applied to treat primary or recurrent infections, among them genital herpes. Year-long once-daily suppressive therapy not only prevents herpes episodes (complete inhibition was achieved in 71,4% of our patients), but significantly cuts the risk of HSV transmission in discordant partners. The rare thymidine kinase negativity resulting in acyclovir resistance can be established by inhibitor assays through virus cultivation or rapidly by using specific primers of PCR. The latter method did not reveal any HSV mutant among non-immunocompromised patients in our study.
Preventive multivalent, combination vaccines against HSV, *Chlamydia trachomatis* and other sexually transmitted infections are under development, but recently chemoprevention and suppressive therapy are the practical ways to combat genital herpes.
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PHOSPHOPROTEOME ANALYSIS ON THE COPPER REGULATION OF METHANE MONOOXIGENASES IN *METHYLOCOCCUS CAPSULATUS* (BATH)

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Methylococcus capsulatus (Bath) is a Gram-negative, coccoid, methanotroph bacterium. For the utilization of methane *M. capsulatus* is able to express two methane monooxigenases (MMO): in the presence of copper ions the particulate MMO (pMMO) and its accessory and transport proteins, responsible for copper uptake, are expressed and the cell morphology changes: extended membrane systems can be seen. In the absence of copper the soluble MMO (sMMO) and its accessory proteins are expressed. sMMO can oxidize a wide range of compounds, from alkanes, alkenes, ethers and haloalkanes to aromatic and even heterocyclic hydrocarbons. Many biodegradation and biotransformation applications for sMMO are currently being investigated.

Although the existence of S, T and Y phosphorylation in prokaryotes was first demonstrated in 1978, our knowledge about S, T and Y phosphorylation in prokaryotes is very limited. In our recent work we studied the copper regulation of MMO enzymes by comparing the phospoproteome of two cultures grown under distinct conditions and screening for proteins the phosphorylation state of which change depending on the available copper.

Cultures for the proteomic experiments were grown either in copper-free (no $CuSO_4$ added) or copper rich-medium (containing 5 μM $CuSO_4$). Phosphoproteins from disrupted cells were enriched by PhosphoProtein Purification Kit (QIAGEN). Enriched phosphoproteins were separated by two dimensional polyacrylamide gel electrophoresis.

After comparing the phospoprotein pattern of the two cultures three spots from copper free sample were submitted to identification by mass spectrometry and bioinformatic analysis. We found that two subunits of sMMO (MMOB and MMOC) are phosphorylated and Elongation Factor TU is only phosphorylated when *M. capsulatus* is cultured in copper-free medium.

VESICULAR STOMATITIS VIRUS INFECTION TRIGGERS APOPTOSIS ASSOCIATED WITH DECREASED ΔNP63A AND INCREASED BAX LEVELS IN THE IMMORTALIZED HACAT KERATINOCYTE CELL LINE

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Oncolytic viruses provide novel modalities for the therapy of malignant tumors. These microorganisms can kill cancer cells selectively, either by serving as replication-defective vectors or by acting as replication-competent biotherapeutic agents. *Vesicular stomatitis virus* (VSV), a member of the *Vesiculovirus* genus of the *Rhabdoviridae* family, has been shown to possess powerful inherent oncolytic activity. VSV elicits a cytopathic effect and apoptosis in cancer cells, whereas normal cells are relatively spared by this virus. Abundant experimental evidence also indicates that VSV should be considered in the future as a powerful biotherapeutic agent effective against a broad histological spectrum of cancers. Since the potential oncolytic activity of VSV has not yet been evaluated in epithelial-derived skin tumors, we set out to investigate the susceptibility of the immortalized HaCaT

keratinocyte cell line to VSV, and analyzed the role of apoptosis in the VSV-mediated induction of cell death. Furthermore, in order to gain an insight into the underlying molecular mechanisms implicated in the apoptogenic properties of this virus, we also determined the effects of VSV infection on the levels of DNp63a, mutant p53 (p53mt) and certain Bcl-2 family member proteins. Indirect immunofluorescence assays, Western blot analyses and plaque titrations demonstrated that the HaCaT cell line was permissive to VSV replication. The results of ELISA for detection of the enrichment of nucleosomes in the cytoplasm of apoptotic cells revealed that VSV infection elicits the apoptotic death of HaCaT cells. Western blot analyses showed that the mock-infected HaCaT cells displayed the endogenous expression of DNp63a, p53mt, Bcl-2 and p21 Bax. The levels of DNp63a and p53mt were decreased; Bcl-2 remained unaffected, while the expressions of p21 Bax and p18 Bax were increased in the VSV-infected HaCaT cells. Together, these data demonstrate that VSV replicates efficiently and triggers apoptosis in the immortalized HaCaT keratinocyte cell line. The VSV-mediated alterations in the expressions of DNp63a and Bax may be implicated in the apoptotic responses of VSV-infected cells and may also sensitize to other apoptotic stimuli. Our findings extend the known spectrum of cell types susceptible to the powerful oncolytic activity of VSV to immortalized keratinocytes and may stimulate further studies aimed at the development of VSV-based virotherapy into an effective modality for the treatment of epithelial-derived tumors of the skin.
Acknowledgments: This study was supported by grants OTKA/T043144 from the Hungarian Scientific Research Fund and ETT/398/2003 from the Hungarian Ministry of Health, Social and Family Affairs. Klára Megyeri is a grantee of the Bolyai János Scholarship of the Hungarian Academy of Sciences.

THE RESULTS OF COMPARING THE OCCURRENCE OF *CAMPYLOBACTER JEJUNI* SEROTYPES IN THE "FROM THE FARM TO THE FORK" PROJECT IN EASTERN HUNGARY: AN OVERVIEW

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Campylobacter jejuni is one of the bacterial pathogens most frequently isolated from human gastroenteritis in developed countries. It is primarily a food-borne infection and in various countries, including Hungary, poultry products are well recognized as being important in the transmission of *C. jejuni* to humans.

To determine the most common serotypes of these bacteria in eastern Hungary, we compared chicken isolates (as the major source of infection) with those strains isolated from patients with diarrhea. We used a passive hemagglutination serotyping method based on the Penner scheme, which classifies these bacteria via the structure of the heat-stable "O" antigen of the cell wall.

Our examinations of 156 human and animal-derived *C. jejuni* strains revealed a broad scale of serotypes. The most common types both in humans and in chickens were the "F (6,7)" (35%), the "A (1,44)" (17%) and the "Y (37)" (11%); the rate of "mixed strains" (more than one Penner type) was 21%. *C. jejuni* serotype "O (19)" is one of the important causes of the Guillain-Barré syndrome, which is an acute inflammatory, demyelinating polyneuropathy leading to a diffuse clinical spectrum of motor and sensory defects. The occurrence of serotype "O (19)" among our isolates was 2.5% (4/156 strains). Further studies monitoring the most common and dangerous serotypes of these bacteria are needed for a clearer picture of the epidemiological background of the prevalence.

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FULLY SYNTHETIC PEPTIDE VACCINES TO THE ECTODOMAIN OF THE M2 PROTEIN PROTECT MICE FROM INFLUENZA INFECTION

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Peptide vaccines are specific and safe alternatives to traditional vaccines but require a special design process to mimic the native presentation of protein antigens and to include T helper cell determinants. We designed and synthesized a series of peptide constructs containing four copies of the ectodomain of the influenza M2 protein and two T helper cell determinants in BALB/c mice. The antigens were spaced to resemble M2 orientation in the transmembrane channel. The B and T cell epitope chains were either synthesized on a single backbone, or two fragments were attached via intramolecular disulfide bridge formation. When these artificial proteins (MW>12,000 Da) together with adjuvants were used as immunogens by the intranasal route in mice, the animals developed significant resistance to virus replication in all sites of the respiratory tract. Significantly, two doses of the peptide vaccine induced a higher level of resistance in nasal and tracheal tissue than two consecutive heterosubtypic infections. The resulting monoclonal antibodies recognized epitopes within a 13-amino acid residue stretch of M2. Since the biological response in peptide construct- and infection-immunized mice appears to be mediated by distinct immune mechanisms, stronger protection can be achievable by combining both protective activities. Additional improvements in antigen spacing and T helper cell determinant selection before preclinical trials may further enhance the immune response. Presumably the utility of the peptide vaccine can be extended to outbreed animal populations and humans.

POXVIRUS INFECTION IN GREAT TIT (*PARUS MAJOR*) IN HUNGARY

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Avian pox is a mild to severe, slow developing disease of chickens, turkeys, pet birds and wild birds occurring in both sexes and all ages. *Avipoxviruses* have been isolated from a wide variety of hosts, and yet little is known regarding the host-virus species variation of the genus *Avipoxvirus*. Poxvirus is a DNA virus belonging to the family *Poxviridae* of the genus *Avipoxvirus*. Avian poxvirus infections have been identified in 232 species of birds belonging to 23 orders, including the Great tit (*Parus major*). Three major groups have been identified: fowlpox virus, pigeonpox virus and canarypox virus. Three great tits presenting nodular proliferative lesions on different areas of the head, suggesting a poxvirus infection, were observed in Pilis Mountains in Hungary. Due to their poor condition two of the birds had to be humanely euthanized and after the necropsy samples were submitted for analysis. Tissue samples were also collected from the third bird, which was released after the surgical intervention, and submitted for analysis. *Avipoxvirus* infection was confirmed by histopathology, electron microscopy and polymerase chain reaction (PCR). Nucleotide sequence analysis of a fragment from the viral 4b core protein gene revealed considerable similarities with other three great tit poxvirus strains isolated in Norway in 2004 and certain divergence among the different viruses in the canarypox group, which could be explained by the host correlation.

The scope of this study was to identify the pathogen that produced the lesions presented by the three birds. Once the pathogen was identified as avipox virus by histopathology, electron microscopy and PCR, the phylogenetic analysis was preformed in order to establish the relationships between the avipox virus strains circulating in Hungary and the avipox strains sequenced so far from *Parus major.* Furthermore, we have established the phylogenetic relationships between the avipox virus strains isolated in Hungary from *Parus major* and avipox virus strains isolated from different species (chicken, turkey, pigeon, canary, and sparrow).

PREFERENTIAL LIGATION DURING CLONE LIBRARY CONSTRUCTION CAUSE BIASED COMMUNITY COMPOSITION PICTURE

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Clone library analysis based on 16SrDNA or 16S-23S intergenic spacer region is still one of the most commonly used methods describing microbial communities. Concerning the community structure, many investigations draw consequences from the composition of the clone library. Although some reports predict biases in connection with the ligation efficiency, this technique was not tested thoroughly for the true quantitative reproduction of the pre-ligation community DNA mixture. Two widely used, commercially avaible TA and TOPO-TA cloning system was tested. Collection strains of bacteria were selected with differing genomic properties and fully known 16SrDNA sequence. DNA from each strain was extracted and amplified with PCR, quantified and mixed at predefined amplicon copy numbers to serve as model community DNA to ligate into the cloning vector. All experiments were run in three parallels. Over 200 clones were analysed with PCR assay from each library. Testing the potential insert length and sequence heterogeneity of 16SrDNA and 16S-23S spacer clone libraries, preferential ligation was detected in all cases, but probably due to different causes. The difference between the performances of the two cloning vector system was also shown.
These findings indicate that members of diverse microbial samples may be excluded during clone library construction because of preferential ligation, this way biasing the true community picture.

COMPARATIVE STUDIES ON THE COMPOSITION AND CATABOLIC PROFILE OF PHYLLOSPHERE COMMUNITIES OF CATTAIL PLANTS GROWN ON UNPOLLUTED AND POLLUTED SITES

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The leaf surfaces of the healthy plants are colonised by a specific epiphytic microbiota composed mainly of bacteria, yeasts and filamentous fungi. The phyllosphere microbiota plays various biological roles.
The bacterial strains isolated from the phyllosphere of cattail (*Typha* sp.) plants grown in natural and polluted habitats could be delineated in the following morphological groups: Gram-negative bacilli and coccobacilli, Gram-positive rod-shaped nonsporogenous and endosporogenous bacteria, cocci and actinomycetes. The proportion of different group of microorganisms on the foliar surface of cattail plants depends on sampling sites. The Gram-negative bacteria and the yeasts were better

represented in the phyllosphere of plants from unpolluted site. The cocci were more frequently isolated from the foliar surface of plants grown on polluted sites. The Gram-positive endospore-forming bacteria could be detected only in the case of polluted plants.
The extraction of 16S rDNA and the amplified rDNA restriction analysis (ARDRA) make possible the delimitation of bacterial strains in 15 ARDRA types. The partial sequencing of 16S rDNA shows that these bacteria belong to the genera *Pseudomonas*, *Sphingomonas*, *Curtobacterium*, *Exiguobacterium*, *Psychrobacter*, *Kocuria*, and *Staphylococcus*.
Using BIOLOG GN microplates, it was shown that the phyllosphere populations of *T. angustifolia* collected from natural habitat were more diversified functionally than those from polluted site. There were differences in the number and chemical nature of utilised carbon sources, as well as in the rate of metabolisation. The microorganisms colonising the foliar surface of unpolluted plants utilised preferentially saccharides as carbon sources, while the phyllosphere community of polluted plants also metabolised amino acids, organic acids and some more complex substance

COMPARATIVE ANALYSIS OF FACTORS INFLUENCING TOTAL PLATE COUNT OF RAW MILK IN SOME DAIRY FARMS IN HUNGARY

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The milk processing plants and dairy farms are interested in the production of basic material (raw milk) of good quality. The importance of the quality of raw milk increased after Hungary had joined to the EU. The 1/2003. (I. 8.) FVM-ESZCSM order prescribes strict conditions about food hygiene and quality of handling and distribution of raw milk. On delivery of raw milk, the microbiological quality, especially total plate count of the milk is very important. Dairy farms have to produce raw milk of extra quality, because the milk processing plants do not accept raw milk of other quality.
The aim of our research was to examine the connection between the total plate count in bulk tank milk and housing and milking technologies of twenty-two farms of different-sizes.
According to the instruction of the MSZ ISO 6610 standard to examine the total plate count of the bulk tank milk, we used TGE-agar and aerobic incubation at 30°C for 72±3 hours. We examined the relation using various statistical methods. For the statistical analysis of the relation between total plate count and each factor, in the case of two variables we used t test or nonparametric Mann-Whitney test. In the case of three variables, we used analysis of variance (ANOVA) or Kruskal-Wallis test. First we examined the factors which influence the total plate count by binary logistic regression, and after this, supported by the results we used loglinear model.
During the examination of the effect of different factors on total plate count of bulk tank milk, we found that the mean total plate count is significantly higher ($P<0.001$) in farms which use tie-stall housing forms, bucket milking, udder preparation with water, and which do not use pre- and post-milking disinfection.
The results showed that besides cooling, the milking procedure and the type of udder preparation had the largest effect on the total plate count. During the loglinear statistical analysis we found that the combination of pipeline milking – tie stall housing system – disinfectant preparation of the udder, and that of milking parlour - loose cubicle housing system - dry preparation of the udder are the most appropriate in the aspect of the total plate count.

DEVELOPMENT OF A SYNBIOTIC AND ITS IMPLICATION IN FOOD PROCESSING

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Functional foods containing physiologically active component contribute to maintain human health and well-being and help to reduce the risk of diseases. Therefore food scientists make efforts to offer innovative new products, which satisfy consumer needs.

Probiotics are viable microorganisms that beneficially influence the health of the host when consumed. Bifidobacteria are widely used as probiotic and play an important role in the maintenance of health. One promising approach to increase the ratio of probiotic bacteria in the intestinal microbiota is the consumption of prebiotics. Prebiotics are non-digestible dietary components that pass through the digestive tract and selectively stimulate the growth and the activity of probiotic microorganisms resident in the colon. Consumption of these non-digestible carbohydrates has been demonstrated to alter intestinal populations. Resistant starch is a special form of high-amylose maize starch which resists the digestion in the upper gastrointestinal tract. This ingredient offers various beneficial properties to the manufacturer and to the consumer: it is a source of dietary fibre, provides techno-functional characteristics, improves viability of probiotic microorganisms in food, protects probiotic bacteria during transit through the gastrointestinal tract, can be used to alter the composition of intestinal microbiota, promotes reduction or elimination of pathogenic bacteria, enhances butyrate production in the colon.

Traditionally probiotics are found in dairy products that are good vehicles to deliver probiotics in humans, but the consumption of dairy products may be limited by allergies and intolerances.

This study is aimed to develop synbiotics: dairy products and non-dairy products which contain the probiotic bifidobacteria and the prebiotic resistant starch. Resistant starch provides protection for the cells during passing through the upper gastro-intestinal tract and they can reach the colon in a viable condition to exert their beneficial effect. Two types of commercially available resistant starch were used. The amylolytic properties of bifidobacteria were examined to complement resistant starch in a synbiotic yoghurt, and to immobilise bifidobacteria by adhesion to starch granules.

To prepare synbiotic yoghurt *Bifidobacterium longum* B6.1 strain and YO-Mix 621 starter culture were used. The optimum concentration of resistant starch with minimal impact on taste was added into the milk. The incubation time of the synbiotic yoghurt has got longer from three at seven hours compared to the product made with starter culture.

Bifidobacteria were adhered to resistant starch granules to develop synbiotic food additives. No correlation was found between the adhesive characteristic of strains and their amylolytic properties. The starch granules were treated with a-amylase to form pores in order to increase surface for the adhesion of bifidobacteria. With this method it was possible to immobilise strains that did not adhere so well. This synbiotic product could be added to various foodstuffs, mostly dry foodstuffs, such as grain products, various kinds of muesli, cornflakes, muesli bars, sweets and snacks.

OGATAEA THERMOPHILA SP. NOV., A NEW METHYLOTROPHIC YEAST SPECIES

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The genus *Ogataea* was proposed by Yamada *et al*. [1] to accommodate some nitrate assimilating methylotrophic *Pichia* species based on the partial 18S and 26S rDNA sequences of the investigated strains. *Candida thermophila*, a species, which clusters in the *Ogataea* clade on the phylogenetic trees constructed from the sequences of the D1/D2 domain of the lsu rDNA, was described byShin *et al*. [2], based on a strain isolated from soil in Korea. Taken into consideration the definitions of Mouchacca [3] and Watson [4], *C. thermophila* is rather thermotolerant than thermophilic, although the upper temperature limit of its growth is above 50°C. Another strain of the species was reported as the causative agent of catheter-related fungemia [5].Next to the two aforementioned strains an additional sporulating, methanol assimilating yeast strain was recovered from rotten willow wood in Hungary. Although several phenotypical differences were observed between the type strain of *C. thermophila* and the Hungarian isolate, taking into account the sequences of its D1/D2 domain of the large subunit (26S) rDNA and its ITS1-5.8S-ITS2 rDNA region, we considered it to be conspecific with *C. thermophila*. As we observed ascospore formation also in the type strain of *C. thermophila*, *Ogataea thermophila* sp. nov., the teleomorph of *Candida thermophila*, typified by the type strain of *C. thermophila*, was proposed [6].

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CLONING AND PARTIAL SEQUENCE ANALYSIS OF THE *GILBERTELLA PERSICARIA* FARNEZYL PYROPHOSPHATE SYNTHASE GENE

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Traditionally, three Zygomycetes fungi, *Blakeslea trispora*, *Phycomyces blakesleeanus* and *Mucor circinelloides*, have been involved in the studies on the carotene biosynthesis. Even the only industrial carotenoid producer fungus, *B. trispora* is a member of this fungal group. Analysis of the carotenoid content of *Gilbertella persicaria*, a fungus closely related to *B. trispora* revealed a carotenoid profile unique among the Zygomycetes as it produced astaxanthin and other keto-carotenoids in detectable amount. Like *B. trispora*, *G. persicaria* produced significant quantities of these pigments only if it was plated as a mixture of two strains with opposite mating types.

In the present work, farnezyl pyrophosphate (FPP) synthase gene (*GpisoA*) encoding a key-enzyme of the isoprene biosynthesis was cloned and analysed. Degenerated primer pairs were designed to conserved regions of known fungal FPP synthase genes and a fragment has been amplified by PCR from the genomic DNA of *G. persicaria*. The resulted amplicon was sequenced and the entire gene was determined by the inverse PCR technique. The *Gilbertella* gene showed high level of similarity to the *isoA* gene of *M. circinelloides* [1].

Carotenoids are isoprenoid compounds derived from the repeated condensation of only a few simple building blocks, what means that the amount of the precursor molecules synthesised in the early isoprenoid biosynthesis can be considered as a limiting step of the final isoprenoid production. Given this fact, we plan the over-expression of the genes encoding the rate-limiting enzymes of the isoprenoid pathway in this. Applicability of the cloned gene for this purpose is also discussed.

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NEW AND FUTURE VACCINES

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The reputation of vaccination rests on a two hundred year old history of success against major infectious diseases. In general, two achievements have been crucial to the success of vaccines: the induction of long-lasting immunological memory in individuals and the stimulation of a herd immunity that enhances control of infectious diseases in populations. However, when one reviews the vaccines now available it is apparent that most successes have been obtained when the microbe has a bacteremic or viremic phase during which it is susceptible to the action of neutralizing antibodies, and before replication in the particular organ to which it is tropic. Both poliomyelitis and infections by capsulated bacteria are examples where vaccination has worked efficiently.

Some success has also been achieved against agents replicating on respiratory or gastrointestinal mucosae. Influenza, pertussis and rotavirus vaccines are examples of such agents, against which it has been possible to induce immune responses acting locally as well as systemically. Control of intracellular pathogens has been difficult, and modern efforts are directed towards pathogens against which cellular immune responses are critical, such as HIV, malaria and tuberculosis.

Nevertheless, great progress has been made recently using both classical and new strategies for vaccine development. Vaccines against pneumococci, rotaviruses, papillomaviruses, meningococci, and zoster open the possibility of controlling the diseases they cause. Better influenza vaccines are also becoming available.

Newer approaches in vaccine production such as nucleic acid immunization, vectors, reverse genetics and additional routes of administration may circumvent prior difficulties. The target of vaccination will shift towards adolescents, adults, patients in hospital and those with chronic diseases, and possibly will extend to therapy as well as prevention. Although some major scientific problems remain to be solved, the biggest problems in the 21st Century will be the increase in anti-vaccination sentiment in the developed world, the financial difficulties in extending vaccination to the poorest countries, insufficient vaccine supplies and the paucity of manufacturers willing to produce vaccines for small markets.

CONNECTION BETWEEN THE OXIDATIVE STRESS TOLERANCE AND VIRULENCE OF *CANDIDA ALBICANS* - BIOMEDICAL AND PHARMACEUTICAL CONSIDERATIONS

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The steadily increasing incidence of different kinds of candidiasis, *e.g.* in immunocompromised patients, the threatening emergence of *C. albicans* strains rapidly developing secondary resistance to currently used antimycotics and non-albicans *Candida* species with natural primary resistance together with the aim to moderate the undesirable side-effects of available medicines all force us to

consider new cellular targets and new drug combinations in future antifungal therapies. In a series of our most recent publications, we shed light on the inhibitory effects of the continuous induction of the antioxidative defence system on dimorphic morphological transitions in *C. albicans*. Moreover, we demonstrated that the decreased virulence of *C. albicans* cells sensing chronic oxidative stress may be an exploitable phenomenon in combating several types of candidiasis.

In more details, the development of oxidative stress tolerant *C. albicans* mutants during chronic oxidative stress caused by exposure to increasing concentrations of *tert*-butylhydroperoxide, a lipid peroxidation accelerating agent, resulted in a reduced germ tube, pseudohypha and hypha-forming capability, a decreased phospholipase secretion and, hence, a considerably decreased virulence in mice (Fekete *et al.* 2007). Such an increased oxidative stress tolerance may therefore be disadvantageous when *C. albicans* cells escape from blood vessels and invade deeper tissues. The oxidative stress tolerant phenotype of the mutants was explained with the continuous induction of the antioxidative defence system by steadily high intracellular concentrations of endogenous oxidants, *e.g.* peroxide, glutathione disulphide, lipid-hydroperoxides, conjugated dienes and thiobarbituric acid-reactive substances (TBARS), *i.e.* with adaptation to persistent oxidative stress [1].

Combinations of corticosteroids, *e.g.* methylprednisolone, which increases the intracellular concentrations of conjugated dienes and TBARS but decreases concomitantly the specific activities of several antioxidant enzymes, with oxidants, *e.g.* menadione, or with oxidative stress-generating antimycotics, *e.g.* amphotericin B, could be considered in the treatment of *C. albicans* infections in patients with prolonged topical application of corticosteroids [2]. It is important to note that corticosteroid-antimycotic interactions should be analysed with care because corticosteroid treatments may even weaken the antifungal effects of certain drugs, *e.g.* lovastatin, with a mechanism of action not including the initiation of oxidative stress in *C. albicans* cells [2,3].

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EFFECT OF BIOFILM CARRIERS ON THE MICROBIAL DIVERSITY IN A "LIVING MACHINE"

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The microbial diversity of a wastewater treatment system applying the "living machine" technology was examined by cultivation-dependent and -independent methods.

The studied construction was a combination of the activated sludge and the fixed-bed system in an aerobic and an anaerobic basin. In the aerobic basin polypropylene filaments were applied to increase the surface area for biofilm growth in the system. On the surface of the anaerobic basin ceramic biofilm carrier beads were used to ensure proper substratum for the settlement of plants.

Germ count and bacterial diversity were compared in the case of the aerobic, anaerobic sludge and the two kinds of biofilm carriers. The value of $MPN.g^{-1}$ dry matter in artificial wastewater broth was determined. The total bacterial diversity was examined by Terminal Restriction Fragment Length Polymorphism (T-RFLP) following the amplification of a region of the 16S rDNA with universal primers. The ammonia-oxidizing bacteria (AOB) were detected by a PCR specific for the ammonia-monooxygenase gene (*amoA*). In the case of nitrite-oxidizing organisms (NOB) regions of the 16S

rDNA specific for *Nitrobacter* and *Nitrospira* spp. respectively, were amplified and the PCR products were subjected to sequence analysis.

The two biofilm carriers showed higher MPN.g^{-1} dry matter values (ceramic beads: 1,74 . 10^7; polypropylene filaments: 6,56 . 10^6) compared to the activated sludge (anaerobic sludge: 1,9 . 10^6; aerobic sludge: 2,71 . 10^6). The T-RFLP results showed that there was great similarity between the pattern of the polypropylene filaments' biofilm and the aerobic sludge. The bacterial diversity of the ceramic beads and the anaerobic sludge differed from the two aerobic samples and varied from each other either. Ammonia-oxidizing bacteria were detected in the aerobic samples and on the ceramic beads either. Nitrite-oxidizing organisms were represented only by the *Nitrospira* genus and only in the two aerobic samples. In the anaerobic sludge and on the ceramic beads uncultured anaerobic bacteria were found by *Nitrospira*-specific PCR. All the organisms detected by *Nitrobacter*-specific PCR proved to be close relatives of this group. In the aerobic sludge *Afipia lausannensis*, on polypropylene filaments *Mesorhizobium* sp. was detected. In the two anaerobic samples *Rhodopseudomonas palustris* was found.

These results show that in this system the application of biofilm carriers has effect mainly on the abundance of bacteria, as providing attaching surface for them. The structure of the total bacterial community and the nitrification ability did not vary considerably in the case of the aerobic activated sludge and the fixed biofilm. The difference among ceramic beads' community structure and the other samples can be explained by the phenomenon that plant roots influence the microhabitats in the rhizosphere by exudates or the release of oxygen.

PROTEOMIC CHANGES OF THE WHEAT APOPLAST ASSOCIATED WITH RESISTANCE AGAINST LEAF RUST

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Upon exposure to a given race of pathogen, a wide variety of genetically determined phenotypic symptoms may be manifested in a host species. At the molecular level, changes in the proteome reflect the plant-microbe interaction involved in the defence against infection: these changes provide a sensitive indication of the processes involved and may be very dynamic. Because the apoplast is usually involved in the first line of defence against pathogens, we surmised that its proteomic composition is also likely to be affected by the genetic background of the host – in particular by certain resistance genes. We therefore analyzed the intercellular washing fluid (ICF) of the susceptible 'Thatcher' and the corresponding near-isogenic Lr1 and Lr9 wheat lines (*Triticum aestivum L.*) resistant to the pathotype 43722 of wheat leaf rust (*Puccinia recondita* fsp. *tricii*) with respect to changes in the protein pattern associated with fungal infection to monitor the differential occurrence of secreted proteins putatively contributing to the resistance reaction. The first leaves of seven-day-old seedlings were inoculated and sampling of ICF was carried out daily over a one-week period. Proteins were separated by one- and two-dimensional PAGE, followed by mass spectrometric identification (MALDI-TOF and LC-MS/MS) of selected protein entities showing differential expression. In response to the infection, the Lr1-resistant line displayed earlier induction and more

vigorous secretion of three ICF proteins, a glucan-endo-1,3-β-D-glycosidase (32557 Da, pI 8.6), a chitinase 1 enzyme (24850 Da, pI 8.6) and a pathogenesis-related protein, PR 1.1 (15211 Da, pI 8.8) than the Thatcher line. Colorimetric β-1,3 glucanase assay indicated an acidic pH optimum at pH 5.2 for all three wheat genotypes (Lr1, Lr9 and Tc) – as expected for apoplast proteins. However, the apoplast of both resistant lines showed an increased level of 1,3-endoglucanase activity as well as specific induction kinetics compared with the susceptible one. Although it has been previously reported that certain glucanases and chitinases responsible for degrading fungal cell wall and producing elicitors are induced in both susceptible and resistant wheat lines affected by leaf rust, they may still serve as protecting agents by their significantly higher expression in some resistant lines. The results here indicate that this is also the case for the isoenzymes differentially expressed in the Lr1 and Lr9 lines playing an active role in seedling resistance. Analysis of the promoter regions of the corresponding homologous genes (most sequence similar isoenzymes) in rice has revealed potential common motifs that may be involved in the differential control of gene expression.

THE CONTRIBUTION OF ENTEROCOCCCI IN FOOD PRODUCTS

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Enterococci are widely distributed in the environment, principally inhabiting the human and animal gastrointestinal tract. Although enterococci isolated from human faeces, they are much less prevalent in livestock such as pigs, cattle and sheep. Enterococci are not only associated with warm-blooded animals, but they also occur in soil, surface waters and on plants, vegetables and insects. They can be found either in food products manufactured from raw materials and heat-treated food products. Enterococci can become an important part of the fermented food microbiota especially in fermented cheeses and meats. The contribution of enterococci to the organoleptic properties of fermented products and their ability to produce bacteriocins are important characteristics for their application in food technology. Moreover they can be used as starter cultures or co-cultures (adjuncts). This paper will review the role of enterococci in food products.

IMMUNE RESPONSE AND CYTOKINE PRODUCTION FOLLOWING IMMUNIZATION WITH EXPERIMENTAL HERPES SIMPLEX VIRUS 1 (HSV-1) VACCINES

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To compare the efficacy of experimental HSV vaccines, Balb/c mice were immunized with the recombinant fusion protein gD1/313 (FpgD1/313, representing the ectodomain of HSV-l gD), with the recombinant *Ori*-binding protein (OBP), with the recombinant thymidine kinase (TK), with the recombinant immediate early (IE) protein ICP27, with the non-pathogenic ANGpath gE-del virus (ANGpathE-3-3) and with the plasmid pcDNA3.1-gD expressing the full length gD1 (FLgD1). As compared to mock-immunized animals, specific antibodies against corresponding antigens (detected by ELISA) reached high titers by 10–20 days after the third antigen dose, the lowest antibody level

being detected following immunization with the DNA vaccine. High grade protection against virulent virus challenge was found after immunization with the pcDNA3.1-gD plasmid and with the gE-del virus. Medium grade, but still satisfactory protection, was noted following immunization with the FpgD1/313, and only minimum protection was seen following immunization with IE/ICP27 polypeptide. No protection was observed in animals immunized with TK and OBP proteins, respectively (despite of prominent antibody titers). A considerable response of peripheral blood leukocytes (PBL) in lymphocyte transformation test (LTT) was found in mice immunized with FpgD1/313, with the pcDNA3.1-gD plasmid and with the live ANGpathE-3-3 virus. For lymphocyte stimulation *in-vitro*, FpgD1/313 was less effective than the purified gD1/313 polypeptide (cleaved off from the fusion protein); both proteins elicited higher proliferation following the 5 mg dose than the 1 mg dose. The splenocyte response in LTT was most efficient with splenocytes from DNA-immunized animals when stimulated with FgD1/313 in the 5 mg dose. The secretion of Th type 1 (TNF, IFN-γ and IL-2) and Th type 2 (IL-4 and IL-6) cytokines into medium fluid was followed in purified PBL as well as in splenocyte cultures; the cytokine levels were determined in pg/ml (using fluorokine multianalytic kit) and were also expressed as relative indexes (antigen stimulated versus non-stimulated leukocytes; stimulated leukocytes from immunized animals versus mock-immunized controls). The PBL from FpgD1/313 immunized mice showed increased secretion of both, Th 1 as well as Th 2 cytokines. The relative index of IL-4 and TNF secretion (as compared from immunized versus mock-immunized animals) was high in leukocytes of FpgD1/313 immunized mice, especially at the 1mg antigen dose. In contrast, splenocytes from FpgD1/313 immunized mice showed a little increase of Th 1 cytokine secretion; an extensive IL-4 production by splenocytes was followed with slightly elevated IL-6 synthesis.

INTERRELATIONSHIP BETWEEN HYDROGEN METABOLISM AND VARIOUS BIOENERGETIC PATHWAYS IN THE PURPLE SULFUR PHOTOSYNTHETIC MICROBE, *THIOCAPSA ROSEOPERSICINA*. A GENOMIC SURVEY.

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Thiocapsa rosoepersicina BBS, a purple sulfur photosynthetic bacterium utilizes reduced sulfur compound for its anaerobic photosynthesis. The cells can grow under photochemolitotrophic, photomixotrophic and microaerophilic conditions in the presence of organic substrates and - in proper conditions - it can fix molecular nitrogen.

T. roseopersicina BBS, as world recorder, contains at least five NiFe hydrogenases having various physiological roles. The strain contains at least one soluble (Hox) and two membrane-associated (Hyn, Hup) [NiF] hydrogenases [1,2]. It was recently demonstrated, that the cells, grown in the presence elevated thiosulfate in the medium, can evolve photobiohydrogen [3]. Inversely, in dark, low thiosulfate concentration stimulates the hydrogen production. In both cases, the so-called NAD+-reducing Hox hydrogenase is responsible for the hydrogen evolution. The HoxYH subunit of this hydrogenase is responisble for the proton reduction/hydrogen oxidation, while the *hoxFU* genes encode for the diaphorase subunits. The fifth subunit, the HoxE, is required for the *in-vivo* but not for the *in-vitro* hydrogenase activity, therefore, this subunit likely has an electron transferring role. Moreover, the *in-vivo* hydrogen evolving capacity could be blocked by using Nuo inhibitors and significantly reduced by photosynthesis inhibitors, hence, it seems that the virtually cytoplasmic Hox

hydrogenase has direct connection to the respiratory chain and photosynthesis. Additionally, the strain has complex sulfur metabolism, accumulates elementary sulfur globules and can synthesize various storage materials like glycogen or polyhydroxyalkanoates. Therefore, this strain seemed to be a good model to study various redox and bioenergetic pathways including photosynthesis, respiration, fermentation, hydrogen, sulfur and nitrogen metabolism. Therefore, sequencing of the approximately 5 Mbp genome has been initiated and a hybrid approach was applied.

Although, the genome annotation is not finished yet, the genes of various redox and bioenergetic processes were found. Taking into account for experimental data, an integrated network could be established, which connected the hydrogen, sulfur and nitrogen metabolism to photosynthesis, respiration, fermentation and metabolism of various storage materials.

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MICROBES FOR THE ENVIRONMENTALLY SOUND ENERGY CARRIERS

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One of the extreme challenges for the current civilization is to find alternative energy sources and carriers in order to satisfy the increasing energy demand of mankind. In line with this goal, the global warming caused by the uncontrolled relaese of the green-house gases has to be also stopped. Virtually, there are few renewable alternative energy sources, but for many cases the final energy source is the Sun. The energy of the Sun can be utilized directly by various chemical compounds and by photosynthetic livings. However, the light energy should be converted to storable, transportable energy carriers, which can serve as environmentally sound fuels for various commercial applications. In these days the most popular energy carriers are the bioethanol, biogas, biodiesel and biohydrogen.

There are numerous – direct and indirect physical, chemical and biological – approaches to convert light energy into transportable/storable fuels. One of the main advantages of the biological systems is that they use really renewable catalysts being either whole cells or enzymes.

The direct utilization of light energy can be performed by photosynthetic microbes like algae, cyanobacteria or anaerobic photosynthetic bacteria. In each case the produced environmentally sound fuel is hydrogen. However, both algae and cyanobacteria perform oxygenic photosynthesis by splitting water and producing oxygen, which process is incompatible with hydrogen evolution. Therefore, the photosynthetic growth and the hydrogen evolution should be separated either in time or in space. In our laboratory, we work with anaerobic purple photosynthetic bacteria, like *Thiocapsa roseopersicina* and few *Rhodobacter* species, which are able to utilize sun in a concomitant evolution of hydrogen. As unique features, *T. roseoperscina* contains the most NiFe hydrogenases and has a special hydrogenase linked to the photosynthesis and respiration. The metabolic map connecting the various bioenergetic processes is going to be established after finishing its ongoing genome project.

In the indirect strategies, first, the light energy is converted into biomass, e.g. energy plants, agricultural, animal or commercial organic wastes, haply into their combinations and this biomass should be utilized for production of technologically sound energy forms. In few cases, there is a common step, where the biomass – containing various kinds of barely degradable biopolymers – should be decomposed into monomers, which – in turn – can serve as substrates for fermentative microbes producing a compound of high energy content, like bioethanol or biohydrogen. For biogas

production, the polymer units have to be transformed into acetic acid and/or carbon dioxide, which are reduced to biomethane by methanogens.
In our workgroup, we study few meso- and thermophilic bacteria and several hyperthermophilic Archaea, which evolve substantial amount of hydrogen during fermentative growth. The basic concept of a system was developed recently, in which various keratinaceous organic waste could be converted into hydrogen in a dual-stage fermentative system. Although, the economic index can be further improved, the fundamental idea has been established. In another approach, various energy plants, crops serve as input material in a two-stage system applying a dark and photofermentation step for production of biohydrogen.
The biogas production might have significant dependence on hydrogen evolving microbes, since the reducing power required by methanogenesis can be principally supplied by hydrogen via interspecies mass transfer. The intensification effect of the hydrogen evolving fermentative bacteria on the biogas yield was recently proved at the molecular level for both mesophilic and thermophilic systems.
As summary, there are few alternative optional energy forms for the near and far future. In a short term the technologcial status of the various energy carriers is the decisive viewpoint, but a long term strategy should seriously consider the environmental impact of the various energy forms.

BIODIVERSITY AND FUNCTIONS OF YEAST IN GRAPE/WINE MICROBIAL COMMUNITY

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Although *Saccharomyces cerevisiae* is responsible for the conversion of grape juice into wine, most fermentations involve contributions of several yeast species of *Candida, Hanseniaspora, Metschnikowia, Pichia, Kluyveromyces* which have a profound impact on wine quality. Because the grapes are a primary source of the indigenous yeast flora, the ecology of natural yeast flora is an important factor influencing wine quality. The composition of yeast flora of grapes is influenced by external factors such as ripening stage of the grape, weather conditions, and viticulture practice as well as internal factors such as availability of nutrients, water and competition with other microbial species. The influence of fungicides on composition of grape berry populations and consequently also on kinetics of spontaneous fermentations, biodiversity of *Sacharomyces* and non-*Saccharomyces* yeasts and their population dynamics will be elucidated. The influence of interactions between yeasts and filamentous fungi (phytopathogen *Botrytis cinerea*) on wine yeasts was studied by *in-vitro* (on different solid media) and semi *in-vivo* (on grape berries) studies. The species that showed the best biocontrol characteristics against the grey mould were *Aureobasidium pullulans, Metschnikowia pulcherrima*, *Pichia guillermondii* which performed better characteristics than commercially available species *Candida oleophila*.
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OPTIMALIZATION OF PULSED-FIELD GEL ELECTROPHORESIS FOR ANTIBIOTIC RESISTANT STAPHYLOCOCCI, ENTEROCOCCI, AS WELL AS *LISTERIA MONOCYTOGENES* AND *STREPTOCOCCUS PNEUMONIAE*

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Pulsed-field gel electrophoresis (PFGE) is an accepted and widely used method to determine the genetic (clonal) relatedness of a group of bacterial isolates. Several protocols are used for Gram-positives, which are time-consuming, expensive, and use overnight cell wall lysis by different enzymes, depending on the bacterial species. The original protocol to be optimalised, lysing a few colonies by 40 $\mu g.ml^{-1}$ lysostaphin for 1 h at 37ºC, is mostly sufficient for methicillin and teicoplanin sensitive staphylococci and vancomycin-sensitive enterococci, however, it proved to be insufficient for this purpose in case of a good number of methicillin-resistant *Staphylococcus aureus* (MRSA), teicoplanin-resistant *Staphylococcus haemolyticus* (TRSH), vancomycin-resistant enterococci (VRE), *Listeria monocytogenes*, and *Streptococcus pneumoniae* strains.

The aim of this methodical study was to optimize PFGE for the analysis of chromosomal DNA restriction patterns of such strains and to shorten the procedure time to less than one day. Several variations of the original method were tried by increasing the amount of bacteria, the concentration of the enzymes (lysostaphin, proteinase-K and *Sma*I) as well as the incubation period, but failed to result in appropriate release of DNA. However, adding lysozyme to the lytic phase significantly improved the lysis of the bacteria and the release of DNA available for the restriction enzyme.

Hydrolyzing the cell wall peptidoglycane of MRSA, TRSH, VRE and *L. monocytogenes* with 80 $\mu g.ml^{-1}$ lysostaphin and 3,2 $mg.ml^{-1}$ lysozyme simultaneously for 3 h at 37ºC, and using lysozyme alone for the encapsulated pneumococci, resulted in sufficient lysis in order to release chromosomal DNA abundantly. One hour longer digestion with 66,6 $\mu g.ml^{-1}$ proteinase-K diminished the grey background of the DNA bands obtained with restriction by 10 U *Sma*I/plug. The optimised protocol applies the same buffers for staphylococci, enterococci, streptococci and *L. monocytogenes*.

Due to the thicker and conformationally altered cell wall structures and envelopes of MRSA, TRSH, VRE, *L. monocytogenes* and *S. pneumoniae*, optimal PFGE procedure needs – beside lysostaphin digestion – to cleave the ß-1-4 glycosidic linkage in the peptidoglycane back bone of the cell walls in these bacteria by lysozyme in order to induce complete cell lysis and optimal release of DNA.

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AVENOINDOLIN: A DEFENSIVE PROTEIN IN OAT GRAINS INHIBIT *IN-VITRO* GROWTH OF *RHIZOCTONIA SOLANI*

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Avenoindolines (especially in oat) are low molecular weight antimicrobial proteins. Their primary structure is highly conserved in *Tritiaceae* and *Avenaceae* also they are found in oat (*Avena sativa* L.) seeds as well as other cereal grains such as wheat, barley and rye by general name of indoline. This study was undertaken to investigate the existence of indolines in Iranian cultivars of wheat and barley using anti-puroinodoline (PIN) monoclonal as well as polyclonal antisera. In addition, we tested the *in-vitro* inhibitory activity of indolines from oat, wheat and barley against *Rhizoctonia solani* AG8, the root rot phytopathogen. 'Western' blot showed the presence of at least two different isoforms of indolines with MWs of 15 and 16 kDa in oat (cv. Hinoat 95), wheat (cvs. Azadi, Karaj2) and barley (cvs. Sararoud, Turkman) seeds. Anti-fungal inhibitory activity of indolines was examined as follows. Seeds were powdered in liquid nitrogen using a pestle and mortar. Indolines were extracted in 52% ethanol, centrifuged and then lyophilized. The final pellet was resuspended in small volume of 52% ethanol and then mixed with 0.5% water agar. The mixture was poured over PDA basal

medium in Petri dishes. Fungal mycelial plugs were placed in the center, plates were incubated at 24°C and the radial growth was measured daily. The results showed that indolines extracts from wheat, barley and oat inhibited *in-vitro* growth of R. solani at 35, 54 and 61%, respectively.

IN-VIVO HYDROGEN PRODUCTION ANALYSES OF TRANSPOSON MUTANTS OF *METHYLOCOCCUS CAPSULATUS* (BATH)

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Methylococcus capsulatus (Bath) is a Gram-negative, methylotrophic bacterium, which utilizes methane as a carbon and energy source. The methane monooxygenase (MMOs) enzyme complexes oxidize methane to methanol and co-oxidize a wide variety of aliphatic, aromatic and halogenated hydrocarbons, therefore they are extremely versatile enzymes for biocatalysis and bioremediation. The *in-vivo* electron donor of the MMOs is NADH, which must be regenerated. For the NADH regeneration H_2 could be an alternative source. Under nitrogen fixing condition the nitrogenase produces H_2 as a byproduct. Hydrogenases are metalloenzymes catalyzing the reversible oxidation of molecular H_2. *M. capsulatus* Bath contains a membrane-bound Hup and soluble Hox hydrogenases. The soluble (Hox) hydrogenase is able to reduce NAD^+ using molecular hydrogen, while the Hup plays an important role in the recycling of hydrogen, and donates the electrons to the quinone pool.
H_2 was proven to be a good energy source for reactions catalyzed by MMOs. H_2-driven MMO activities of hydrogenase deletion mutants were measured to obtain information about the *in-vivo* function of the hydrogenases. Surprisingly, the Hup hydrogenase - which is unable to reduce NAD^+ directly - was required for the H_2-driven activity of MMO; while deletion of the soluble Hox hydrogenase did not abolish the H_2-driven MMO activity. We focused on the role of Hup hydrogenase in the hydrogen-metabolism and on the mechanism of providing reducing power for the MMOs. In order to understand the role of Hup hydrogenase in hydrogen-metabolism it is important to find all genes affecting the Hup hydrogenase activity.
The Δ*hupSL* and Δ*hoxH* mutants were generated and a *M. capsulatus* random mutant library was made by transposon mutagenesis. Several Hup- phenotype mutants were isolated, in some of them the transposons were found in the structural gene (for example: large subunit, *hupL*), in an accessory gene (for example: *hupD*) of Hup hydrogenase, or in other genes: e.g. conserved hypothetical protein for NADH ubiquinone/plastoquinone complex and TonB-dependent receptor-like putative protein.
The wild type, and the various mutant strains were grown under nitrogen fixing and nitrogenase repressed conditions to compare their *in-vivo* hydrogen production capacities. The Hup hydrogenase of the wild type *M. capsulatus* consumes the H_2 from the gas phase and the Δ*hupSL* mutant showed the highest hydrogen production. The transposon mutants had higher hydrogen consumption activity than that of the Δ*hupSL* mutant both under nitrogen fixing and nitrogenase repressed conditions.
Further analysis of the mutants is necessary to establish what is the real physiological role of the enzymes disrupted by the transposon insertion in the hydrogen and methane metabolism.

TAXONOMICAL AND PHYSIOLOGICAL INVESTIGATION OF *BACILLUS* STRAINS ANTAGONISTIC TO OYSTER MUSHROOM PATHOGENIC PSEUDOMONADS

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Oyster mushroom (*Pleurotus ostreatus*) is one of the most extensively cultivated mushrooms in the world, but significant loss of crop and quality arises from blotch diseases caused by different bacterial pathogens. The yellowing and the brown blotch disease, caused by *Pseudomonas tolaasii* are well known. Moreover the blotch diseases other fluorescent pseudomonads such as *Ps. agarici*, *Ps. constantinii* and *Ps. gingeri* etc. could cause similar symptoms as well. Sixty strains of an infected *Pleurotus ostreatus* farm in Hungary were investigated, belonging either to the fluorescent or to the non-fluorescent groups. The strains were isolated on *Pseudomonas* selective S-1 medium, from prefermented oyster substrate and water samples. As a result of the *in-vitro* antagonism tests 40 isolates had mushroom growth inhibitory character. To suppress these strains and additional *Ps. tolaasii* type strains we isolated appropriate *Bacillus* strains. The isolation was carried out with a heat treatment of the compost samples at 90°C for 15 minutes which could guarantee the gaining high number of *Bacillus* strains selectively. Forty strains were isolated with this method and 20 of them had no inhibitory effect to the mycelium growth of *P. ostreatus*. After further analysis (ARDRA, rpoB-RFLP, *in-vitro* tests), 10 strains were selected, which suppressed the growth of the harmful pseudomonads in dual cultures. The physiological tests of the strains confirmed high correlation between the enzyme production ability and the pathogenicity. The molecular characterization of these strains was performed by ARDRA and the rpoB-RFLP methods. On the basis of the investigations we could establish that the most powerful antagonists were *B. subtilis* and *B. amyloliquefaciens* strains. These isolates could be used as biocontrol agents, instead of chemical pesticides.

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OXIDATIVE STRESS STUDY OF *SACCHAROMYCES CEREVISIAE* MUTANTS CARRYING GENES DELETED IN SPHINGOLIPID AND ERGOSTEROL BIOSYNTHESIS

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The aim of our study is to examine *Saccharomyces cerevisiae* mutants with genes deleted in sphingolipid and ergosterol biosynthesis while exposed to oxidative or heavy metal stress and their sensitivity to various antibiotics.

Sphingolipids are abundant components of many membranes in eukaryotic cells, but particularly the plasma membrane. In *S. cerevisiae* they comprise about 30% of the phospholipids or about 7% of the mass of the plasma membrane. Yeast membranes comprising the endoplasmic reticulum, Golgi, vacuole and endosomes also contain sphingolipids, with lesser amounts in mitochondrial membrane. It has been unclear whether or not sphingolipids act as signalling molecules in *S. cerevisiae*. The lipid rafts in *S. cerevisiae* cells are enriched for sphingolipids and ergosterol and these two types of lipids are essential for raft formation [1]. Many papers have suggested that rafts play a role in a wide range of important biological processes, including numerous signal transduction pathways, apoptosis, cell adhesion and migration, organization of the cytoskeleton [2].

In our experiments, we have worked with the mutants below containing deleted genes. *ERG2*, *ERG4*, *ERG5* and *ERG6* genes all have an important role in ergosterol biosynthesis while *SUR2*, *SUR4*, *LAG1*, *LAC1*, *CSH1*, *CSG2*, *SUR1*, *IPT1*, *LCB3* and *YSR3* genes are important in sphingolopid biosynthesis. We have determined the minimal inhibitory concentrations (MIC) of these mutants

under the influence of H_2O_2, lipid-peroxide (t-BOOH), menadione (superoxide-radical inducing agent), chromate(VI) and cadmium. We have also determined their sensitivity to amphotericin B and miconazole. Just as expected, mutants carrying deleted genes in the ergosterol synthesis became more tolerant to antibiotics. The MIC of mutants containing damaged ERG6 genes became twice as tolerant to miconazole and six times as tolerant to amphotericin B but 2.5 times more sensitive to t-BOOH. Similarly, *erg4Δ* and *erg5Δ* mutants became more sensitive. The enzyme encoded by *ERG6* gene (encode C-24 sterol methyltransferase) is responsible for transforming zymosterol to fecosterol. Some mutants containing genes damaged in sphingolipid biosynthesis became more tolerant to antibiotics, whereas *csg2Δ* mutant became four times more sensitive to miconazole. *CSG2* encoded enzyme is responsible for transforming inositol-phosphoceramide to mannose-inositol-phosphoceramide. With respect to these results, we have chosen mutants carrying *erg6Δ* and *csg2Δ* mutants for our further experiments. To explain these phenomena, high pressure liquid chromatography must be used to examine whether the lack of some genes influences the composition of cell membrane fatty acids. We are also planning to study how various antibiotics change membrane rigidity with electron paramagnetic resonance spectroscope.

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2. Munro S. 2003. Cell 115, 377.

ANTIMICROBIAL ACTIVITY OF TURNIP EXTRACTS

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Turnips are among the most commonly grown and widely adapted root crops. Turnip roots are used raw or cooked as a vegetable and tops are used as potherb like spinach. Turnip roots are also grown for feeding livestock during fall and winter. In folk medicine, the powdered seed is said to be a remedy for cancer and breast tumors, while a salve derived from can help to treat skin. In this research, the antimicrobial activity of methanol, ethanol, n-hexane and chloroform extracts of turnip was evaluated with bacteria and mold. Alcoholic extracts of turnip were prepared and their antimicrobial activity was tested using an agar diffusion method. The highest antimicrobial activity was observed by methanolic extracts on *Micrococcus* spp. while mold was resistant to this extract. Other alcoholic extracts also showed higher activity on *Micrococcus* spp.

REVERSAL OF MULTIDRUG RESISTANCE BY ESSENTIAL OIL COMPONENTS

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The rate of antibiotic resistance of bacteria and chemotherapeutic resistance of tumour cells shows an increasing tendency, which is a great burden for the treatment of microbial infections and neoplastic diseases. This necessitates the development of new anti-infective agents, chemotherapeutic drugs or resistance modifiers. The kingdom of plants provides a great source for the development of new drugs; one quarter of prescription drugs have been developed from herbs. Thus compounds of herbal origin are worth to study in terms of their multidrug resistance (MDR) reversal properties.

The following essential oil components were studied in *in-vitro* models: p-cymene, eucalyptol, β-cariophyllene, carvacrol, limonene, linalool, α-pinene, β-pinene, sabinene, α-terpinene, γ-terpinene, borneol, cariophyllene-oxyde and thymol. The antimicrobial activities (minimal inhibitory concentrations, MICs) were determined on four bacterial and four yeast strains and the plasmid curing action on the metabolic plasmid of *Escherichia coli* F'lac K12 LE140 laboratory strain, which serves as a model of antibiotic-resistance gene carrier plasmids. Carvacrol and thymol exerted the most pronounced antimicrobial effects, carvacrol and linalool eliminated the metabolic plasmid of *E. coli* F'lac in 45,5% and 61,2% respectively. The antiproliferative activities were examined on human MDR1 gene transfected mouse lymphoma cells alone and in combination with doxorubicin. The MDR reversal activities were also studied in the rhodamine 123 exclusion assay on the mouse lymphoma cells, and the fluorescence activity ratios were evaluated according to the fluorescence activities measured by flow cytometry compared to verapamil control. Based on the morphology of the cells after treatment, four compounds were selected to establish their apoptosis-induction activities. β-pinene and borneol were slightly active in this respect, they caused 8,87-8,83% total apoptosis in the mouse lymphoma cell population in the annexin V assay.
Since essential oils are used in medicine, complementary medicine, cosmetic- and food industry, there is a demand on a profound knowledge of their versatile biological profile. Our experiments aimed at the evaluation of MDR modulator activity of essential oil components and we may conclude that, since some of the compounds exerted MDR modulating action, those essential oils which are made up of the compounds in question, seem to be promising in the treatment of superficial bacterial and neoplastic diseases.

VARIATION OF HUMAN *CAMPYLOBACTER JEJUNI* STRAINS ISOLATED IN THE SOUTHERN REGION OF HUNGARY

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Campylobacter jejuni is the leading cause of bacterial gastroenteritis in the developed world. Although this bacterium has been isolated from a diverse range of domestic and wild animals, poultry is the main reservoir and the commonest source of human infections. Infections are typically sporadic leaving several questions concerning the epidemiology of this disease to be answered. Several typing methods have been applied in the past to identify infection-causing strains of this highly variable micro-organism. In the year of 2006 we established a collection of strains consisting of 190 independent human *C. jejuni* isolates. The aim of this study was to investigate the diversity and to reveal any possible clonality using a *flaA* specific PCR-RFLP test among human *C. jejuni* isolates in the region. As expected, the overwhelming majority of the isolates appeared to be very heterogeneous. However, besides of a few smaller clusters, a large one consisting of 19 strains, was identified exhibiting over 95 % similarity by PCR-RFLP. The possible epidemiological implications of the phenomenon will be discussed.

DETERMINATION OF ZEARALENONE IN MAIZE SAMPLES FROM CROATIA BY TLC/HPLC METHOD

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Fusarium species are world-spread microfungi which can colonise the maize before harvest. They produce various mycotoxins, including zearalenone (ZEA). This toxin shows utherotropic, estrogenic and anabolic properties in domestic animals and there is a possible impact on human health. The objective of this study was to determine frequency of *Fusarium* species and ZEA in maize (N=30) stored in Croatian households (Koprivnica N=15, Karlovac N=13, Vinkovci N=2). The maize was harvested in 2004 and samples were collected and analysed in 2005. *Fusarium* sp. (87%) dominated in samples, followed by species of *Rhizopus* (63%), *Mucor*, *Trichoderma* and *Penicillium* (20%). Extraction and TLC qualitative detection of ZEA were performed [1]. Quantitative determination of ZEA was done using HPLC/UV-VIS method [2] with minor modifications. ZEA was found in 26% of samples in concentration range from 6.03 to 29.43 $mg.kg^{-1}$, mean 13.93 mg/kg. Mean concentrations of ZEA in samples from Koprivnica (3/15) and Karlovac (4/15) were 14.65 and 9.62 $mg.kg^{-1}$, respectively. One sample from Vinkovci contained 29.03 $mg.kg^{-1}$ ZEA. In 2006, 30 samples of animal feed were analysed on ZEA content, and this toxin was not detected in any of samples. According to FAO/WHO (2003) ZEA is now regulated in food in 16 European countries, with limits varying from 0.05 to 1 mg/kg. Relatively high concentrations of ZEA found in maize samples from Croatia could be due to favourable environmental conditions for ZEA production, particularly temperature oscillation. In addition, extraction method in a combination with TLC and HPLC/UV-VIS detection of ZEA may be used for naturally contaminated samples.

1. Balzer et al. 1978. JAOAC., 61, 584.
2. Frisvad et al. 1987. J. Chromatogr., 404, 195.

DIVERSITY OF AVIAN *PASTEURELLA MULTOCIDA* ISOLATES IN HUNGARY

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Pasteurella multocida is the etiological agent of fowl cholera, a widely distributed and economically important disease of various avian species (especially chickens, turkeys, duck and geese). Strains of serogroup A are recognised as the primary cause of fowl cholera whereas isolates of serogroup B, D and F are less frequently associated with the disease. At the same time, serogroup A is also associated with respiratory diseases in cattle, swine and other ruminants. It is supposed that *P. multocida* strains of serogroup A have divers DNA background. Wide range of molecular studies pointed out the extreme diversity of these strains, particularly that of avian isolates. The outer membrane proteins (OMP) of gram-negative bacterial pathogens serve as an interface between the bacterium and the host cells and play essential role in the disease processes. Analyses of OMP try to identify proteins that may be useful virulence markers and thus support epidemiological surveys.

The aim of the study was to investigate ERIC-PCR (enterobacterial repetitive intergenic consensus sequencer polymerase chain reaction) pattern and OMP diversity among avian *P. multocida* strains isolated from poultry (goose, duck, turkey, chicken, pheasant) in Hungary. Earlier we analyzed our strains, with traditional fermentation probes, *P. multocida* specific PCR, capsular multiplex PCR and so-called biovar test.

Based on traditional characterisation, biovar 3 and 1 were dominant among our isolates and the majority of the strains belonged to *P. multocida* subspecies *multocida* that was independent from the host species. With capsular specific PCR we detected high prevalence of serogroup A while the rate

of capsular type F strains was very small. Because these techniques gave neither proper degree of discrimination within species nor data to host specificity, we used further molecular methods for studying our isolates. With ERIC-PCR we were able to detect relationships between the isolates and discriminate *P. multocida* isolates from different hosts. Analysis of OMP diversity will contribute to our understanding of host-pathogen interaction in fowl cholera, because the similar pattern could refer to unique immunological features.

IMPROVED DIAGNOSTIC METHOD IN FUNGAL SEPSIS BY REAL-TIME PCR

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The significance of nucleic acid amplification techniques such as PCR assay is rising. Polymerase chain reaction method is a rapid and reliable solution for identifying and differentiating fungal pathogens. In the case of real-time PCR, identification and differentiation can be performed by using specific (TaqMan, FRET) or aspecific dyes (SYBRGreen I). Most of the real-time PCR equipments, after the last PCR cycle make it possible to determine the melting temperature of the amplicon or the probe when using SYBRGreen I or FRET probes. This melting-point analysis is applicable on differentiate fungal pathogens in genus or species level. After using FRET probes, some of the rare *Candida* species requires further differentiating methods. Our idea was to use consensus primer pair and so the discrimination was achieved through consecutive melting-point analysis with the help of the non-specific fluorescent dye SybrGreen I. In this way, all of the fungal species are detectable, and the most frequent and important *Candida* species can be differentiated. The problematic aspect of the method is that the different master mixes not linearly shift the melting-points. Therefore, it is required to optimize carefully and control is necessary. Here, we report the melting-point changes in the case of different master mixes such as self-developed, BIO-RAD IQ SuperMix, SIGMA SYBR® Green Taq ReadyMix and Roche LightCycler-DNA Master SYBRGreen I.

B-LACTAM RESISTANCE AMONG ANAEROBES

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Anaerobic pathogens are often undervalued in infectious diagnostics mainly because of the complicated culturing and special knowledge requirements. However, they can cause severe infections that are difficult to treat. The antibiotics most frequently used in human infections are the β-lactams, which can be administered to treat anaerobic infections with limitations in respect of the infecting organisms and the type of antibiotic.

It is a rough general rule that the susceptibility of anaerobic pathogens is inversely related to their clinical significance. β-lactam resistance has been documented in all groups of these pathogens, mainly members of the Gram-positive cocci, *Bilophila*, *Clostridium* and Bacteriodaceae, though with widely differing prevalences. The main resistance mechanism in these cases is the production of β-lactamases. *Bacteroides*, *Prevotella*, *Porphyromonas* and *Fusobacterium* spp. often produce Class A

cephalosporinases. In this group of species, a β-lactamase conferring cephamycin resistance can also be found. The resistance gene (*cfxA*) is coded on a mobilizable transposon responsible for the horizontal spread. This gene can also be found on plasmids of aerobic relatives of *Bacteroides*. The most important β-lactamase of *Bacteroides* is the metallo-β-lactamase, CfiA, of *B. fragilis*. It is chromosomally coded, and for its expression, insertion sequence elements are needed as in many antibiotic resistance mechanisms of *Bacteroides*. It destroys all β-lactams except monobactams, belongs in Class B and requires Zn^{2+} for its activity. *cfiA*-positive *B. fragilis* strains form a subgroup of the species. Another carbapenemase has been reported to occur among *B*. (*Parabacteroides*) *distasonis*. In the cefoxitin resistance of *Bacteroides* spp., the role of penicillin-binding proteins has been demonstrated. *C. difficile* is inherently resistant to cephalosporins and imipenem, but not to meropenem and amoxicillin.

DIFFERENTIAL DIAGNOSTIC OPPORTUNITIES IN FUNGAL SEPSIS BY REAL-TIME PCR

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The significance of invasive fungal infections caused by *Candida* and *Aspergillus* species is rising due to intensive medical intervention. The fungal infections represent the fourth most common nosocomial blood-stream infection. Although, *Candida albicans* remains the species most commonly isolated other newly emerging and antifungal resistant *Candida* species such as *C. glabrata*, *C. krusei* and *C. lusitaniae* are increasingly common. The rapid identification of these fungal species may facilitate optimal antifungal therapy and patient management. The current methods involving biochemical and phenotypic tests may require up to 96 h for species identification from the initial time of blood culture bottle positivity. The DNA amplification assays rapidly detect the fungal pathogen but the postamplification analysis step commonly based on electrophoretic migration or hybridization with specific nucleotide probes is time-consuming. These procedures involve a potential risk of contamination and are labour-intensive, too. The solution is to use real-time assays to detect and identify the different fungal species. The previously described real-time multiplex PCRs built up by species-specific primer pairs or universal primers with species-specific probes. These methods concentrate on just some of the most frequent *Candida* species and so the independent general PCR method was necessary for detecting the rare fungal species. One versatile solution is the real-time PCR with universal primers and a general probe. Thus, it is possible to detect and differentiate more fungal species with the help of the melting-point analysis and the appropriate probe. Unfortunately, some of the rare *Candida* species requires further differentiating methods, too. Our idea was to use consensus primer pair and so the discrimination was achieved through consecutive melting-point analysis with the help of the non-specific fluorescent dye SybrGreen. In this way, all of the fungal species are detectable, and the most frequent and important *Candida* species can be differentiated.

BIAS OR NOT: THE DIVERSITY OF FLAVOBACTERIA

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The occurrence of members of the *Bacteriodetes* genus *Flavobacterium* in a wide range of environmental samples has been well recorded by analyses of 16S rRNA gene sequences of isolates and from DGGE bands and clones of following PCR amplification of environmental DNA. In order to analyze the function of prokaryotes in the formation of tufa in a 400 m long hardwater rivulet (Harz Mountain, Germany). About 1000 isolates from the spring and three downstream sites and a randomly selected portion of 100 strains were analyzed by MALDI-TOF. More than 50% of the isolates are members of the genus *Flavobacterium* and 16S rRNA gene sequence analysis pointed towards the presence of many novel genospecies. While the cell number of bacteria as well as the diversity of flavobacteria on the spring sampling site was low the numbers and diversity increased with the water flow. As the river flow is rather rapid the question about the origin of the flavobacteria was further elucidated. Soil samples from 10 different sites adjacent to the rivulet were taken, DNA extracted and subsequently amplified with *Flavobacterium*-specific primers. Each soil sampling site possessed a rich (and by and large unique) *Flavobacterium* community. Sequence analysis of 80 DGGE bands verified the specificity of the primers as, besides a few *Bacteriodetes* sequences, only flavobacterial DNA was amplified. Comparison of the short DGGE-derived sequences with the partial sequences of the isolates from the rivulet indicated a low degree of identity (~15%). However, as compared to the type strains of the genus Flavobacterium (~8%), the overlap with the isolates was significantly higher.

Both methods, culture-dependent and culture-independent are well described for their bias. While, among other factors, medium composition, temperature, growth rate and picking strategy influence the selection of isolates, PCR amplification, operon microheterogeneity, and lack of visibility of taxa occurring as part of the minority population are, among other factors will influence the DGGE patterns. Consequently, a significantly higher portion of the isolates need to be investigated in order to determine a statistically significant origin of the rivulet flavobacteria from the soil. This work is presently in process but results will be presented. The richness of flavobacteria in water and soil stands in contrast to the flavobacterial composition in a tufa core sample, taken from a downstream site. Applying the same isolation techniques than for the water samples to a series of eight horizontal core layers (~4-6 mm thick) the proportion of flavobacteria and the phylogenetic diversity is much smaller. Representatives of this genus were isolated from a few layers only, including the top biofilm layer. DGGE analysis of flavobacteria-specific DNA reflects this picture in that the complexity of well resolved banding patterns diminishes with depths. Whether or not flavobacteria play a decisive role in the formation of stromatolites will be addressed on future metagenome studies.

NUCLEIC ACID AMPLIFICATION TECHNIQUES IN THE DIAGNOSIS OF STIS'

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Nucleic acid amplification techniques (NAATs) are the most recent and important advantage in the field of diagnosis of chlamydial and gonococcal infections and enable the detection of a low number

of organisms in different specimen types in men and women with a high sensitivity and specificity. The number of NAATs, which are FDA approved for both, chlamydial and gonococcal diagnosis, has increased during the last years. In addition to DNA amplification by PCR (COBAS Amplicor) and by strand displacement amplification (ProbeTec), the amplification of chlamydial and gonococcal RNA is used in the highly sensitive and specific Transcript Mediated Amplification assay (TMA). The Gen-Probe APTIMA Combo 2 Assay is a second generation NAAT that utilizes target capture, transcription mediated amplification of RNA and dual kinetic assay technologies. This assay qualitatively detects *Chlamydia trachomatis* (APTIMA CT) or *Neisseria gonorrhoeae* (APTIMA GC) or both (APTIMA Combo 2) in endocervical and urethral swab specimens as well as in urine samples from symptomatic and asymptomatic individuals and is already FDA approved for testing vaginal swabs. The advantage of the NAATs is their ability to detect organisms even with a low target concentration in specimens. This especially occurs in genital samples of asymptomatic individuals and their contact persons without signs of inflammation. The use of noninvasive specimens such as first void urine (FVU), vaginal swabs, and introital specimens contaminated with *C. trachomatis* or *N. gonorrhoeae* is an important approach for screening possibilities in individuals at risk for being infected. Furthermore, the number of organisms present at atypical infection sites such as rectal or pharyngeal regions might be low and amplification tests may therefore be recommended as the preferable diagnostic technique. While for chlamydial diagnosis NAA assays are now recommended as the gold standard method, molecularbiological techniques for gonococcal diagnosis are recommended in case of transport or storage problems as well as when culture is not performed in the appropriate way.

EFFECT OF HIV-1 VIRAL PROTEIN R (VPR) ON CELLULAR FUNCTION OF FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*

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All organisms must have specific and precisely balanced internal conditions for optimal growth and function. The internal environment of the cell is maintained to promote proper operation of the cell; however fluctuations in the external milieu can result in a variety of cellular perturbations that can disrupt the internal environment. Human immunodeficiency virus type 1 (HIV-1) expresses various accessory proteins Nef, Vif, Vpu, and Vpr, that collectively influence different host-cell processes in order to promote viral replication, persistence and immune suppression. Many important functions related to HIV-1 pathogenesis have been qualified to these accessory proteins. Particularly, viral protein R (Vpr) has been implicated in long terminal repeat transactivation, nuclear import of the pre-integration complex, induction of cell cycle G2/M arrest, and apoptosis of the host cells. However, little is known at present about specific roles of Vpr relate to oxidative stress induced by reactive oxygen species (ROS). Fission yeast *S. pombe* is then likely to be a tractable model system in which to study these functions of Vpr. In this study, *S. pombe* parental strain (Sp223) and RE007 (NL4-3) strain which carries a single integrated copy of Vpr under the control of an inducible nmt1 promoter were used. To validate Vpr inducibility under Vpr-inducing and -repressing conditions cell morphology, Vpr induced G2 arrest, conventional septation index were determined at different time points of early log and early stationer phases. In parallel, ROS, including superoxide anions, hydroxyl radicals, and hydrogen peroxide levels were determined. Based on determined activities and protein

levels of antioxidant enzymes, such as catalase (CAT), superoxide dismutases (SOD), glucose-6-phosphate dehidrogenases (G6PD), glutathione-S-transferases (GST), glutathione reductase (GR), glutathione-peroxidase (GPx) and glutathione (GSH) we concluded that, HIV-1 Vpr protein might influence generation of oxidative stress. To validate enzyme assays real-time PCR studies are currently in progress.

MULTIDRUG RESISTANCE REGULATION IN YEAST

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Multidrug resistance, or PDR, is defined as the cell's ability to become resistant to a multitude of structurally and functionally different cytotoxic compounds. It is caused by the overexpression of membrane associated protein pumps due to gain-of-function mutations in genes encoding transcriptional regulators implicated in the control of multidrug resistance. Molecular mechanisms of multidrug resistance are best understood in the yeast *Saccharomyces cerevisiae*. Pdr1p and Pdr3p are two zinc cluster proteins that have been named the master regulators of drug resistance in baker's yeast. Mutational analysis of Pdr3p revealed several amino acid substitutions that increased the activity of transcriptional activator, enhanced the expression of major drug efflux pump Pdr5p and resulted in multidrug resistance in mutant yeast cells. Using a recently elaborated genetic screen for positive selection of loss-of-function *pdr* mutants two *pdr3* mutant alleles containing point mutations were isolated. They failed to complement the drug hypersensitivity in the *Dpdr1Dpdr3* mutant strain and surprisingly upon overexpression they enhanced the drug susceptibilities both in the wild type and drug resistant yeast strains. Molecular characterization of loss-of-function *pdr3* mutant alleles revealed that the C-terminal activation domain and the Asp residue at position of the 853rd amino acid are essential for the transactivation function of Pdr3p.
The understanding of molecular mechanisms of multidrug resistance regulation in baker's yeast is of interest in providing information towards designing new strategies to overcome infection caused with drug resistant pathogenic yeast species.

THE TIME-PROFILE OF CELL GROWTH IN FISSION YEAST: MODEL SELECTION FOR WILD-TYPE CELLS

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In the first half of the 20th century yeasts have become model organisms in different fields of cell biology. Since the late '50s *Schizosaccharomyces pombe* (also known as fission yeast) has been spotlighted through its favourable physiological features, for example, its symmetrical division enables good synchronisation techniques, which are necessary for cell cycle studies. The cylindrically shaped fission yeast cells grow exclusively at their tips almost from birth to division by maintaining a constant diameter, therefore cell length is approximately proportional to cell volume. As a consequence, cell length is an easily measurable parameter, which characterizes cell age, i.e., progression through the cell cycle. Length growth patterns may therefore indicate connections

between volume changes and cell cycle events. The classical method to study the growth of individual cells is time-lapse microphotography; cells are growing on the surface of an agar pad in a thermostated photomicroscope, and later on we can study the growth pattern of cell length simply by a projector. In different cell types, there is considerable controversy concerning the exact growth profile of size parameters during the cell cycle. Linear, exponential and bilinear (i.e., two linear segments with a rate change point) models are commonly considered, and the same model may not apply for all species. Selection of the most adequate model to describe a given data-set requires the use of quantitative model selection criteria, which are suitable for comparing differently parameterized models. Length increase data from two individual fission yeast cells (one wild-type and one *wee1*Δ mutant), measured on time-lapse films have been reanalyzed using these model selection criteria. To fit the data, a recently introduced linearized biexponential model was developed, which makes possible a smooth, continuously differentiable transition between two linear segments. (This function might be even more realistic than using two linear segments with a breakpoint.) Essentially all the quantitative selection criteria considered here indicated that the bilinear model was somewhat more adequate than the exponential model for fitting these two fission yeast cell data. Although the bilinear model seems more adequate, especially in the case of the *wee1*Δ cell, the statistical evidence is not strong enough to favour one model clearly over the other. Using these model selection criteria, we have also studied length growth patterns of 30 wild-type cells; their analyses and our conclusions will be presented at the conference.

ASSOCIATION OF BETA-LACTAMASE PRODUCTION WITH OTHER PARTICULARLY QUINOLONE RESISTANCE MECHANISMS

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Resistance to beta-lactam antibiotics by beta-lactamases is an increasing problem for clinical therapeutics. Such resistance in *Klebsiella pneumoniae* to third-generation cephalosporins is typically caused by the acquisition of plasmids containing genes that are encoded for extended-spectrum beta-lactamases (ESBLs), what is more, these plasmids often carry other resistance genes as well.
ESBL-producing strains show high levels of co-resistance to aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin. The reasons for that, the plasmids bearing the genes encoding ESBLs frequently carry genes encoding resistance to aminoglycosides and trimethoprim/sulfamethoxazole as well. There have also been increasing reports of plasmid-encoded decrease in susceptibility to quinolones, frequently in association with plasmid-mediated cephalosporin resistance. Although quinolone resistance commonly results from chromosomal mutation, recent studies indicate that such resistance can also be transferred on plasmids carrying the gene responsible, *qnr*. The Qnr proteins, capable of protecting DNA gyrase from quinolones. AAC(6′)-Ib-cr, a variant aminoglycoside acetyltransferase capable of modifying ciprofloxacin and reducing its activity, seems to have emerged more recently. Both mechanisms provide low-level quinolone resistance that facilitates the emergence of higher-level resistance in the presence of quinolones at therapeutic levels. Even when plasmid-encoded decrease in quinolone susceptibility is not present, there is a strong association between quinolone resistance and ESBL production. Much remains to be understood about these genes, but their insidious promotion of substantial resistance, their horizontal spread, and their co-selection with other resistance elements indicate that a more cautious approach to quinolone use and a reconsideration of clinical breakpoints are needed.

TESTS OF MICROBIOLOGICAL HYDROCARBON DEGRADATION ON CONTAMINATED GROUNDWATER SAMPLES

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The effectiveness of microbiological clean up methods is discussed that were applied in practice at hydrocarbon contaminated sites in Hungary. The main goal that we focused was the *in-vivo* applicability of the microbes to degrade hydrocarbon compounds. The experiment was carried out using contaminated groundwater from three sites in Hungary: pipeline brake, Ópusztaszer (site #1); oil refinery, Zala county (#2); former oil base, Szarvas (#3). Non pathogen, selected Bacteria strain was used to inoculate the contaminated groundwater, i.e. the CHB-20, CHB-15, AK-37, and AK-40 laboratory strain. Using groundwater samples from these three hydrocarbon polluted sites, an *in-vitro* test was carried out to prove the real hydrocarbon degrading ability of four laboratory strains. Three parallel solutions were made from the contaminated groundwater sample. The Control (K) contained nothing else only the sample. In the OIR labelled solution nutrients were given to the sample. In the "4" labelled test the same nutrients and the 4 hydrocarbon degrading microbes were given to the contaminated groundwater sample. Laboratory methods were worked out to avoid the dilution of the starting concentration. After one week of shaking together the TPH content of the solutions were analytically measured. It was found in site #1 and #2 that 4 strains could reduce the hydrocarbon concentration in samples within a short time. In Szarvas (site #3) we found that the 4 strains can reduce the contamination to the quite same level like OIR labelled solutions. So the sampling and experiment was repeated on the same groundwater well sample, but some test portions were sterilized to show the differences between the ability of the 4 strains and the autochtonous microbes.

First portion of the sample was the non-sterile control (NSK) to show the loss of contaminants during the shaking phase. In the second test only nutrients were given and the sample was not sterilized (ONS) to show the degrading ability of the autochtonous microbes. In the third test the sample was only sterilized (SK) to show the measure the abiotic loss during the sterilizing and shaking method. For the "S4" labelled test the 4 strains were given to the sterilized sample to show the ability of degradation without autochtonous microbes. In the last test (OS4) the 4 strains and nutrients was also given to the sterilized sample to show how the nutrients could help the degradation of hydrocarbons. After one week of shaking the quantity of TPH contaminations in the water wasOS4: 9260 $\mu g.l^{-1}$, S4: 14300 μgl^{-1}, SK: 20400 μgl^{-1}, ONS, NSK:>100000μgl^{-1}. It means that the 4 laboratory strains have the ability to degrade hydrocarbon compounds in polluted areas, but the success of the inoculation is depending on the features of the site.

As a result of our paper it can be established that the on site applicability of *in-vitro* well degrading inocula always depends on the characteristic of the contaminated sites.

OUTBREAK OF COLONISATION BY EXTENDED SPECTRUM B-LACTAMASE (ESBL) PRODUCING *SERRATIA MARCESCENS* STRAINS IN A NEONATAL INTENSIVE CARE UNIT

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The ESBLs of Gram-negative rods are responsible for the most rapidly evolving mechanisms of resistance in pathogenic bacteria under the selection pressure of antibiotic use. It is well known that the mortality of nosocomial infections caused by ESBL producing strains is significantly higher when carrier status remains unexplored and the patient is treated with cephalosporines. The prevalence of extended spectrum beta-lactamase (ESBL) producing Gram-negative pathogens in University of Debrecen shows an increasing tendency.
ESBL producing *Serratia marcescens* strains were isolated from different specimens of 32 newborns in Neonatal Intensive Care Unit of University of Debrecen during one year period (between April 2006 and 2007). The isolates were recovered as follow: throat (45 cases), tube (29 cases), conjunctiva (3 cases), ear and nose (2-2 cases) and pharynx (1 case). The strains were identified using routine criteria and VITEK 2 system. All isolates showed ESBL producing phenotype using E-test and VITEK 2 system. On the basis of disk diffusion results the strains showed *in-vitro* susceptibility only to carbapenems and fluoroquinolones. The phage typing and macrorestriction profile analysis of isolates showed that all of them belonged to the same genetic clone. The *bla*SHV gene was detected in representative strains using a multiple PCR method. Direct sequencing demonstrated the presence of SHV-5 type ESBL. The *Serratia marcescens* caused only colonisation in newborns.
We could not find the source of outbreak, the screening studies on nursing staff and the environment were not successful.

ETIOLOGICAL ROLE OF HPV AND TTV INFECTION IN ORAL CARCINOGENESIS

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Oncogenic viruses, such as mucotropic human papillomaviruses (HPV) may play a role as an etiological factor in the development of oral squamous cell cancer (OSCC). However, the virus infection alone is not sufficient for tumorgenesis, other factors, e.g. physical and chemical carcinogens or other infective agents need to malignant transformation. Coinfection with genogroup 1 torque-tenovirus (TTV) may influence tumorgenesis or disease progression. In a previous study we found significantly poorer clinical outcome of laryngeal cancer in patients infected with HPV and genogroup 1 TTV simultaneously. Our aim was to evaluate the prevalence of HPV and genogroup 1 TTV in OSCC and oral premalignancies compared to healthy individuals.
HPV detection and genotyping was performed using the MY/GP consensus PCR and restriction enzyme analysis of the amplimers. Genogroup 1 TTV DNA was detected by NG059/063 and NG061/063 primer pairs in a nested PCR. Prevalence data were analyzed with chi-square and Fisher exact tests. HPV positivity was 50.1% (28/55), 40.5% (15/37) and 32.7% (32/98) in OSCC, oral leukoplakia (OL) and oral lichen planus (OLP), respectively, while only 4.2% (3/72) in the control group. Dominantly high risk genotypes were found in all patient groups. Genogroup 1 TTV was found in 23.6% (13/55) in OSCC patients. Contrastingly, only 5.4% (2/37), 8.2% (8/98) and 1.4% (1/72) of clinical samples carried TTV DNA in OL, OLP and control population, respectively. HPV-TTV coinfection was detected in 6 of 13 OSCC patients, but we did not find significant difference in the prevalence of dual infection in OLP and OL groups compared to controls.

Analyzing the prevalence of viruses in the apparently healthy mucosa of the patients, HPV prevalence was significantly higher in OSCC and OL groups, while TTV DNA was detected with significantly higher frequency only in OSCC patients. We did not find differences in HPV, TTV and coinfection prevalence in the healthy mucosa in any other comparisons.
These data support the possible role of HPV in oral carcinogenesis and raise the possibility of the enhancing effect of genogroup 1 TTV on development of oral malignant tumours.
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EFFICIENCY COMPARISON OF FUNGICIDES AGAINST FUNGAL PATHOGENS OF CULTIVATED MUSHROOM

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The cultivation of common mushroom (*Agaricus bisporus*) is threatened most strongly by fungal pathogens. The crop loss in Hungarian mushroom cultivation caused by mycoparasites can reach 70 - 80%. The most important fungal pathogens are *Verticillium fungicola*, *Mycogone perniciosa*, *Dactylium dendroides*, *Trichoderma agressivum*. However, the loss caused by competitive moulds like *Scopulariopsis fimicola*, *Papulospora byssina*, *Botryotrichum piluliferum*, *Penicillium* spp., *Mucor* spp., *Aspergillus* spp. may be also considerable under favorable conditions.
There protection against the pathogens is markedly difficult: (i) the number of pesticide preparates permitted in mushroom cultivation is fairly low, (ii) the microfungi can rapidly develop resistance against the fungicides, (iii) some important pesticides were withdrawn by EU regulation. The biological protection tools with good laboratory performance proved not to be enough efficient and reliable.
The aim of our work was to elaborate a chemical protection regime that is based on pesticides having permission from EU regulation bodies, efficiently protecting mushroom against the pathogens, and having lower environmental risks. Large collection of different mushroom pathogens was established after a countrywide isolation procedure. In vitro fungicide sensitivity and tolerance increase potential studies were achieved both with regular pesticides used in the mushroom cultivation practice and with other fungicides having permission for other cultures. A small plot trial was carried out with selected fungicides under working conditions of regular cultivation plant. The phase delay, the occurrence of symptoms as well as the crop quality and quantity were observed after provoked infections.
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NOVEL VACCINE TECHNOLOGIES

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With the continuing emergence of new infectious diseases, the development and potential destructive use of bio-warfare agents, the increase in international transmission of current epidemic diseases due to globalization, there are compelling reasons to focus resources, people and attention on improving

the capabilities of vaccines, decrease the development cycle time and develop more efficient manufacturing methods.
Majority of vaccines have been and continue to be developed through the classical approach of generating an attenuated or inactivated pathogen as the vaccine itself. This approach does not take advantage of the explosion in our understanding of host-pathogen interactions and immunology. Rather, it remains an empirical approach that consists of making variants of the pathogen.
For "Hit-and-Run" pathogens (e.g., Yellow Fever vaccine), current vaccines are mostly effective, although the technologies used today were developed in the 1960's. For "Hit-and-Hide" pathogens (e.g., HIV, TB, HCV, PRRS), it is difficult to develop vaccines as these pathogens have developed mechanisms to escape the immune system and cause chronic infection. Pathogenic microorganisms, such as bacteria and viruses, have molecular structures that are not shared with their host, are shared by many pathogens and evolutionarily conserved. Collectively, these molecular structures are referred to as pathogen-associated molecular patterns (PAMPs). Successful pathogens, especially those that can establish chronic infections, have molecular patterns that allow them to evade, or escape, the body's immune response. Effective vaccines against these pathogens are difficult to develop using traditional vaccines composed of attenuated, killed or genetically modified pathogens, since the immune response is directed by their particular molecular patterns.
In light of today's concerns over national security, rising health care costs, and the rise of antibiotic-resistant-strains, we need to develop, test, and assess both preventive and curative vaccines and immune enhancers faster than ever before. There are new developments in the selection methods of antigens, such as bioinformatics and biopanning, etc. New and more efficient adjuvant molecules have been developed, such as different TLR agonists molecules. There are also new developments in vaccine carriers, which allow us to take advantage of the readiness of the immune cells in the mucosal and skin surfaces. This presentation will give an overview of these new developments.

SEQUENCE COMPARISON OF RNA-DEPENDENT RNA-POLYMERASES INDICATES DIFFERENT ORIGIN OF BEET CRYPTIC VIRUS 1 AND -2

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Viruses with dsRNA genomes are widespread in nature and infect hosts ranging from bacteria and fungi to plants and animals. Their genome is often arranged in multiple segments. The most widely distributed dsRNA viruses of higher plants are the cryptic viruses. Cryptic viruses present several peculiar features (they are seed and pollen transmitted, symptomless, not graft transmitted) that makes their study rather challenging. The genome of *Beet Cryptic Virus 1* and *-2* (BCV1 and -2) is composed of two dsRNA segments. To replicate their genomic RNA, viruses encode an RNA-dependent RNA polymerase (RDRP). This enzyme must have played a vital role early in evolution and also has crucial functions in contemporary biology (genome replication, mRNA synthesis, RNA recombination, etc).The other viral protein encoded by practically all viruses is the coat protein (CP) which encapsidates the genomic RNA.
The aim of our work was to characterize the genomic dsRNA of BCV1 and BCV2 viruses by cDNA-cloning and sequence analysis and to analyse the relatedness and possible origin of the viruses by sequence comparison. As expected, in both cases one of the genomic segments contained a contig of a single continuous open reading frame encoding the putative RDRP. BCV1 RDRP had a molecular

weight of 65.9 kDa, the BCV2 enzyme was slightly smaller (54.2 kDa). Both deduced amino acid sequences contained characteristic sequence motifs (motifs III-VIII) found in genes that encode putative RNA-dependent RNA-polymerase (RDRP) of RNA viruses. According to multiple RDRP alignments, BCV1 and BCV2 belong to clearly different groups of cryptic viruses. BCV1 RDRP shows very high homology to Vicia Cryptic Virus, White Clover Cryptic Virus 1 and to some fungal viruses of the Partitiviridae family, whereas BCV2 resembles a group of cryptic viruses, which, to our present knowledge, occur only in plants (Raphanus sativus Cryptic Virus 2, Pinus sylvestris partitivirus, Beet Cryptic Virus 3, Pepper cryptic virus 1, Pyrus pyrifolia partitivirus).

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IN-VITRO PROBIOTIC PROPERTIES OF NON-STARTER LACTIC ACID BACTERIUM *LACTOBACILLUS CASEI* SUBSP. *CASEI*

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Lactobacilli (LAB) play an important role in the human microflora and they are also present in several fermented dairy products such as yoghurt or cheese. They exert their beneficial effects via the production of various antimicrobial metabolites, like acids, bacteriocins or hydrogen peroxide. Furthermore some strains can adhere to the intestinal surfaces which help them to colonize the gastrointestinal tract. Adhesion ability is also a prerequisite to exert certain beneficial effects, such as the exclusion of enteropathogenic bacteria. LAB are able to stimulate the immune system, modify the balance of the cytokine production of the intestinal epithelial cells. Intestinal epithelial cells, however, induce high levels of interleukin-8 (IL-8) secretion in response to the colonization of enteropathogenic bacteria such as *Salmonella (S.*) *enteritidis*. In addition, when gut epithelial cells are exposed to various stresses, like heat, cold or infection they express protective proteins, heat shock proteins (Hsp).

The aim of the study was to find out whether *L. casei* subsp. *casei* or their spent culture supernatant could protect intestinal epithelial cells against *S. enteritidis* infection. For these purposes IL-8 and Hsp70 production of intestinal epithelial cells were determined. Furthermore the adhesion ability of lactobacilli was also tested. As an *in-vitro* model a human colon adenocarcinoma cell line called Caco-2 was used, because this cell line shows structural and functional characteristics of small intestinal villus-like cells.

It was found that the lactobacilli induced IL-8 expression was significantly lower compared to the amount of IL-8 induced by *S. enteritidis*. Pre-treatment of *S. enteritidis* 857 with the spent culture supernatant from lactobacilli inhibited the IL-8 expression and induced Hsp70 production in Caco-2 cells. *L. casei* subsp. *casei* treated Caco-2 cells also expressed Hsp70. Although the *Lactobacillus* strain investigated exerted immunomodulatory properties, it showed poor adhesion ability to Caco-2 cells. In conclusion the beneficial effect of LAB on the intestinal inflammation might be in connection with the decrease of IL-8 levels and adhesion is not certainly required.

These results show that this effect of LAB is mediated at least in part via the secreted antimicrobial products, which work directly against the enteropathogenic bacteria or indirectly through the synthesis of Hsp70.

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SERUM-FREE CULTURE MEDIA BASED ON CYCLODEXTRIN/LIPID COMPLEXES

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Due to the increasing incidence of the occurrence of virus and prion related contaminants in certain biotechnological processes and products using animal-derived additives, has motivated authorities in the pharmaceutical industry to try to minimize the probability of the occurrence of such contaminants in biotech products.

Serum-free cultivation systems are complex media containing all necessary components that cells require for their normal growth and propagation, except that certain animal serum components are not present. Serum-free media may also describe a class of media that do not require supplementation with serum. They may contain discrete proteins or bulk protein fractions. Proteins such as albumin serve as carriers and protective agents for other molecules present. Successful serum-free media development results in commercially available media formulations which require no serum additives for many relevant cell types.

Lipids, cholesterol, fatty acids essential to the proliferation, growth and survival of all cells are solubilized and stabilized against oxidative degradation and free radical generation by serum proteins, like BSA, in conventional culture media. However, in the serum-free culture media the solubilization and protection of lipids have to be provided by a suitable additive of non-animal origin. This kind of additive can be a plant-originated encapsulating agent like cyclodextrin, which provides a dual function in one: it effectively solubilizes lipids and protects them against surroundings by molecular encapsulation. The cyclodextrin selected for molecular encapsulation is a randomly methylated derivative containing 7 glucose units in a molecule (RAMEB). The product is a freely soluble powder with 2-5% lipid/fatty acid content. The solubility of the lipid/fatty acid is >3 $mg.ml^{-1}$

Lipid/fatty acid	Active ingredient content
Ascorbic palmitate	2.0-2.5%
Cholesterol	4.5-5.0%
b-Estradiol	4.5-5.0%
Ergocalcipherol	3.5-4.0%
Lauric acid	2.5-3.0%
Linoleic acid	2.5-3.5%
Linolenic acid	2.0-2.5%
Myristic acid	5.0-5.5%
Octanoic acid	8.0-9.0%
Oleic acid	3.5-4.0%
Palmitic acid	2.0-2.5%
Na palmitate	2.0-2.5%
Retinyl acetate	1.5-2.0%
Tocopherol	3.0-3.5%

A careful optimization of culture media additives based on CD-solubilized lipids has become a routine method to replace serum albumine in cell-and tissue culture media and they have been successfully used in practice.

REGULATION OF CD23 EXPRESSION BY DNA METHYLATION AND HISTONE ACETYLATION

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The human leukocyte differentiation antigen CD23 (the low-affinity receptor for IgE) is a key molecule in normal B cell differentiation and activation, Epstein-Barr virus (EBV) induced B cell immortalization, and regulation of the IgE response to allergens and to parasitic infection. Two isoforms, CD23a and CD23b are transcribed from two alternative promoters (CD23ap and CD23bp) and differ by only six amino acids in the cytoplasmic N-terminus. CD23ap is flanked by several long repetitive elements and both the region immediately upstream of the transcription initiation site and the 5' end of the first intron regulates it's activity. CD23 expression is tightly regulated throughout B cell development and both promoters are transactivated by the type III latency proteins of EBV. As DNA methylation plays an important role in the control of many developmentally regulated genes, we analyzed the DNA methylation patterns of CD23ap and CD23bp with bisulfite sequencing in a panel of B cell lines representing the various differentiation stages of B lymphocyte development. We also analyzed the histone acetylation state of CD23ap in some of the EBV positive cell lines with different latency types. Our results showed that DNA methylation does not play a central role in the regulation of the CD23 promoters during B cell development or in the transactivation by EBV. The level of histone acetylation at CD23ap, however, significantly correlates with the expression of CD23a.

INVESTIGATION OF THE BACTERIAL AND FUNGAL COMMUNITY CHANGES DURING OYSTER MUSHROOM (*PLEUROTUS* SP.) SUBSTRATE PRODUCTION

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Oyster mushroom is the second largest commercially produced mushroom in Hungary, and it gives 25% of the total world production of cultivated mushrooms. The substrate production method has three phases: (1) chopped and moistened straw composted in open air for six days, turned once a day; (2) pasteurized at 65°C for 18 hours in tunnels; (3) conditioned at 50°C. The produced amount of oyster mushroom depends on the quality of the substrate, which is partially determined by the bacterial and fungal communities. We focused on the molecular characterization of the substrate with fingerprinting methods and on the identification of the dominant microbes with culture-independent methods. Further aim was to investigate, if there is a significant correlation between the structure of the microbiota and the mushroom yield.

After PCR amplification of the 16S rDNA of the substrate bacterial communities (from the beginning, the end of the first phase, and from the end of the third phase), T-RFLP (Terminal Restriction Fragment Length Polymorphism) was carried out to get relative abundance of bacterial species. In order to identify dominant bacteria in the fingerprints, clone libraries of 16S rDNS genes

were developed. The clones were grouped according to their T-RFLP patterns and the group representatives were sequenced. In case of fungi the ITS-region were amplified for two reasons. First, F-ARISA (Fungal-Automated rRNA Intergenic Spacer Analysis) was applied to obtain community-fingerprints. Second, F-RISA (Fungal-rRNA Intergenic Spacer Analysis) was carried out on polyacrylamide gel in order to excise the main bands and identify the dominant fungal species in the community. The analysis of the T-RFLP results shows significant differences in the bacterial composition among the different phases, but there were no significant differences observed among the different production series. The first phase was dominated by Proteobacteria (e.g. *Sphingomonas*, *Pseudomonas* sp.), but some Actinobacteria occurred as well (e.g. *Saccharopolyspora* spp.). The representative bacteria of the third phase were autochthonous thermophile Firmicutes and Actinobacteria (e.g. *Thermobacillus xylanilyticus*, *Microbispora bispora*). *Thermobacillus xylanilyticus* is a thermophilic, xylan-degrading bacterium, which can play an important role during the composting, since the parallel chemical measurements revealed a moderate decrease in the amount of hemicellulose through the substrate production. In case of fungi a considerable patchy structure evolved in spite of extensive homogenization of the substrate during maturation, which explains, that at the fungal community no clear trend was observable at F-ARISA. In the first phase *Pyrenophora tritici-repentis* was found to be the most abundant species in our samples, *Candida membranifaciens* and a species from *Lewia* genus were found, too.

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GLUCOSE INDUCED RESPIRATION IN CLAY SUBSTRATES DURING SUCCESSION ON A COLLIERY HEAP

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Few studies investigated the chronosequence of post mining sites in relation to soil community development. We studied the active microbial biomass and the labile C and N pools of substrate in four stages ranged 1 - 43 years in the top (0-5 cm) and bottom (10-15 cm) layers for their indicative values on soil development.

The study area was located near Sokolov (North-Western Bohemia, Czech Republic) on colliery spoil heaps. Four serial stages were observed: The "Initial" stage (1 year) was represented by freshly heaped spoil substrate without vegetation. The herbaceous vegetation has started to develop in surface layer on "Early" stage (11 years). "Mid" stage (21 years) was characteristic with rapid development of herbal as well as shrub species. "Late" stage (43 years) was covered by well-developed vegetation with trees. Samples were taken in four localities at each succession site from two layers, 0-5 cm and 10-15 cm depth. Microbial biomass was measured by substrate induced respiration (SIR), water-extractable organic carbon (WEOC) and water-extractable nitrogen (WEN) were analysed by TOC/TN analyser.

There was a significant effect of spoil age on the pH, SIR, WEOC and WE C:N (P<0.05) but not on the WEN. The effect of soil depth was also significant on the measured variables excluding WEOC. Soil age x soil depth interaction had significant effect (P<0.05) on all variables. The significant decrease in pH during chronosequence was much more evident in the 0-5 cm layer than in 10-15 cm. WEOC in the top layer at LATE stage was significantly higher than at other stages, while in the bottom layer all stages showed no significant difference. The WEN was significantly higher at MID and LATE stages than at INITIAL while the EARLY did not differed from either INITIAL or MID in

the 0-5 cm. The relatively high WEOC and WEN content in the substrate being at INITIAL stage was in agreement with another study showing relatively high content and rich spectrum of fatty acids, probably representing a non-viable-fossil microbial community, and other fossil materials, which could be important C and energy sources for the pioneer heterotrophic community that colonises the heaped mine spoil.
SIR as a measure of active part of the soil biomass increased in the chronosequence of spoil succession. This change observed first in the top (0-5 cm) and later in the bottom (10-15 cm) layer relating probably to the vegetation and soil fauna succession. SIR better than by the total organic C can indicate soil succession, because this latter was partly originated from the fossil organic matter.

FERTILIZER EFFECT ON CARBON DYNAMICS OF THREE DIFFERENT TEXTURE SOILS UNDER TOMATO

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We studied the soil C and N cycling in tomato crop. Microbial biomass C and microbial biomass N as an important indicator of soil labile N pool together with *in-situ* C and N mineralization activity of soil microbiota have been investigated under tomato nutritioned with slow release fertilizer (SRF) and split N application and drip irrigation during one growing season in field lysimeters filled with three different texture soils. Nitrogen treatment was applied at 4 levels: 0, 60, 120, and 240 $kg.ha^{-1}$ of N in 2006 year. Experimental design was completely randomized block with 3 replicates representing 36 lysimeters altogether. The first portion of fertilizer (60 $kg.ha^{-1}$) was added one week after planting in the form of SRF. Further portions, as topdressings in the form of NH_4NO_3 were applied later. Soil texture and N fertilization rate significantly affected plant carbon and nitrogen accumulation, consequently C input into the soil by tomato roots. Soil texture had significant effect on both microbial biomass C and N, they were significantly smaller in sand than in alluvial and clay loam soils, while they were not differed between alluvial loam and clay loam in June and also in October sampling. Effect of N fertilization rates was significant neither on soil microbial biomass C nor on microbial biomass N at the end of tomato growth. Microbial biomass C and N was slightly increased at sand while significantly reduced at alluvial loam and clay loam soils from June to October. A decrease in microbial C: N ratio from June to October suggests a regression of the microbial population due to N deficit since fertilizer N recovered by crops completely by the end of growing season. Furthermore, microbial C: N ratio decrease may be resulted from the shift of microbial community composition from fungal to bacterial dominance. The effect of soil texture on the decomposition rate was significant in all periods. The rate of cellulose decomposition was accelerated by N fertilization at 120 and 240 $kg.ha^{-1}$ N additions during crop growth reflecting competition for soil inorganic N between the decomposer microbial population and crop roots. Ion exchanger resins placed at 0.3 m depth showed no increase in inorganic N leaching resulted from N fertilization.

HPV VACCINES AND THEIR APPLICATION IN GYNECOLOGICAL PRACTICE

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Globally, Human papillomavirus types 16 (HPV-16) and 18 (HPV-18) are responsible for the etiology of epithelial cell cancers of the female lower genital tract including the uterine cervix, vagina and the vulva. Moreover, HPV-6 and HPV-11 cause approximately 90% of all anogenital condylomas in both sexes. The global disease burden of cervical cancer is estimated at 490 000 new cases and 250 000 deaths annually with almost 80% of cases in the developing countries. Recently, HPV vaccines based on L-1 virus-like particles have been introduced to protect against viral infection and development of precancerous lesions. Initial studies comprising up to 50 000 female participants in their reproductive ages provided convincing evidence that three doses of a quadrivalent (HPV-6/11/16/18) or a bivalent (HPV-16/18) vaccine induce virus neutralizing antibody titres in the serum that are still present around the level of antibodies induced by natural infection after 5 years of follow-up. As demonstrated in a smaller group of participants, an antigen challenge of quadrivalent vaccine was shown to stimulate an anamnestic response in vaccinated persons suggestive of a long-lasting anti-viral protection. Clinical studies with quadrivalent and bivalent vaccines demonstrated a 95-98% efficacy in preventing high-grade cervical lesions in young women previously not exposed to HPV types of the vaccines. In addition, the vaccine targeted to prevent HPV-6/11-associated anogenital lesions had similar efficacy in a 3 years follow-up. These data suggest that widespread immunization of female children and adolescents may result in a substantial decrease in HPV-16 and HPV-18-related cervical disease including cervical cancer.

CHRYSEOBACTERIUM HUNGARICUM SP. NOV., ISOLATED FROM HYDROCARBON CONTAMINATED SOIL

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The taxonomic position of a *Flavobacterium* strain isolated from kerosene contaminated soil at a former military air-field in Hungary was examined by a polyphasic approach. The isolate, designated CHB-20p, was clearly assigned to the genus *Chryseobacterium* (Flavobacteriaceae) on the basis of morphological and chemotaxonomic data. The outstanding oil degrader strain CHB-20p. is a Gram-negative, non-spore-forming, rod-shaped bacterium which produces yellow pigmented, slimy colony type. Based on the results of the experiments on growth conditions strain CHB-20p is an aerobic, mesophilic microbe with a 30°C temperature optimum. By the use of transmission electron microscope of a negatively stained samples of type strains of *C. defluvii*, *C. daecheongense*, *C. scophtalmum*, *C. indoltheticum* and strain CHB-20p, identical, rod shaped (1,2x0,5 μm) cells were found without flagellation. An almost complete 16S rRNA gene (rDNA) sequence was obtained for the test strain and compared with those of representative type strains of *Chryseobacterium*. 92% to 97% similarity was measured among them. The 16S rDNA sequence data not only supported the taxonomic position of our isolate but also regretted the possibility for a new taxon within the *Chryseobacterium* genus. The result of the most adequate DNA-DNA hybridization method also confirmed this finding: the DNA-DNA relative homology values between CHB-20p and the closest relative *C. caeni*, was lower than 70%. Moreover, indole production was also found as a diagnostic phenotypic property which distinguishes CHB-20p from its closest relative, the indole negative *C. caeni*. On the basis of phenotypic, chemotaxonomic and genotypic data, isolate CHB-20p is assigned to a new species within the genus *Chryseobacterium* under the name *Chryseobacterium hungaricum* sp. nov. The type strain is CHB-20pT.

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PREVALENCE OF TTV AND HEV IN SWINES AND PIGLETS IN HUNGARY

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Primers specific for swine TTV were used to test the prevalence of TT virus in 13 piggeries from different parts of Hungary. 27 of 83 swine sera proved to be positive. We received at least one positive sample from almost all piggeries. PCR products were cloned and sequenced.. Piglet sera are tested for the presence of HEV-RNA by nested RT-PCR. No specific products were obtained so far. Commercially available anti-HEV ELISA was used to detect cross-reacting antibodies to human hepatitis E virus. The protocol provided with the kit was followed except that instead of the labelled anti-human-IgG, Protein A of *Staphylococcus* origin conjugated with horse radish peroxidase was used to detect the swine antibodies to the hepatitis E virus antigens. 69 swine sera from various piggeries were tested. Cross-reacting antibodies to human hepatitis E virus were found in 13 of them.

DETECTION OF THE MICROSPORIDIAN PARASITE *NOSEMA CERANAE* IN EUROPEAN HONEYBEE (*APIS MELLIFERA* L.) COLONIES IN HUNGARY

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Microsporidia are intracellular parasites with wide range of hosts. The microsporidiosis (nosema disease) of the European honeybee (*Apis mellifera L.*) is present worldwide. These unicellular parasites may have much negative effect on the colony, and may cause heavy economic losses in apicultures. The causative agent (*Nosema apis*) is a parasite living in the ventriculus of adult honeybees. An other microsporidium species, *Nosema ceranae*, was reported to infect the asian honeybee (*Apis ceranae*) which can be infected also with *Nosema apis*. *N. ceranae* was also detected in European honeybee, and in Europe the presence of the *N. ceranae* has been reported first in Spain in 2006. It is difficult to distinguish *N. ceranae* and *N. apis* morphologically. Using a rapid accurate method developed to differentiate *N. apis* and *N. ceranae* based on PCR-RFLP of partial LSUrRNA we found, that *N. ceranae* is very widespread in the Hungarian honeybee colonies, so now it seems to be a frequent parasite of *A. mellifera* across most of the world. In this study we tried to detect *N. ceranae* in European honeybee with molecular diagnostic methods on 20 samples which were found nosema-positive in previous parasitological investigations but species identification of the *Nosema* was not performed. PCR specific primers were designed able to attach to the LSU rRNA region of *N. apis* as well to *N. ceranae* based on the sequences deposited in GenBank Database. The nucleic acid sequence of the PCR products was determined, and compared to the sequences deposited in the GenBank. The sequenced products were identical to *N. ceranae* sequence. After the PCR we have investigated our samples using RFLP analysis. The restriction enzyme MslI digests the nucleic acid of the amplicon of *N. ceranae*, but not that of *N. apis*. Using the method we determine the occurrence

rate of *N. ceranae* in Hungarian bee samples. Up to now all our positive samples were identified as *N. ceranae,* which means that this species is far more widespread in Hungary nowadays than the *N. apis*.

EFFECT OF TEMPERATURE INCREASE ON BACTERIA COMMUNITY OF A SLUDGE DIGESTER DETECTED BY CHEMOTAXONOMICAL METHODS

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Large amount of sludge formed during waste water treatment is a common raw material of biogas producing. The aim of the present study was to monitor the changes of Bacteria community structure in a pilot sludge digester during a heating up process. A mesophilic (35°C) digester was heated up to 70°C through 40 days gradually. Abiotic parameters (temperature, pH, gas yield, concentration of volatile fatty acids) were daily measured. Samples were taken four times a week. Above 60°C gas yield started to decrease and stopped at 70°C. As chemotaxonomic markers of the bacterial community cellular fatty acids and respiratory quinones were measured. Apolar components of samples were extracted in organic solvents. After purification, fatty acids and quinones were measured by high performance liquid chromatography (HP 9001, ODS Spherisorb column) and gas chromatography (HP 5890, HP1 capillary column), respectively. These molecules are characteristic to the taxa producing them, thereby are indicators of changes in community structure.
Though diversity indices of fatty acid markers showed decline during the whole heating up period though local fluctuations were also observed. Local maxima of general marker diversity overlapped with local pH minima. Despite general decline, diversity of fatty acids showed a short term maximum at 55°C. Yield of biogas were maximal at the same time. Our results affirm the experiences about good efficacy and narrow ecological tolerance of thermophilic (55°C) biogas producing communities. Reaching 50°C a culmination could be detected in relative amount of cyclopropil- (markers of anaerobic fementative bacteria) and branched-chain fatty acids (markers of Gram-positive bacteria in general). At this temperature, the start of a rapid decrease could be detected in pH while strong increase started in amount of volatile acids in sludge. The reason of decline of pH values might be the production of acidic metabolites of fermentative bacteria. Concurrent increase could be detected in characteristic fatty acids of some sulphate reducer bacteria (15:1, 17:1) and biogas yield. A few days later, around 52°C culminations of former parameters were coincident with minimal pH values and maximal volatile acid concentrations as well. By the time of pH minimum relative amount of fatty acids of fermentative bacteria were suppressed, it indicates a considerable development of other groups living on their products. Although sulphate reducing bacteria and methanogenic Archaeal prokaryotes often compete for H_2 in different habitats, in this case H_2 produced by fermentative microbes might be sufficient for both bacterial groups.

TOPO-OPTICAL INVESTIGATION OF SUGAR CHAINS AND SIALIC ACID ON BACTERIAL CELL SURFACE DURING THE PHAGOCYTOSIS OF ENCAPSULATED AND NON-ENCAPSULATED *KLEBSIELLA PNEUMONIAE* IN MOUSE

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Polarisation optical technique gives an opportunity to perform sub-microscopic investigation on structures containing sterically highly ordered molecules like the cell envelope of prokaryotic cells. Such structures can evoke optical anisotropy and birefringence i.e. the refractive indices are measurably different in different axes of the structure. This natural birefringence can be enhanced or modified by different dyes or reagents yielding further possibilities to specifically investigate the composition and structure of bacterial surface compounds.

The bacterial capsule is built up from repeating subunits, consisting of four to six sugar molecules. This capsular polysaccharide (CPS) covers the surface of *Klebsiella* strains in a massive layer. Beyond the capsule the peptidoglycan layer also contains carbohydrates in highly ordered manner. Fischer and Emődy (1976) described the changes in birefringence of encapsulated *Klebsiella pneumoniae* during the process of phagocytosis by rat peritoneal cells applying the "classical" ABT reaction developed by Romhányi et al. (1975). In this study we give an account on our findings raised in a mouse intraperitoneal phagocytosis model using encapsulated and non-encapsulated *Klebsiella*, and applying more recently developed topo-optical methods.

NMRI mice were intraperitoneally inoculated with 5 x 10^8 cells of either the encapsulated *Klebsiella pneumoniae* No. 591, K2:O1 strain or its non-encapsulated derivative. Samples were taken from the abdominal cavity at different time intervals (5',1, 2, 4 h) by peritoneal lavage. Smears were formalin fixed and subjected to the following reactions: aldehyde-bisulphite-toluidine blue (ABT), periodic acid–borohydrid-potassium hydroxide (PB-KOH-ABT), potassium hydroxide - ABT (KOH-ABT), KOH-sialic acid specific, 9-O-acyl sialic acid specific and chlorpromazine-eosin staining. The preparations were evaluated under the polarisation microscope.

With ABT, KOH-ABT, PB-KOH-ABT, and KOH-sialic acid reactions, the encapsulated bacterial cells showed intensive metachromatic staining and linear negative birefringence with respect to the cell surface (orientation of carbohydrate chains parallel to the radius) before being phagocytosed. Applying the ABT and KOH-ABT reactions the non-encapsulated derivative stained less intensively than the encapsulated strain and presented with a birefringence of opposite orientation i.e. linear positive with respect to the cell surface. In case of PB-KOH-ABT and KOH-sialic reactions the non-encapsulated derivative gave linear negative birefringence with respect to the cell surface. Both encapsulated and non-encapsulated bacterial cells changed their original pattern of their birefringence after being phagocytosed. Starting one hour after inoculation the birefringence gradually decreased then disappeared and by the end of the four-hour incubation time it reappeared with an opposite orientation. The molecular background of these changes is discussed.

IN-VITRO EXAMINATION OF THE INHIBITION EFFECT OF DIFFERENT MATERIALS ON SEED BORNE BACTERIAL DISEASE OF TOMATO AND PEPPER

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In ecological farming systems farmers can not use chemicals against pests. In plant protection the aim is to prevent diseases; if it is not possible the use of allowed materials are permitted. Until now there have not been enough effective and environment friendly materials for seed treatment in organic farming. Seed borne diseases of tomato and pepper can cause serious losses in yield, so finding appropriate inhibitors has a great importance. In tomato (*Lycopersicon esculentum* L.) production of Hungary bacterial cancer (*Clavibacter michiganensis* subsp. *michiganensis)* and bacterial spot (*Xanthomonas campestris* pv. *vesicatoria)* of tomato cause high yield losses; bacterial speck (P*seudomonas syringae* pv. *tomato)* is just sparse problem. In conventional farming generally kasugamycin agent chemicals are used against seed borne diseases of tomato for seed dressing in Hungary. In ecological farming just maximum 1,5% sodium-hydroxide (NaOH) is allowed to use against these diseases, but it is a disinfectant so seed dressing materials has not been allowed in Hungarian ecological farming. Different materials were tested with agar diffusion cup plate method against these bacterial strains in this study. *In-vitro* trials have shown that vinegar, cider vinegar, red wine vinegar and white wine vinegar have inhibiting effect against the *Clavibacter michiganensis* subsp. *michiganensis B. 01778, B. 01779, Pseudomonas syringae* pv. *tomato B.01277, B.0 1682, B.01538* strains. These materials also have inhibiting impact on the *Xanthomonas campestris* pv. *vesicatoria B.01771*. The most effective concentration was 5% but in some case lower (0,5%) concentrations also seemed to be effective. Seed dressing with alkaline materials is used in agriculture, and the mode of action of it, that it is changed the pH on the surface of seed. The vinegar is also changing the pH, so this new circumstance won't be favourable for bacteria. In our examination the treatment with (natural alkaline material) sodium hydrogen carbonate ($NaHCO_3$) had no effect on examined bacterial strains.

Among examined essential oils cinnamon oil proved to be the most effective, but all oils decreased germination ability. Germination test has shown that examined vinegar types do not decrease germination ability. Vinegar types seem to be environmental friendly, cheap, and perspective materials in ecological seed treatment.

We plan to test further materials and carry out *in-vivo* experiments on the fields with the most effective ones. Using different combination of materials and treatments it could give better effects against bacterial canker, bacterial speck of tomato and bacterial spot of pepper.

DEVELOPMENT OF PSEUDORABIES VIRUS-BASED TRANSSYNAPTIC GENE DELIVERY VECTORS

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In this study we have developed various recombinant pseudorabies virus (PRV) strains for labeling multisynaptic neural pathways. PRV exerts cytotoxic effects on the infected cells, which is inconvenient if we want to investigate cellular physiology of the transsynaptically labelled neurons. To circumvent this problem, we have eliminated the most important virulence factors of PRV, which did not abolish the spreading capability of the virus. The deleted genes were as follows: the virion host shut-off (*vhs*, which is a ribonuclease) and early protein 0 (*ep0*, which is a transactivator playing a role among other in the decision of the type of virus infection) genes. In addition, we have inactivated antisense transcript (AST) promoter (ASP), which also found to be significantly contributed to PRV virulence. In addition, attenuated viruses have been shown to spread in a reliably specific manner than virulent strains. Strain PRV-Bartha (Ba) is a widely used tracer strain. We

found, however, that PRV-Ba provides a highly instable genetic background for foreign DNAs with large size or complex structure. In addition, in some experimental paradigm PRV-Ba is too attenuated, and a more virulent strain would be desirable. Therefore, we have constructed a wild type (strain Ka)-based retrograde tracer by eliminating the gE and gI glycoprotein genes, which are required for the anterograde spread of PRV. Furthermore, in order to visualize the stage of viral infection, we inserted the mRFP (monomeric RFP) and mem-GFP (a membrane-bound GFP) genes to the genome of PRV-Ba. The mRFP has a much longer maturation time than that of GFP, therefore, neurons without mRFP signal indicate a very early stage of infection and hence, an intact physiology. We have also constructed recombinant viruses expressing fluorescence proteins with various colours. These viruses have currently been tested in mouse retina. Following V1 (primary visual cortex) inoculation, they reach the retina and label the connections of individual retinal ganglion cells.

MICROBIAL COMMUNITY ANALYSIS OF TCE CONTAMINATED SITES AND TECHNOLOGY IMPROVEMENT FOR ENHANCED BIOREMEDIATION

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Knowledge on the relationship of microbial community structure and hydrogeochemistry in chlorinated solvents contaminated aquifers is required to develop tools for predicting possibilities and monitoring natural attenuation or enhanced bioremediation. In this study analysis of aliphatic chlorinated compounds and water-chemical parameters were conducted in parallel with culture-independent profiling of microbial communities present in several TCE contaminated sites in Hungary. Microcosm studies were performed to assess dechlorinating activity under a variety of electron donor amendment conditions. Two molecular methods were used to characterize the bacterial and archaeal community structure of samples: clone library construction and terminal restriction fragment length polymorphism (T-RFLP) analysis.

In the laboratory microcosm experiments we used organic industrial by-products as electron donors. Microcosms showed evidence of methanogenesis. Sulphate reducing bacteria were stimulated, FeS production was visible in the microcosms. In microcosms from samples J18/2, J18 and K7 trichloroethene almost completely degraded to cDCE, and VC, and in microcosm of sample J18/2 ethene was also detected. The bacterial community analyses over a period of 150 days revealed a clear population shift influenced by the electron donor amendment. Clone library and T-RFLP analyses suggested that in effective amended microcosms populations belonging to Firmicutes, like *Clostridium* spp., *Trichococcus* sp, *Leuconostoc* sp. dominated. In addition, *Sporomusa*-like bacteria were identified, which most likely act through their homoacetogenic metabolism. *Sulfospirillum multivorans* could also be detected.

Four examined organisms were detected, which are *Dehalococcoides ethenogenes*, *Dehalobacter restrictus*, *Desulfomonile tiedjei* and *Desulfuromonas chloroethenica*. The presence of these organisms was monitored both on the contaminated sites and in the microcosms studies. Where the degradation of trichloroethene by dehalorespiration was observed, *Dehalococcoides ethenogenes* was always demonstrated as well, however it was not confirmed in the case of the three other bacteria. In spite of the fact, appearance of *Dehalococcoides ethenogenes* was not coupled occasionally with full dechlorination. Based on sequence analyses the *Dehalococcoides* group is divided in two subgroups.

Our results indicated that the used electron donor is a promising, effective substrate to help remediating at a wide range of chlorinated aliphatic hydrocarbon concentrations at different contaminated sites. The use of adequate laboratory-scale studies of electron donor utilization and

substrate transformation is recommended to give a more accurate assessment of the prospect of a bioremediation technology of specific pollutants in aquifers contaminated by TCE.

ESCHERICHIA COLI CYTOLETHAL DISTENDING TOXIN (CDT) TYPE I AND TYPE IV ARE ENCODED BY RELATED PROPHAGES

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Five types of cytolethal distending toxin (CDT- I, II, III, IV and V) have been identified in *Escherichia coli.* In the present study we have cloned and sequenced *cdt-IV* operon and flanking region from a porcine extraintestinal pathogenic *E. coli* (ExPEC) strain of serogroup O75. CDT-IV was shown to block HeLa cells at the G2/M transition and induced phosphorylation of histone H2AX, a sensitive marker of DNA double strand breaks. The *cdt-IV* genes showed high nucleotide sequence similarity with *cdt-I* genes and were framed by lambdoid prophage genes. Since the flanking regions of *cdt-I* genes were not available in database, we cloned and sequenced the *cdt-I* operon flanking regions from an ExPEC strain of serotype O18:K1:H7. The *cdt-I* genes were also flanked by homologous prophage genes but the phage organization within the hyper-variable tail region, was not conserved. For instance, PCR studies proved that a gene coding for a putative protease was always associated to *cdtC-IV* gene, but was absent or not associated to *cdtC* genes in strains producing CDT-I, III, and V). Our results suggest that *cdt-I* and *cdt-IV* might have evolved from a common ancestor acquired by phage transduction. These lysogenic bacteriophages have the potential to carry non-essential "cargo" gene or "moron" and therefore play a crucial role in the generation of genetic diversity within ExPEC.

MICROBIAL COMMUNITY OF THE HIGH PURITY WATER SYSTEM OF A POWER PLANT

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Microbial contamination can lead to corrosion even in high purity waters which are characterised by low salt and nutrient concentrations.

Scanning electron microscopic (SEM) investigations were carried out on the pipeline "biofilms" of a power plant. Then cultivation (using oligotrophic - R2A, 0.1% TSA, M27 - media) of cooling water samples (feed and product pipelines of ion exchange units as well as a volume compensatory tank) was evaluated. Isolated bacteria were grouped based on their fatty acid profile, phenon representatives and all ungrouped strains were subjected to 16S rDNA sequencing. Simultaneously, 14 litres of product pipeline water sample was filtered and direct DNA isolation was carried out for molecular cloning. At the same time direct DNA isolation was performed for T-RFLP analysis from all water samples, biofilms of pipelines, ion exchange resins and feeding chemicals to trace the origin of bacteria in the water.

The SEM examination revealed diverse microbial communities in the biofilms, among them budding forms of *Hyphomicrobium* were detected. Cultivation results showed the dominance of aerobic, chemoorganotrophic β- (*Delftia, Chromobacterium, Sterolibacterium,* etc.) and α-proteobacteria (*Bradyrhizobium, Chelatococcus, Afipia,* etc.) of different water samples. Nevertheless, members of γ-proteobacteria (*Serratia, Enterobacter,* etc.) and Gram positives could also be detected. Different, so far uncultivable bacteria could be isolated. Molecular cloning confirmed the results of cultivation as the dominance of α- and β-proteobacteria could be detected but several other taxa (*Chloroflexi*, *Synechococcus*, *Meiothermus,* etc.) as well as uncultured clones were also demonstrated.
T-RFLP method indicated a complex microbial community structure in each sample. Diversity indexes (Shannon-Weaver) based on the TRFs of the samples varied among 3.605 - 4.456, the highest was in one of the ion exchange resin samples.
The profile of the two pipeline water samples was similar, differences could be detected mainly in the ratio of TRF areas. Making a hierarchical cluster analysis from all TRFLP results it became obvious that the origin of bacteria in the water body is mainly the biofilm and ion exchange resin, chemicals less contributed to microbial contamination of waters.

COMPARISON OF BACTERIAL GENE EXPRESSION AND PRODUCTION OF INFECTIOUS PARTICLES IN HEP-2 CELLS TREATED WITH PENICILLIN AND INFECTED EITHER WITH A RESPIRATORY OR A VASCULAR STRAIN OF *CHLAMYDOPHILA PNEUMONIAE*

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Chlamydophila pneumoniae (*C. pneumoniae*) is a respiratory pathogen in humans, causing pharyngitis, bronchitis, and pneumonia. It has also been suggested that this bacterium plays a role in the etiology of atherosclerosis. Different strains of different origin of *C. pneumoniae* may have dissimilar ability for persistent infection in various cell types in humans. There are several ways *in-vitro* to induce persistent chlamydial infections, including withdrawal of amino acids or glucose from the culture medium, or treatment of the infected cultures with interferon-g or certain antibiotics.
The molecular characteristics of the persistent chlamydial infections are not well defined. In our study, as an *in-vitro* model for persistent infections, susceptible HEp-2 cells were infected either with *C. pneumoniae* TW-183 or with *C. pneumoniae* CV-6 strains and treated with penicillin. TW-183 strain is a respiratory strain although it was isolated from conjunctiva, the CV-6 strain is a vascular isolate. We compared the expression profile of some of the important chlamydial genes at different time points by real-time PCR. The following bacterial genes were investigated: the 16S ribosomal RNA gene (bacterial house keeping gene), the *ompA* gene (encoding the major outer membrane protein), the *omcB* gene (encoding a cystein-rich protein), the groEL-1 cistron (encoding a 60 kDa heat-shock protein), and the *ftsK* gene (involved in binary fission).
The productions of elementary bodies by the two strains with or without penicillin treatment as well as the time course of the reactivation of the bacteria after penicillin removal were also determined.

GENETIC CHARACTERISATION OF *BOTRYTIS CINEREA* POPULATIONS IN THE EGER WINE DISTRICT

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Botrytis cinerea (teleomorph: *Botrytinia fuckeliana*) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants by infecting various tissues. In grapevine, the frequent occurrence of *B. cinerea* prior to harvesting results serious losses of fruits and deterioration of wine quality. Information about the populations of plant pathogen fungi is essential for the effective and economic protection. A plant pathogenic fungal population with high level of genetic variation is likely to adapt more rapidly to fungicides or resistant host plants than populations with little or no genetic variations, and information on the level of migration between populations and on the presence or absence of sexual reproduction within a population may indicate how rapidly will novel (fungicid resistant or more pathogenic) genotypes spread between populations. Application of the tools provided by recent advances in population genetics and biology are crucial in gathering those information. Based on these considerations, a first-of-its-kind study to characterize *B. cinerea* populations of Eger wine district was undertaken. In the initial stage, 100 isolates of grapevine berry-growing *B. cinerea* from various locations of the Eger wine region, respectively, were collected. Individual strains were obtained by single-spore isolation. Characterization of their genotype was done by analyzing MSB1 minisatellite and fragment of the translation elongation factor TEF1 sequences and by the determination of the presence or absence of transposon elements.

In general, sequence analysis revealed a high degree of genetic diversity and the combination of alleles suggests the presence of sexual reproduction in the area, while the disperse distribution of the genotype indicates high migration rate.

Based on the absence or presence of two transposable elements *Boty* and *Flipper, vacuma*, *transposa* and *flipper* type isolates were identified.

MAPPING OF THE LEFT END OF MURINE HERPESVIRUS 72 (MHV-72) GENOME

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Murine herpesvirus (MHV-68), belonging to *Murid herpesvirus 4* (MuHV-4) species, is the best-characterized prototype murine herpesvirus; it is related to human gammaherpesviruses, such as Kaposi's sarcoma-associated herpesvirus (HHV-8) and Epstein-Barr virus (HHV-4). All MHV isolates (60, 68, 72, 76, 78, 4556, 5682, and SUM) originate from free-living mouse rodents captured at the territory of the Slovak and/or Czech Republic. To date, only two strains – MHV-68 and MHV-76 have been fully sequenced; their sequence comparison showed the presence of a 9,5 kbp long deletion at the left end of the strain MHV-76 genome. We analyzed the complexity of the left end of the clone h3.7 DNA, obtained from the MHV-72 isolate, as compared with that of MHV-4556 strain, isolated from a different mouse species (*Apodemus flavicollis*). To identify the MHV-specific gene block (M1, M2, M3 and M4 genes) as well as the viral tRNAs sequences encoded by the left end of MHV-72 genome, which had not been found in strain MHV-76 DNA, the MHV-72 restriction maps

of *Bam*HI, *Eco*RI, *Hind*III, *Sac*I, *Sph*I, *Mlu*I and *Not* I were analysed. The presence of relevant genes in purified restriction fragments was confirmed by gene-specific PCRs. Then, the primary structure of fragments containing the "left end" genes was determined by walking sequencing.
We confirmed that the MHV-72 clone h3.7 DNA has no left end deletion such as described within the MHV-76 strain genome. Moreover, the length of MHV-4556 restriction fragments carrying the "left end" genes similarly as full length sequencing of the M3 gene allowed the prediction that the region in question within the MHV-72 clone h3.7 genome is similar to that of the prototype MHV-68 strain DNA.

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ROLE OF OXIDATIVE DAMAGE IN THE PATHOGENESIS OF ACUTE AND LATENT HERPES SIMPLEX VIRUS TYPE 1 INFECTIONS OF THE NERVOUS SYSTEM

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The molecular mechanisms by which herpes simplex virus type 1 (HSV-1) infections cause tissue injury in the nervous system are not well understood. HSV-1 encephalitis in mice is associated with nucleic acid damage due to hydroxyl radical attack, lipid peroxidation and protein damage mediated by the lipid peroxidation by-product 4-hydroxy-2-nonenal (HNE). Oxidative damage affects both neurons and non-neuronal cells and localizes to brain regions that show histopathologic and immunohistochemical evidence of HSV-1 infection and reactive inflammation. Interestingly, HSV-1 latent infection in mice also is associated with modest but consistently detectable oxidative nucleic acid and protein damage and lipid peroxidation. Oxidative damage appears to affect latently infected neurons and possibly uninfected neurons and glial cells. The relative contributions of direct viral toxic effects and that of reactive inflammatory processes in the pathogenesis of HSV-1-induced oxidative nervous system injury are not well understood. While it is likely that immune and inflammatory responses of the host play an important role, *in-vitro* studies suggest that direct HSV-1 toxicity may also cause oxidative neural injury. In cultured P19N neural cells, HSV-1 infection increases reactive oxygen species levels and also leads to an increased release of lipid peroxidation byproducts HNE and malondialdehyde (MDA) into the culture medium. HSV-1 replication in P19N cells is inhibited by the antioxidant compound ebselen and high concentrations of HNE, but is increased by low concentrations of HNE. These findings indicate that oxidative damage is a mechanism by which acute and latent HSV-1 infections damage the nervous system and raise the possibility that oxidative damage is a mechanism of bystander cell injury during these infections. These observations also suggest that moderate oxidative stress may promote HSV-1 replication in neural cells, while massive oxidative stress appears to have an opposite effect. Thus depending on its extent, oxidative stress induced by the host immune and inflammatory responses may either promote or limit HSV-1 replication. An enhanced understanding of the role of oxidative damage in HSV-1 infections of the nervous system may lead to therapeutic strategies that would reduce tissue damage during viral infection without impeding the antiviral host response.

POLYPHASIC TAXONOMY OF *ASPERGILLUS* SECTION *NIGRI*

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Species of *Aspergillus* section *Nigri* are among the economically most important fungal groups. Many species cause food spoilage, and several are used in the fermentation industry to produce hydrolytic enzymes. Some black *Aspergillus* species also produce ochratoxin A, a mycotoxin with nephrotoxic properties. In this study, we examined black Aspergilli using a polyphasic approach. Phylogenetic analysis of ITS, b-tubulin and calmodulin gene sequences, analysis of extrolite profiles, and morphological and physiological examinations were carried out. During this study, 4 new species were identified from different sources. All these species can be distinguished from other black Aspergilli based on b-tubulin and calmodulin gene sequences, and two of them also by ITS sequence data. *Aspergillus brasiliensis* sp. nov. was originally isolated from Brasilian soil, and can be distinguished from other black Aspergilli based on ITS, b-tubulin and calmodulin gene sequences, by AFLP analysis and by extrolite profiles [1]. *A. brasiliensis* isolates produce naphtho-g-pyrones, tensidol A and B and pyrophen in common with *A. niger* and *A. tubingensis,* but also several unique compounds justifying their treatment as separate species. The new species is most closely related to *A. niger*, and was isolated from soil from Brazil, Australia, The United States, The Netherlands, and from grape berries from Portugal. Two new species have been discovered during a survey of the mycobiota and ochratoxin A contamination of green Arabica and Robusta coffee beans came from coffee growing areas of Northern and Southern Thailand. One of them was found to be related to *A. carbonarius* and *A. ibericus*. This species produces orange coloured sclerotia and is unable to grow at 37ºC. The other new species is related to *A. aculeatus*, but has smaller conidia, and produces species-specific combination of secondary metabolites (neoxaline, aculeasins, secalonic acid D and F). The fourth new species, *Aspergillus uvarum* sp. nov. was isolated from grapes in the Mediterranean region. This species is also related to *A. aculeatus*, but can be distinguished by calmodulin and b-tubulin sequence data and by AFLP analysis, and produces unique secondary metabolites including sulochrin, geodin and erdin. None of the newly discovered species produce ochratoxin A, making them promising candidates for enzyme or organic acid production for the biotechnhological industry.

1. Varga et al. 2007. Int. J. Syst. Evol. Microbiol. (in press).

PROKARYOTE DIVERSITY OF TCE CONTAMINATED SITES IN HUNGARY

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Halogenated hydrocarbons are unfortunately common contaminants in soil and groundwater in Hungary. Conventional pump-and-treat technologies have limited effectiveness in remediating groundwater pollution. Stimulation of dechlorinating microorganisms is potentially the most promising and cost-effective technology for remediating contaminated sites.

Under anaerobic conditions, the stepwise reductive dehalogenation of perchloroethylene to trichloroethylene (TCE), dichloroetylenes, vinyl-chloride, ethene and ethane has been observed. The bacterial and archaeal consortia in soil and groundwater obtained from a contaminated plume were assayed using PCR-amplified 16S rRNA genes. The diversity was investigated by TRFLP. The contaminant concentration was between 3000-300000 ug.L^{-1} TCE on the selected samples. The sulphate concentration varied between 690 and 60 mg.L^{-1}. Comparison of 16S rDNA-based TRFLP profiles of Bacteria and Archaea in soil and groundwater samples revealed a clear difference between the microbial community structures at different contaminant sites.
Based on TRFLP analysis we detected in groundwater samples *Acidovorax* sp., *Pseudomonas* sp., *Clostridium* sp., but we were unable to detect any dechlorinating bacteria. Based on clone library analysis we could detect *Anaeromxyobacter dehalogenans*, other uncultured Myxococcales clones and an uncultured clone from a TCE contaminated site. *Dehalococcoides* sp. was detected by nested PCR using primers targeted to unique regions of *Dehalococcoides* 16S rDNA in groundwater samples from site *"J", "K",* but it was not detected in the samples from site *"T"* and *"A"*. From sites *"J"* and *"A"* aerobe cultivation has been also done. The identified isolates belong to *Kocuria* sp., *Leifsonia* sp., and *Arthrobacter* sp. from the group Actinobacteria, *Brevundimonas* sp., *Acidovorax* sp. *Pseudomonas* sp. and *Lysobacter* sp. from the group Proteobacteria.

AN EU FUNDED STUDY ON THE HEALTH RISK OF BATHING WATERS – EPIBATHE YEAR 2

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Epibathe is an international project funded by the EU involving partners from UK, Spain, Hungary and WHO. Aim of the project is to (1) assess the potential health risk of recreational bathing waters that comply with current EU standards (2) complement the existing data on dose-response relationship of bacterial indicators and health effect. Previous studies were organized in UK marine beaches and German bathing lakes. This project targets conducting 8 trials in the course of 2 bathing season in Hungary and Spain.
Studies are organized as randomized control trials. 500 volunteers are recruited for each trial. Participants are randomly divided into a bather and a non-bather group. On trial day, bathers spend 10 min in the water, submersing their head 3 times. Water samples are taken at the site in 6 zones, at every 20 min. Personal exposure is estimated as counts of intestinal enterococci and *E: coli* at the exact place and time of bathing. Health outcome is assessed by questionnaires. Four questionnaires are completed by each volunteer, 3 days before the exposure, on bathing day, 1 week and 3 weeks after exposure. In year 2007, presence of human pathogen viruses is also included in the Hungarian studies as a potential indicator of health effects.
Hungarian study sites in 2006 were Danube dead-legs: Dömsöd on Ráckevei-Duna (July 16th, 2006) and Dombori on the Fadd-Dombori dead-leg (August 13th, 2006). Spanish trials were conducted on the Mediterranean coast of Salou on September 23rd and 30th. 2007 trials in Hungary are organized on riverine sites on Tisza (Csongrád, July 1st, Tiszakécske, August 5th).
To date, all trials were conducted successfully. Target volunteer numbers for 500 volunteers per trial were met and surpassed, in 2006, a total of 2300 participants were involved. All sites qualified as

excellent bathing water on the day of trial, bacterial counts were low. No severe health outcome was observed. Final result will e concluded on the completion of all 8 trials.

RAPID METHODS AND AUTOMATION IN MICROBIOLOGY: A QUARTER CENTURY OF DEVELOPMENTS- PROMISE, PROSPECTS AND CHALLENGES

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The detection, enumeration and characterization of various pathogens, and toxins in, a reliable, cost effective and timely manner is critical in meeting challenges of food safety assurance. Despite limited usefulness of conventional methods, the microbiology food safety testing has largely relied on conventional culture methods, chromatography, Immunoassays or bioassays (live animal tests). Microbiologists throughout the world have been searching for rapid methods and alternative approaches for the analysis of foods for microbial contaminants. This has lead to the development of a wide range of test methods over the past 25 years. The methods are based on a number of different principles, and vary in scope, sensitivity and specificity, not to mention reliability and industry wide applicability. Developments in miniaturization, mechanization, automation, and computerization as well as advances in immunology and molecular biology have lead to many novel approaches for rapid and accurate detection of the target organism, toxin, metabolite or gene sequence. These include new generations of media, automation and miniaturizations, immunoassays, methods based on chemiluminescence and fluorescence as well as methods based on hybridization and amplification of nucleic acid, e.g. polymerase chain reaction (PCR), methods involving the use of a specific nucleic acid fragment (genetic sequence) with or without amplification of sequences and the so-called biosensors and microarray assays. Many methods are already available commercially; other promising technologies and methods will be commercialized in the near future. The promise of rapid and accurate detection of the target organism, toxin, metabolite or gene sequence is realized and the new techniques for rapid microbiology now available present unprecedented choices to microbiologists. However, there are challenges and issues including sampling, concentration and enrichment of target organism or analyte, correlation with the "official" methods, validation and regulatory acceptance preventing their wide spread adoption in microbiology laboratory and food industry. This presentation is designed to review developments in rapid methods and discuss the promise, prospects and challenges of rapid microbiological methods.

DECOMPOSITION RATES OF BT CORN AND NON-BT CORN RESIDUES IN THE SOIL

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Soil biological and microbiological methods were used to study the environmental risk of genetically modified corn expressing the *cry1ab* gene from *Bacillus thuringiensis* ssp. *kurstaki*. During two consecutive years decomposition of transgenic *Bt* (DK-440-BTY) and isogenic non-*Bt* (DK-440) corn was compared in the field in

two plots of soil, where previously the transgenic and the isogenic plants were grown, at the experimental station of the Plant Protection Institute of the Hungarian Academy of Sciences.
Beside litter-bag assessment of the decomposition process (1 mm and 40 mm mash-size) culturable microbes (heterotrophic, oligotrophic, spore-forming bacteria, micromycetes) [1], microbial biomass, and total microbial activity [2] were also measured in the second year.
There were significant differences found between the decomposition rates of the two plants and between the decomposition processes going on in the two plots of soil. Comparison based on the bags with different mash-size showed that in the soil of the isogenic corn the contribution of soil animals to the decomposition process was higher. Total microbial activity measured by FDA hydrolysis and microbial biomass in the soil where previously *Bt* corn was grown was significantly greater than in the soil of non-*Bt* corn. No differences were found in the numbers of culturable bacteria (oligotrophic- and spore-forming) and fungi between the soil of *Bt* and non-*Bt* corn except that the abundance of heterotrophic microbes was higher in the soil of *Bt* corn.
Acknowledgement: Supports of the Hung. Research Fund (T0 46610) and the bilateral agreements between RAS-HAS, BOKU-RISSAC and CSIC-HAS have been highly acknowledged.
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2. Schnürer at al. 1982. *Appl. Environ. Microbiol*. 43, 1256.

INVESTIGATION OF POTENTIAL BIOLOGICAL IMPACT OF DISTINCTIVE PESTICIDES AND THEIR DEGRADATES

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Pesticides sprayed on the soil surface are exposed to effect of UV photons resulting in decomposition of the molecule. The toxicity of the given pesticides is investigated during their registration process, but the toxicity of the degradation products to the soil microorganisms is unexplored yet.
The objective of our work was to acquire information about the microbiological activity of the basic compounds and the degradates of five photosensitive pesticides (carbendazim, acetochlor, simazine, chlorpyrifos, EPTC) of different type and behaviour. Six representative soil microbes were applied as test organisms throughout our microbiological model-experiments. Three bacteria (*Bacillus subtilis, Pseudomonas fluorescens, Mycobacterium phlei*) and three filamentous fungus species (*Fusarium oxysporum, Penicillium expansum, Trichoderma harzianum*) were applied as test organisms. The antimicrobial effects of the pesticides and their degradates were assessed with filter paper disk method. The antimicrobial effect of the degradation products exhibited marked differences in terms of pesticide types, irradiation time, and the test organisms. Acetochlor and its photolytic degradation products were found to be more toxic to bacteria than fungi. All the three bacteria proved to be sensitive to the basic compound and its degradation products as well. The end-product of carbendazim was weakly antibacterial against *P. fluorescens* and *B. subtilis* but strongly antifungal against *T. harzianum*. Chlorpyrifos and its end-product inhibited neither test organisms, but the degradates hindered the growth of four of them. The basic compound of EPTC and the degradates of simazine had significant toxicity to the test bacteria.
It might be claimed that the pesticide photodegradation may result in significant changes in soil microbiota, as well as formation of biologically harmful degradates.

UTILIZATION OF PREBIOTICS BY VARIOUS BACTERIAL STRAINS

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The intestinal microbiota plays an important role in our health and well-being. Modification of the intestinal microbiota by exogenous and endogenous substrates may change various physiological functions of the human body. Novel approaches include the application of prebiotics in different combinations or in nutrients to different bacterial groups and to different parts of the intestine.
For a long time, only changes in the faecal microbiota composition – induced by consumption of a prebiotic – have been studied to assess the efficacy of prebiotics. In the past, the bifidogenic effect (encouraging the growth of bifidobacteria) of a prebiotic was considered proof of its efficacy.
In this study commercially available prebiotics were applied as growth substrate for a selection of bacterial strains. Our aim was to evaluate bifidogenic behaviour of the individual prebiotic preparations and to investigate the utilization of prebiotics by bacterial strains present in human gut or in foodstuff. Dietary modification of the human gut microbiota is a popular area of the nutritional sciences. This is driven by the fact that the gastrointestinal tract, particularly the colon, is heavily colonized and that the composition of the microbiota can be changed. One of the promising approaches is the application of prebiotics as ingredients in food. Therefore eight different types of prebiotics were screened in basal media for their effects on the growth of the probiotic *Bifidobacterium* cultures. Glucose was used as carbon source in control trials. Ten *Bifidobacterium* strains were tested in the experiments. Growth of these probiotics was investigated by measuring optical density over a period of 24 h. The results of this study showed that all tested prebiotics have supported the growth of *B. adolescentis* strain more efficiently than glucose did. Similar good results were registered in the case of *B. lactis* Bb-12 strain, which is widely applied in dairy industry as starter culture, but it has not shown any growth on Raftiline®. Based on the results of cultivation experiments it can be concluded that prebiotic utilization highly depends on the stain applied. The utilization of the prebiotics was also investigated by two *E. coli* strains, one *Enterococcus faecalis,* one *Enterobacter cloacae* and one *Clostridium sporogenes* strain. Results have revealed that all tested prebiotics were utilized by the selected bacterial strains. One of the *E. coli* strains and the *Enterococcus faecalis* has utilized some of the tested prebiotic preparations better than glucose.
To highlight the efficacy of the prebiotic oligosaccharides mixed culture fermentations were carried out. To prove the prebiotic effect *E. coli* O157:H7 strain was chosen as a partner and the other partner was either *B. adolescentis* or *B. lactis* Bb-12 strain. The prebiotic effect of the Raftilose® was proven in both trial, but selectivity of the xylo-oligosaccharide P 95 preparation was not clear.
Results help the design of synbiotic components for food processing. This model system using mixed culture is applicable for the evaluation of various prebiotics with different chemical structures.

RAPID IDENTIFICATION OF BACTERIAL PATHOGENS IN FOOD AND WATER: ASSIGNING AN ID NUMBER TO BACTERIAL PATHOGENS

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Rapid identification and typing of pathogenic microorganisms have become major objectives over the past decade in food and water microbiology. The recent terror attacks have made it clear that food and water supplies may be a terror targets. Advances in molecular biology have resulted in the development of numerous DNA-based methods for discrimination among bacterial strains. Here we describe the use of variation in mutable SSR loci for accurate and rapid genotyping of Bacterial

strains. *In-silico* genome-wide screening for simple sequence repeats (SSR, Microsatellites or VNTR) in published bacterial genomes showed that the majority (88– 90%) of the SSRs are MNRs, 5 to 9bp in length. These MNRs tracts are evenly distributed throughout the genomes with an average appearance of every 241bp, 225bp, 110bp, 224bp and 225bp in *E. coli* K12, *E. coli* O157:H7, *L. monocytogenes*, *V. cholerae* and *V. vulnificus* genomes, respectively. Sequence analysis of the MNR-containing loci showed high variation level, either as polymorphisms in the MNR itself or as single nucleotide polymorphisms (SNPs) in their flanking sequences, allowing discrimination among strains of these species. These variable sequences were further used as probes for a custom-made allele specific oligonucleotide optical fiber microarray, dedicated to rapid high-throughput typing of bacterial strains based on their MNR-MLST signatures. Amplified MNR-containing loci of *E. coli* strains were hybridized to the array and clearly showed discrimination between all strains. In addition, analysis of long SSRs (L-SSRs) with core motif longer than 3bp, a method also termed MLVA, revealed high discrimination among isolates of *V. cholerae* and *V. vulnificus*.
In conclusion, DNA sequences of MNR loci together with L-SSR loci can be used are a ready source of high variation content, which can be utilized for accurate strain identification. Multi-locus sequence typing (MLST) analysis of loci harbouring mononucleotide repeats MNR-MLST approach can be used to assign an "identity card" to any given strain and can be integrated in a high-throughput method for bacterial identification and typing.

MOULD AS A PROBLEM OF THE PANEL-BUILDING RECONSTRUCTION – 2. HUMAN ASPECTS

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The effective panel technology project in the blockhouse construction was very popular in the 70's. By now these buildings became old and need renovation. It is well known that a lot of technological and execution lacks had been in this project already at the beginning. As a consequence of the inadequate realization of these buildings the occupants have got several problems. The main reason of these problems was the air pollution caused by moulds, since the conditions which were developed due to the construction failures and the life-mode in these flats allowed the colonization of fungi on the walls and other surfaces.
Unfortunately the new renovation and panel reconstruction project does not take into consideration all of the reasons and sources of the mould-pollution, therefore the conditions of mould development will not be eliminated. So the problems and the possibility of health threats still remain, and we have to investigate further this topic in order to pay attention to the incorrect solutions.
The leading health problems and diseases are caused by moulds of three main groups: i. toxic (>300 species, by thermostable metabolites also); ii. invasive (>200 species, living, growing in tissues); and iii. hipersensitivity (allergic) type (>150 verified species, dead fragments of the agent are enough). All have specific routes to enter the human body. Mould-conidia or mycelium fragments can enter into the human body through the respiratory tract. The effect which is induced by this attack depends on the size of conidia. Small (<2 μm diameter particles can invade the lower (alveolar) region and can cause true invasive infections (alveolitis allergica), but the bigger (2-4 μm) irritate the upper respiratory tract generating allergic (bronchial asthma) reactions, while the biggest ones (>10 μm) cause the top reaction (hay fever, rhynitis). However, it has to be considered funny that in the common knowledge and practical mind plant pollens are believed more harmful to humans than the moulds. The truth is the following. In the period of 1995-2001 the highest pollen density has been found with ragweed (*Ambrosia*) but this value never exceeded 500 pollens . m^{-3}. On contrary the

fungal load of indoor air in 96 living quarters with molded wall could reach more than 40 000 fungal propagules . m^{-3} (average 2 100 . m^{-3}). The outdoor presence of pollens show seasonality, on contrary the indoor fungal pollution is present all over the year. Because of the thick polysaccharide cell wall, frequently incrusted with melanin of high chemical resistance, all fungi could have been considered as allergenic one, however only 150 of them are used as *in-vitro* and *in-vivo* diagnostic means. Many of these antigens are glycoprotein metabolic enzymes. These give a broader spectrum of pathogenicity than the plant pollens. The antigenic cross-reactions among certain allergenic molds cauuse dangerous exposition of hyposensitized persons. However the same cross-reactions mediate the multispecific desensitizing.