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INTENSIFICATION OF BIOGAS PRODUCTION FROM SEWAGE SLUDGE

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One of the biggest problems today is the depletion of non-renewable energy sources. We must turn our attention to alternative energy sources, such as biogas. The easiest and most effective way to produce energy from biogas is to burn the gas away in gas motors. In this case we can get both electric and heat energy. Biogas is naturally composed of 75 % methane and 25% carbon dioxide. We need a special consortium of bacteria for the production of biogas. This consortium is separate and contains three different levels. The first is the level of hydrolysing bacteria hydrolyse the biomass (cellulose, starch) into smaller compounds that can be used by other bacteria, the acetogens. These bacteria produce fatty acids and hydrogen for the methanogens, which finally produce methane and carbon dioxide. The production of biogas from this unconventional source (sewage sludge), the enhancement of gas production and the creation of an optimised way to gather energy were the aim of this study. The experiment started with the addition some specialised hydrogen producing bacteria to the consortium. Another way is to pretreat the sewage sludge using for example cellulolytic, proteolytic and lipolytic bacteria. To achieve this, it is important to characterise these bacteria, and especially their exoenzymes, which is difficult and time-consuming. For the experiments, we used photometric, gel electrophoretic and batch fermenting methods. We measured the volume of the produced gas by cubic content overflow measurement, and determined the enzyme activity with a specially designed photometric assay. Another important part was to optimise a continuous fermenting process, which is more similar to the methods used in the industry.

THE ROLE OF FPMK3, A HOG1-TYPE MAP KINASE ENCODING GENE OF *FUSARIUM PROLIFERATUM* IN MULTIPLE ABIOTIC STRESS TOLERANCE

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Fpmk3, a mitogen activated protein kinase (MAPK) encoding gene was cloned by fungal MAPK subgroup-specific nested and single oligonucleotide nested (SON) PCRs from the plant pathogenic fungus, *Fusarium proliferatum* (*Gibberella intermedia*). The deduced amino acid sequence of Fpmk3 gene showed significant homology to other fungal YSAPK (*y*east and *s*tress *a*ctivated *p*rotein *k*inase) MAPK proteins. FpMK3 was found to contain all the YSAPK MAPK, MAPK and Thr/Ser PK specific signature motifs including a TGY dual phosphorylation/activation site and a C-terminal common docking (CD) domain. Reverse transcription PCR (RT-PCR) and real time PCR data suggest that Fpmk3, in contrast to the YERK2-type Fpmk2 MAPK gene, is constitutively expressed in microconidia, during conidial germination and mycelial growth. In microconidia, multiple MAPK-dependent stress resistance mechanisms were active. The microconidia of a Δ*Fpmk3* mutant showed increased sensitivity towards UV-C (220-280 nm), monochromatic UV-C (254 nm) and UV-B (312 nm) irradiations, as well as to hydrogen peroxide treatments. On the other hand, the ΔFpmk3 mutants had and increased tolerance to dicarboximide (vinclozolin) and phenylpyrrole (fludioxonil) fungicides. Hyperosmotic (mannitol) and salt (sodium chloride) stresses caused severe mycelial growth inhibition in the mutants. In-gel kinase assays revealed moderate, but reproducible kinase activation kinetics within minutes after exposure to these stressors.
Comparison of acute and adaptive phase responses of the wild type and its ΔFpmk3 mutants during

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sodium chloride treatment supports the idea that FpMK3 MAPK is essential for cell survival and growth arrest abolition under stress conditions.

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EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) STRESS ON THE SURVIVAL OF *LISTERIA MONOCYTOGENES*

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Listeria monocytogenes is of particular concern for the food industry due to the severity of the illness as well as the wide distribution of the pathogen in the environment and consequently its presence on raw and minimally processed foods. *Listeria monocytogenes* is able to survive and proliferate in adverse environmental conditions.

Also in industrial setting, microorganisms are confronted with very high pressures, as the use of high pressure processing is increasing in food preservation. High hydrostatic pressure (HHP) exerts diverse effects on microorganisms, leading to stress response and cell death. Sublethal HHP stress response and its effect on adaptation and cross-protection is less understood. In this study, the HHP stress response of *Listeria monocytogenes* 4ab (avirulent strain) and *Listeria monocytogenes* (pathogen strain) was characterized in pure cultures.

We examined the effect of HHP treatments (200-600 MPa for 5, 10 and 20 min) in itself and in combination with mild heat treatment (48 °C, 30 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 without NaCl.

THE PROBLEM OF ANTIBIOTIC RESISTANCE: CAN MICROBIOLOGISTS SOLVE IT?

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Antibiotic resistance in bacteria responsible for both hospital and community infections is now considered to be a major threat to successful healthcare in the future. Although antibiotic resistance is usually perceived to be a relatively simple problem, it is actually exceedingly complicated and our failure to either control or reverse resistance has shown that we seldom understand the difficulty. Resistance is often defined as the ability to overcome therapy resulting in clinical failure, this is an easy concept to understand but far less simple is the process by which this has been achieved and what the causative factors have been. This will become a crucial component of microbiology in the future, particularly if new antibiotics come into clinical use.

We are finally beginning to understand some of the individual stages that a bacterial strain has gone through to become multi-drug resistant; did it acquire resistance gene by mobile genetic elements and why are some strains more capable of achieving this than others of the same species? However, we are still largely incapable in predicting the emergence of individual resistance mechanisms, most forecasts have been based on previous knowledge and usually fail when trying to envisage clinical resistance to a new antibiotic. We must improve our capability to do this if we are to reduce the impact of resistance in the future. It is usually considered that antibiotic use is the direct cause of resistance and, although it must contribute, it is unclear why the usage of an individual antibiotic does

not always correlate with the amount of resistance. The link between usage and resistance must be more clearly understood if we are to control resistance without unnecessarily restricting the use of antibiotics. It is also clear that some antibiotics have been very much more capable of selecting multi-resistant bacteria than others; examples often cited are the fluoroquinolones and the cephalosporins, but it is not always obvious why this should be the case.

If we ask whether microbiologists can solve the problems of resistance, the answer is probably only as part of a multidisciplinary team. The role of the microbiologist is certainly to give an individual identification of the causative organism and its likely sensitivity but also to show how this organism contributes to the overall problem. In this respect, the microbiologist not only needs to be able to generate robust data but also to show whether resistance is being disseminated by the spontaneous emergence of individual resistant bacteria or if individual strains are spreading clonally as has occurred with MRSA and multi-resistant *Streptococcus pneumoniae*; the causes of spontaneous emergence and clonal spread are likely to be quite different as the latter is a cross-infection problem. Once the causative organisms have been characterised, the microbiologist will be able to advise the prescriber and the medical staff how to manage the problem. The microbiologist has a crucial role with this advice and is in the front line to control the spread of resistance. If global antibiotic resistance continues to increase, microbiology will not be solely responsible but surely we must accept some of the guilt.

SPECIFICITIES OF THE MICROBIAL SENSITIVITIES TO CHLORSULPHURON IN VITRO AND IN SOIL-INCUBATION EXPERIMENT

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The effects of the herbicide Glean (80% chlorsulphuron) rates were studied in a soil-incubation pot-experiment, carried out for a three-month period *in vitro*. The four herbicide concentrations (0.001, 0.01, 1 and 10 mg/kg soil) represented 1, 10, 1000 and 10.000-fold rates, which are generally used in agricultural practice. The pots were incubated for three months at 28 °C at 60 % of the field water capacity. Samples were taken regularly and countable microbial parts were assessed on selective plates (Nutrient, Congo Red Ashby agar, arginine-glycerine agar etc.) by a modified plate-dilution method of Angerer et al. [1]. The colony-forming units (CFU/ml) of the heterotrophs, free-living nitrogen-fixing bacteria, the actinomycetes and spore-forming *Bacillus cereus* var. *mycoides* were estimated after three-weeks (shorter) and a three-month period (longer), incubated at 28 °C. The chlorsulphuron (0; 0.001; 0.01; 1 and 10 mg/ml) sensitivities of some authentic strains, originating from culture collections were also assessed in liquid nutrient broth after a 14-hour incubation period *in vitro*. The log10-transformed data of the mean values were calculated and significant differences in LSD 5% were shown in the study.

Different sensitivities were found as a function of the herbicide doses and the studied microbial groups. Such sensitivity however was highly influenced by the test-method used. In the liquid broth, less tolerance of chlorsulphuron could be detected in comparison with the soil, however, differences were found between the authentic and the isolated strains. As it was expected from earlier reports [2], the nitrogen-fixing bacteria were showing the highest sensitivities to the herbicide rates with both test-methods. The growth or microbial abundance of those bacteria could be eliminated at higher rates of chlorsulphuron, soon after the shorter incubation period or under the *in vitro* conditions. At smaller doses (i.e. 0.01 mg/kg or mg/L chlorsulphuron), however, the microbial counts of some other bacteria, such as the *Actinomycetes* and *Bacillus* sp., were found to be stimulated. Due to the different

sensitivities of the various microbial groups *in vitro* and *in vivo*, the microbial structure composition in the chlorsulphuron-amended soil may be shifted by regular use in agriculture.

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DOUBLE STRANDED DNA PLASMIDS IN THE MITOCHONDRIA OF *TRICHODERMA* STRAINS ASSOCIATED WITH GREEN MOULD DISEASE

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Trichoderma species are common fungi found in many cultivated and natural soils, and used as biological control agents of fungus-associated plant diseases. However, green mould epidemics on commercially grown mushrooms caused by *Trichoderma* species spread in the last two decades both in Europe and North America.

Double-stranded DNA plasmids were found in the mitochondria of numerous filamentous fungi. The plasmid-carrying fungi generally do not show any symptoms, but special phenotypes are associated with their presence in certain cases. Little is known about the extrachromosomal genetic elements of *Trichoderma* species. Although plasmid ladders derived from circular mitochondrial plasmids have already been reported in some *Trichoderma* isolates, the complete sequence of only a single 2.6 kb plasmid detected in the *T. harzianum* strain – T95 – is known.

In the present study, we investigated the presence of extrachromosomal dsDNA molecules in *Trichoderma* isolates derived from Hungarian *Agaricus* compost and *Pleurotus* substrate samples as well as from the CBS culture collection. In addition to a large DNA band, two smaller bands, 1.7 and 5.0 kb in size, and two bands in the very-high-molecular-weight region (>23 kb) were observed in the undigested total DNA preparation of strain *T. aggressivum* f. *aggressivum* CBS 450.95. These fragments were resistant to S1 nuclease and RNase treatments, indicating their double-stranded DNA nature. Hybridization experiments carried out using the 5.0 kb fragment as probe revealed that these fragments exhibit sequence homology with each other. The DNA samples derived from the isolated mitochondria of the strain also contained the fragments, indicating the mitochondrial localization of the plasmid molecules. Similar-sized bands exhibiting sequence homology with the labelled 5.0 kb fragment were observed in the undigested total DNA preparations of strains *T. aggressivum* f. *aggressivum* CBS 100527, *T. aggressivum* f. sp. *europaeum* B1, and *Trichoderma* sp. DAOM 175924 C15 as well. It was pointed out in the case of *Neurospora* and *Fusarium* that mitochondrial plasmids seemed to be highly mobile and their horizontal transfer occurred frequently in nature. A similar mechanism could have caused the widespread appearance of these plasmids in these green mould associated *Trichoderma* strains.

Further studies are in progress to clarify whether these plasmids have any effect on the virulence of the harbouring strains.

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TOWARDS AN EFFICIENT AND INTEGRATED BIOGAS TECHNOLOGY

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Most biogas production technologies that are in use today are based on the biological activity of microbial consortia developing spontaneously during the start-up phase of the anaerobic digestion process. This usually provides satisfactory results in spite of the obvious fact that these microbial consortia are not optimized for maximum biogas production. Biogas is the ultimate excrement of these consortia and the natural equilibrium prefers optimal growth rather than optimal biogas production. Among recent significant advances in understanding the ecology of anaerobic biodegradation of organic wastes is the recognition of the close syntrophic relationship among the three distinct microbe populations and the importance of H2 in process control. The regulatory roles of hydrogen levels and interspecies hydrogen transfer optimize the concerted action of the entire population. The concentration of either acetate or hydrogen, or both together, can be reduced sufficiently to provide a favourable free-energy change for propionate oxidation.

During anaerobic biodegradation, hydrogen concentration is reduced to a much lower level than that of acetate. In addition, hydrogen partial pressure can change rapidly, varying by an order of magnitude or more within a few minutes. This is related to its rapid turnover rate. The energy available for acetate-consuming methanogens is independent of hydrogen partial pressure, whereas that of the hydrogen-producing and hydrogen-consuming species is very much a function of it.

We have shown that under these circumstances the addition of hydrogen producers to the system and thereby shifting the population balance brings about advantageous effects for the entire methanogenic cascade. The decomposition rate of the organic substrate increases and both the acetogenic and methanogenic activities are remarkably amplified. In laboratory experiments, some 2.6-fold intensification of biogas productivity was routinely observed and the same results were obtained in scale-up experiments.

Proper management of the bacterial population is expected to facilitate the start-up of fermentation. In order to reduce the costs of this treatment, supplemented bacteria are grown in diluted industrial wastewater. In contrast to the commonly used factor of 0.6-0.8 used to estimate biogas yields, the integrated technology, using intensified microbiological biomass decomposition, should yield a two-three fold increase when using 15% solid content biomass. The integrated technology uses sugar accumulating energy plants (e.g., sweet sorghum, Jerusalem artichoke) to increase the biodegradable content of the substrate biomass.

FIRST DETECTION AND GENETIC CHARACTERIZATION OF USUTU VIRUS IN HUNGARY

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Usutu virus (USUV) is a member of the *Flavivirus* genus, family *Flaviviridae*. Viruses related to USUV exhibit considerable veterinary- and public health impact (i.e. West Nile Virus, Japanese Encephalitis Virus, and Murray Valley Virus). The agent was first isolated in South Africa, from *Culex neavei* mosquitoes in 1959. Later it was also isolated in several African countries from mosquitoes, wild birds, rodents, and once from a man with fever and rash. The virus emerged in

Austria in 2001 and caused serious die-offs around Vienna in wild birds, especially in blackbird (*Turdus merula* L.) populations. The virus was presumably introduced from Africa by migratory birds, and it could successfully adapt to the Central European host and vector species, and became a resident pathogen in Austria. In the following four consecutive years, enzootics of USUV in wild birds were observed mainly between the middle of the summer and the middle of the fall in the eastern federal states of Austria. Compared to previous years, the wild bird mortality rate in 2005 declined; and seropositivity against USUV was detected in several individuals.

Considering the Austrian observations, particular attention was paid to the emergence of USUV in Hungary. Although we have already received information in 2003 about the reduced number of blackbirds in urban parks at the Austrian border, the few investigated blackbird samples were found negative for flaviviruses in reverse-transcription polymerase chain reaction (RT-PCR) analysis. More than 130 wild bird corpses, which were collected in the central region of the country between 2003 and 2005, were also tested negative for the presence of USUV nucleic acid. In August, 2005 the Central Veterinary Institute received a dead blackbird from the eleventh district of Budapest. Histopathology investigations revealed lymphocytic encephalitis in the brain of the bird. The flavivirus-specific RT-PCR assay gave a positive result, and, according to the sequence of the amplification product, USUV was detected in the sample.

To reveal the origin and relationship of the virus, the complete genome was amplified by RT-PCR employing overlapping primer-pairs, and the amplicons were sequenced. The nucleotide and putative amino acid sequence of the Hungarian genotype showed the highest identity (99%) to the Austrian strain emerged in 2001, while identity to the African reference strain was lower. Our results indicate that after its emergence in Austria, the virus spread to the Hungarian wild bird populations. Although the emergence of the virus in Hungary was not unexpected, it is surprising that the first positive case was diagnosed about 200 kilometres from the closest known Austrian focus of USUV infections (Burgenland). The observations of the 2006 epidemic season and the preliminary results of our investigations on the occurrence of the infection in Hungarian birds are also discussed in the lecture.

THE INCIDENCE OF GENETIC HUMAN PRION DISEASE IN HUNGARY

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We performed the analysis of the prion protein gene (PRNP) in 27 out of 109 Hungarian confirmed prion disease patients between 1994 and 2004. E200K mutation was found in 17 cases. Another 10 patients, lacking PRNP analysis, showed positive family history. The mean annual incidence (0,27/million) and proportion (25,6%) of genetic prion disease is unusually high in Hungary and might be related to the migration of ancestors from the Slovakian focus.

MICROBIOLOGICAL MONITORING OF LANDFILLS IN THE BEREG REGION

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Quantity and quality of the contaminants distributed from communal and industrial landfills is significantly diverse and possesses the characteristics of the given landfill in its main aspects. Generally, rich ruderal vegetation, abundance of organic matter and a characteristic microflora can be found in landfills. This especially concerns microorganisms living on the surface of the leaves of furrow-weeds growing in large quantities. Our main objective was to study the surface microflora of predominant plants occurring in large quantities on landfills and polluted areas; and to identify the factors affecting them.
Landfills located near small settlements in the Bereg region served as the sampling areas: Beregsurány, Beregdaróc, Gelénes, Nagybégány, Muzsaly, control: Asztély forest, Beregdaróc forest. Studied plants: nettle (*Urtica dioica*), common burdock (*Arctium lappa*), catch weed (*Galium aparine*) and sow thistle (*Sonchus asper*). Randomly selected 3 leaves of 5 plants each were touched with both sides onto the surface of agar plate containing meat-peptone medium in the spring and autumn of 2004 and 2005. The plates were incubated for 1-5 days at 26 °C and colonies of different morphology were isolated from the plate. Colony counts were calculated to 1 cm^2 leaf area, strains were characterized by biochemical tests, including API.
In 2004, Gram-negative and Gram-positive microbes, and in 2005, mainly Gram-positive microbes were isolated from the surface and reverse sides of the leaves. The members of *Bacillus* genus dominated on the surfaces of plants in 2004. The *Bacillus* genus also occurred in large quantities in 2005 but the Coryneform bacteria dominated that year. On the surface of the leaves of *Arctium lappa, Corynebacterium* accounted for 50% and the genus *Bacillus* accounted for 18% of the isolated strains; in case of *Urtica dioica,* the percentages were 79 and 22%; in case of *Galium aparine,* they were 35 and 65%; and in the case of *Sonchus asper,* 50% and 33%, respectively. Identified species of the genus *Bacillus* were *Bacillus cereus, B. subtilis*, *B. megaterium, B. licheniformis, B. pumilus. Corynebacterium aquatile*, *Corynebacterium spp*. species dominated among the Gram-positive Coryneform bacteria. *Aeromonas*, *Pseudomonas*, *Erwinia* and *Klebsiella* bacteria were also isolated from the upper and lower surfaces of the studied plants, and from soil, water and air.

DETECTION OF PRRSV ANTIGENES USING A BROAD SPECTRUM IMMUNOHISTOCHEMICAL METHOD ON FORMALIN FIXED, PARAFFIN EMBEDDED TISSUE SAMPLES

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Porcine reproductive and respiratory syndrome (PRRS) is a widespread disease causing significant economic losses. The relatively favourable epizootiological situation of previous years shows a worsening tendency, and although reliable data on the occurrence rate of the infection are not available, 20-25% percent of our large scale pig farms may be considered seropositive in Hungary. The virus causing PRRS is a member of the *Nidovirales* order, *Arteriviridae* family, *Arterivirus* genus. Its genome is a positive, single stranded, approximately 15000-nucleotide-long RNA molecule, comprised of 9 open reading frames (ORF's). Based on antigenical and nucleotide sequence analyses, the strains are classified into European (type 1) and American (type 2) genotypes. The strains show high sequence variability even within the genotypes; genotype 1 shows higher genetic variation.
Our research team demonstrated the presence of the American type in a herd in North-Western Hungary hence the continuous development of diagnostic methods is essential to detect all possible

PRRSV strains in the investigated samples. To ensure the detection of any (European or American) genotypes present in the samples, we developed a peroxidase-based immunohistochemical reaction, and demonstrated the presence of the virus in formalin-fixed and paraffin-embedded tissue samples. The virus is mostly detected in the alveolar or in the interstitial macrophages of the lungs causing brownish red, diffuse discoloration of the cytoplasm. Our method proved to detect strains of both genotypes. In our investigations, the specificity of the immunohistochemical method was tested by RT-PCR on the appropriate samples and sequencing of the amplicons.

GENETIC SUBTYPES OF HIV-1 IN CHILDREN IN HUNGARY

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HIV-1 infection is characterized by a high degree of genetic diversity among viral variants due to a high mutation rate, rapid viral turnover and recombination of viral genomes. This diversity is highest in the *env* gene. HIV-1 variants can be classified into three groups: the major (M), the outlier (O) and the new (N) groups. Group M viruses are further classified into ten subtypes or clades (A-K). The aim of our study was to determine the genetic subtypes of HIV-1 in children in Hungary. Proviral DNA isolated from peripheral blood mononuclear cells (PBMCs) of 9 HIV-1 infected children was classified into subtypes by sequencing of the *env* (C2V3), *gag* (p24), *LTR* and *nef* regions of the viral genome. In the case of the *env* region, the heteroduplex mobility assay (HMA) was also used for subtyping. Five children were infected via nosocomial transmission and 4 vertically. We found, based on the env, gag, LTR and nef sequences, that 1 vertically infected child and 5 nosocomially infected children carried subtype F HIV-1 strain. Subtype B strain was found in 2 vertically infected children. In addition, in 1 vertically infected child we found a recombinant HIV-1 strain: the env, LTR and gag regions corresponded to subtype B, while the nef gene of this child showed the highest homology with subtype F sequences.

FUNCTIONAL ANALYSIS OF THE HUPHI GENES IN THE OPERON OF THE HUP HYDROGENASE IN THE PURPLE SULPHUR PHOTOSYNTHETIC BACTERIA *THIOCAPSA ROSEOPERSICINA* BBS

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Thiocapsa roseopersicina is a phototrophic purple sulphur bacterium belonging to the family of Chromatiaceae. There are at least three hydrogenases in the cells, which differ in their composition and biochemical properties. Two of them are membrane-associated [NiFe] hydrogenases (HynSL-Isp1,2 and HupSLC), while the third one is a soluble hydrogenase (HoxEFUYH). HynSL is resistant to proteolytic enzymes, detergents, oxygen and heat. The Hyn and Hox enzymes can catalyze both H2 uptake and H2 evolution *in vivo*, however the other membrane-associated hydrogenase, Hup, plays a role in the hydrogen uptake only (hup = hydrogen uptake).

The *hup* locus consists of seven genes (*hupSLCDHIR*). The *hupS* and *hupL* code for the hydrogenase small and large subunit, respectively, while the genes (*hupCDHI* and *hupR*) identified downstream of

the structural genes code for proteins likely linked to the biosynthesis and/or function of the Hup hydrogenase. It was shown that HupC encoded a cytochrome b-type protein involved in the transmembrane electron transfer, while HupD was an endopeptidase that played a role in the proteolytic removal of the C-terminal tail from the large subunit (HupL). The HupR is a regulatory protein being a component of a hydrogen dependent signal transduction pathway. However, little is known about function of *hupH* and *hupI* genes. It was suggested that HupH protein - by interacting with the small subunit - was required for the translocation of the H2ase to the membrane, while *in silico* analysis indicated that HupI was a rubredoxin-type protein probably involved in the electron transfer processes.
Reverse transcription coupled PCR analysis showed that *hupSLCDHI* genes were transcribed together but further experiments were needed to determine whether the *hupR* gene is a part of this operon or not. In order to investigate the role of *hupH* and *hupI* genes, in frame deletion mutants were created and the effect of the mutations on the *in vivo* and *in vitro* hydrogenase activity was examined. Moreover, an expression cassette was constructed for homologous expression of the HupH protein N-terminally fused to a Flag/Strep II oligopeptides. Using this system, it is possible to purify the HupH protein with its interaction partners, which can be identified by mass spectrometry.
Acknowledgement: The work has been supported by EU 5th and 6th Framework Programme projects (QLK5-1999-01267, STREP SOLAR-H, HYVOLUTION) and by domestic sources (NKFP, DEÁK-KKK, KN-RET, ASBÓTH, BAROSS). International collaboration within the EU network COST Action 841 is greatly appreciated.

TRACING *LEGIONELLA* COLONIZATION IN DRINKING WATER SYSTEMS

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Legionellae, including *L. pneumophila*, the causative agent of Legionnaire's disease are known to inhabit a variety of man-made water environments from cooling towers to jacuzzis and communal hot water systems. They are able to persist and proliferate in biofilms on natural or artificial surfaces. Drinking water systems – both cold and hot water lines – are prone to colonization by different *Legionella* species. Our aim was to (1) assess the diversity of Legionellae within a hot water system at both species and subspecies level, (2) identify the most efficient method for strain level identification of *Legionella* isolates.
Water samples were taken at 5 to 10 points of cold and hot water systems of hospitals, hotels and other communal buildings. Samples were processed by membrane filtration, Legionellae were cultured on BCYE and GVPC media. Legionellae were enumerated on the membrane filters, at least 3 typical colonies were isolated from each sample. Isolates were characterized by morphology, serotyping, ribotyping, repetitive element (rep-) PCR using ERIC primers, *Legionella* genus and *Legionella pneumophila* species specific PCR. A subset of isolates was also typed with MALDI-TOF MS. Ribotyping was shown to differentiate Legionellae at species level. Identity of group representatives was confirmed by 16S rDNA sequence analysis. *L. pneumophila* was the most frequently detected species, though currently used culture methods may be biased for the isolation of *L. pneumophila*. Serotyping, rep-PCR and protein profiling with MALDI-TOF MS were all suitable for subspecies typing of Legionellae. Grouping based on serotyping and rep-PCR was identical. Several strain types, and occasionally more than one Legionella species, were identified within a water system. Both qualitative and quantitative distribution of Legionellae was heterogeneous within the water lines.

DISCOVERY OF THE NOVEL FUMONISIN MYCOTOXINS BY ION-TRAP MASS SPECTROMETRY

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Fumonisins are a group of structurally related mycotoxins that are mainly produced by *Fusarium verticillioides* (Sacc.) Nirenberg, formerly known as *F. moniliforme* Sheldon, which is one of the most common moulds colonizing maize crops throughout the world prior harvesting, during the time between harvesting and drying, and during storage.
Fumonisins were produced in a *Fusarium verticillioides*-infected rice culture and were analyzed by RP-HPLC/ESI - ion-trap MS2 immediately after the extraction of the culture material without any sample clean-up. Beside already known fumonisins, numerous new fumonisin mycotoxins and fumonisin-like compounds were detected. On the basis of the ion-trap MS2 data, detailed fragmentation pathways, including new mechanisms, were proposed for the different series of fumonisins. The retention times, the molecular and fragment ion masses including the backbones, the characteristic neutral mass losses from the molecular ions of the new compounds suggested their structures (applying the well-known designation): iso-FA1a,b, iso-FB1a-d, iso-FB2,3a-e, PHFB2a-c, PHFB4a-d, FB5/iso-FB5a-d, FBK1 2TCA, FBK4 2TCA, FC2, iso-FC2,3, PHFC4, FD and FBX series. The relative quantities of fumonisins and fumonisin-like compounds found in the sample extract were expressed as percentages of FB1 (0.02-100%). The backbone of the compound denoted FD contained fewer carbon atoms than the well-known fumonisins with the C19 or C20 backbone and may well be a precursor of the longer compounds. For the compounds denoted FBX (12 compounds), one or two OH groups attached to the fumonisin backbone were esterified by carboxylic acids other than tricarballylic acid, such as *cis*-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid.
Acknowledgement: This research was supported by a Hungarian State Research grant (OTKA 46739) and a GAK grant (ALAP1-00073/2004).

OCCURRENCE OF *LISTERIA MONOCYTOGENES* IN FOOD AND THE DIFFICULTIES OF IDENTIFICATION

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Listeria monocytogenes is a Gram-positive, non spore-forming, rod shaped, motile bacterium present everywhere in the environment. It can be detected in raw products of animal origin, such as milk, meat, poultry and fish. The presence of *Listeria monocytogenes* in a food product is a problem partly because it presents health hazard in higher numbers for the consumer, and it also indicates faults in the processing. Based on our current knowledge, it can be concluded that the risk of contamination with *L. monocytogenes* can be minimised, however, the microbe cannot be totally eliminated either from end products or from the environment. Pasteurisation and cooking destroys the bacterium, but recontamination may also occur in ready-to-eat foods, cheese, ice cream and meat products (pâté) as well as on fresh fruits and vegetables. An interesting feature of the bacterium that worries most food-microbiologists is that it is able to grow at refrigerating temperature. In most countries, the regulation is very strict, "zero-tolerance" is a norm for ready-to-eat-foods, though the critical concentration presenting health hazard is above 100 living cells. In Hungary, we have studied the spoilage microflora of commercially available, fresh, ready-to-use sliced mushroom, sliced carrot and strawberries. *Listeria monocytogenes* could not be detected in any of the examined samples.

The new legislation on the detection of *Listeria monocytogenes* coming into force in 2005 requires the use of Agar *Listeria* according to Ottaviani and Agosti (ALOA agar) as the detection medium. The medium was tested according to one of the producers on more than 200 food samples, and only two microbes other than *Listeria* were capable of growing on it, although without giving the colour reaction. However, in our studies, the first product applied to the new media was proved *Listeria* positive on ALOA agar for 28 out of 31 samples. The number of Listeria-suspected colonies varied from 10^2 to 10^6 CFU/g. This product was red paprika powder. The detected microbes also gave positive results on Oxford agar. At the biochemical examinations, the microbes did not prove to be *Listeria*, and the microbes were eliminated in the process after the *Listeria* selective enrichment (Half-Fraser, Fraser broth). Nevertheless, it is important to realise that, for some types of food, high numbers of microbes other than *Listeria* giving the same colour reaction might make the enumeration of *Listeria* impossible on ALOA agar.
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TRANSCRIPTIONAL REGULATION BY MEDIATOR SUBUNITS IN *SCHIZOSACCHAROMYCES POMBE*

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The first step in the expression of a protein coding gene is transcription, when an RNA copy is synthesised on the DNA template by RNA Polymerase II. This is a highly regulated process, which involves both gene-specific transcription factors and general transcription regulators such as Mediator. The Mediator complex acts in eukaryotic cells as a bridge between the gene-specific transcription factors and the RNA Polymerase II complex. Much has been revealed about the structure of the Mediator complex in yeasts. Most of its subunits have been identified but little is known about the functions of the subunits. Two subunits of the *S. pombe* Mediator complex were investigated in this project. In previous reports, we identified and characterised two genes that code for the Mediator subunits Sep10/Med31 and Sep15/Med8. Their mutations impaired the separation of sister cells at the end of cytokinesis, which changed the unicellular yeast morphology to hyphal morphology. A genome-wide transcription profiling (by DNA microarrays) of the mutant and the wild-type cells revealed that the inactivation of these genes modified the transcription level of large groups of genes involved in diverse processes and functions. The major functional categories were: transport processes, carbohydrate metabolism and amino acid metabolism. Numerous Sep1-Ace2-dependent cell separation genes were also down-regulated in the mutants, which may account for the inability of the cells to separate at the end of the cell cycle. The results indicate that the Sep10/Med31 and Sep15/Med8 subunits participate in the regulation of large subsets of the gene pool of *S. pombe*.

HEAVY METALS INFLUENCED THE MICROBIAL PROPERTIES, ENZYMATIC ACTIVITIES AND RESPIRATION RATE IN ALFALFA SOIL RHIZOSPHERE

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It is accepted that accumulated heavy metals reduce soil microbial content and change the various enzymatic activities, leading to a decrease in the functional and structural diversity of the microbial content in the soil as well as the development of metal tolerant microbial populations. In this study, the effect of heavy metal (Cd, Cu, Ni and Pb) contaminated soil on some soil properties (pH, organic-C and available-P), microbial content (total counts of aerobic heterotrophic bacteria, and spore-forming, actinomycetes, microscopic fungi, cellulose decomposers and phosphate solubilisers), the ratios of Gram negative to Gram positive bacteria and enzymatic activities of fluorescein diacetate hydrolysis, dehydrogenase, catalase, urease, protease, aryl-sulphatase, phosphatase, β-glucosidase) and soil respiration rate were investigated after 10 weeks in containers filled with one of four different concentrations of metal contaminated soil from Gödöllő, Hungary.
The results indicated that the soil organic C content showed statistically (at $P < 0.05$) significant correlation with the total microbial contents, enzymatic activities, soil respiration at low concentrations of Cd, Cu and Pb, and with higher concentration of Ni. There was a statistically negative correlation between heavy metal concentrations and the catalase activity and soil respiration and microbial biomass-C. The results showed that soil respiration decreased linearly with increasing heavy metal content. Also, the activity of the enzymes related to nitrogen, phosphorus and sulphur turnover cycles was more influenced by the heavy metal polluted soil. Overall, the investigated heavy metals could have long-term effects on the microbiological properties of the soil. The ratio of microbial biomass –C/total organic C illustrated a very similar pattern as the microbial biomass C. This ratio is useful to be used as a heavy metal soil pollution index. It appears that the organic C, soil respiration, dehydrogenase, catalase, fluorescein diacetate hydrolysis are reduced more by the heavy metal contamination in agro ecosystem, which reflects a reduction in soil quality. Due to these facts, the microbial population densities, microbial biomass and enzymatic activities can be considered as sensitive indicators of soil contamination with heavy metals and soil quality. Further investigations should be carried out on the effect of heavy metal polluted soil on soil biochemistry in different soil types (e.g., acidic, alkaline, arid, semi-arid, agricultural, sand, clay, etc.) using different sources of heavy metals, and general conclusions should be made about the changes.

SYMBIOTIC INTERACTION BETWEEN *MEDICAGO SATIVUM* - *SINORHIZOBIUM MELILOTI*: MODEL FOR RHIZOREMEDIATION OF HEAVY METALS

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Root microbes are used in agro ecosystems in the form of bio inoculants (biofertifilizers, bio stimulants, bioprotectants or/and bioremediants). Specific root microbes have considerable potential to alter the composition and activity of the rhizosphere microbiota, such as *Rhizobium*. Root microbes that promote nodulation of legumes by *Rhizobium* are referred to as nodulation promoting rhizomicrobes (NPR). The beneficial effects of these rhizomicrobes have been variously attributed to their ability to produce various compounds, such as phytohormones, growth factors such as vitamins or other organic compounds, and toxins or antibiotics to suppress harmful root microbes.
The results of antagonistic experiments showed no antagonistic effect among the interactions between *Sinorhizobium meliloti* (MSH-21 and MSH-22 strains) and NPR strains of *Azotobacter* sp., *Bacillus* sp., *B. subtilis*, *Enterobacter* sp., *Flavobacterium* sp., *Pseudomonas fluorescens, P. putida, Saccharomyces cerevisiae, Streptomyces* sp., *Trichoderma harzianum* and *T. viride* strains isolated

from *Medicago sativum* and other plant rhizosphere. The ability of the selected rhizomicrobes to influence the nodulation of alfalfa by *S. meliloti* strains was assessed in sterile polluted soil by different concentrations of Cd, Cu, Ni and Pb at semi-field scale.
The colonization intensity of the selected rhizomicrobes in the seedling rhizosphere and the nodulation potential were dependent upon the mixture rates between selected rhizomicrobes. The results indicated that the simultaneous presence of *Sinorhizobium* strains with other rhizomicrobes was necessary for enhanced alfalfa nodulation. The best nodulation was obtained when *S. meliloti* (MSH-21) was applied to the seedling rhizosphere combined with *Bacillus* sp., *Enterobacter* sp.*, P. putida, S. cerevisiae* and *T. harzianum*.
The results showed that plant dry weight, length, root biomass, root-hair proliferation and lateral root formation increased in response to inoculation with the bioinoculant combination. The plant productivity and nodulation potential were higher in the plant inoculated with the bioinoculant containing MSH-21 than the one containing MSH-22 strain. After 10 weeks, the detected amount of soil-polluting metals was 20 and 30% lower than the initial value, and metal accumulation in plant tissues was higher than in the control. The model productivity was not significantly reduced statistically at the highest metal concentration. This means that in the model system bioremediation of the soil is performed through the macro- and microsymbionts. It was found that Cd and Pb had a nearly equal effect, while Cu was the most toxic and Ni was the least toxic metal of the model. These preliminary results demonstrated the potential of root-associated rhizomicrobes other than the microsymbiont to alter the dynamics of the alfalfa-*Sinorhizobium* symbiosis. However, these results need confirmation with non-sterile soil and field application studies.

TRENDS IN FOOD MICROBIOLOGY

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In the second half of the 20th century, the main focus of food industry was to provide enough food of good quality. With the passing of time, quality became the important point, and with world-wide globalisation accompanied by mass food products, different national, ethnic and exotic foods, have broadened the choice. The eating and shopping habits of people have changed over time, we eat out more often, buy food for longer periods, prefer kitchen-ready, or ready-to-eat, deep frozen, pre-packed, modified atmosphere-packed food. The food industry had to keep up with the increasing demand of consumers for fresh, or fresh-like, health-promoting and, most of all, safe products. Microbial safety is of utmost importance, since it has not only a direct and short effect on our health, but may also influence the quality of life.
New preservation technologies have been developed in the last half-century, including irradiation, high hydrostatic pressure, pulsed electric field and different packaging and storage techniques, etc. They can be applied alone or in combination to minimize the effect on sensory food quality. The goal of the treatments is to preserve food in good quality (to hamper the enzymatic, physiological and microbiological spoilage of food), and to eliminate food-borne pathogens. The spectrum of food-borne diseases has changed over time and we face new challenges, partly due to the effect of human activity on the environment.
Food safety is the main issue currently, with the focus on microbiological safety. To fulfil the requirements, we have to learn more and more about the effect of newly emerging food technologies on the microbes in foods, and also about their survival in the environment. The biofilm formation ability of microbes and their role in the food industry have also gained great attention recently. The role of microbial databases and efforts to predict the possible survival/growth of microbes in certain food commodities under given conditions open a new possibility to apply and/or develop appropriate food technology. The lecture will give a survey on the work done in the fields of food

microbiology/food preservation carried out in the last thirty years in the Central Food Research Institute. I would also like to express my gratitude to my teachers in this field and thanks to my colleagues during all these years.

INTEGRATION OF BIOHYDROGEN FERMENTATION AND MEMBRANE GAS SEPARATION

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Hydrogen was produced by fermentation using *Escherichia coli* XL1-blue and MC4100 strains in this work. The hydrogen gas formed should be recovered by an effective separation method, like a membrane technique (gas separation). In the anaerobic fermentation system, hydrogen (25 %) and carbon dioxide (20 %) are formed. Besides, nitrogen was used as inert gas. Thus the task was to separate biohydrogen from a three-component gas mixture. A two-stage membrane system was designed and built to separate biohydrogen. The first membrane was a porous HDPE membrane, where separation was achieved by the Knudsen-mechanism and major part of the CO2 content of the gas stream was separated from the initial gas mixture, resulting in a hydrogen-rich permeate. This nitrogen-hydrogen gas mixture was then brought to the shell side of a hollow fiber gas separation membrane module (material: polyethersulfone-polyimide). Hydrogen content of the collected permeate was over 70 %, which is suitable for fuel cell applications.

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COMPARISON OF DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) AND HETERODUPLEX ANALYSIS (HDA) FOR THE DISCRIMINATION OF *CAMPYLOBACTER* ISOLATES

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Human health has high priority, thus determination of the sources of infections and the routes of transmission are in the centre of attention. The epidemiology of *Campylobacter* infections has been investigated by several microbiological methods, but despite the large number of different identification and typing systems, only a few comparisons of the methods and their utility have been published. The aim of our work was to examine and compare the power of two PCR-based electrophoresis systems for the detection and discrimination of *Campylobacter* isolates originating from different (human and animal) sources.

A total number of 38 isolates were studied by two different methods: (1) denaturing gradient gel electrophoresis (DGGE) and (2) heteroduplex analysis (HDA). We used the CF02-CF03 primer-pair for the amplification of the *Campylobacter flaA* gene sequence. In the case of DGGE, a GC-clamp was attached to the reverse primer. The gradient of the chemical denaturant was adjusted to 15-45% and an 8% of polyacrylamide gel with a parallel chemical denaturing gradient was used. For HDA, heteroduplexes were generated by denaturing and renaturing the mixtures of the amplified sequences of the strains and these were loaded onto a 5% polyacrylamide gel to analyse differences in mobility.

DGGE analysis differentiated the amplified fragments on the basis of their sequence-dependent melting temperatures. The addition of a GC clamp to the reverse primer further enhanced the sensitivity of the technique. Differences were found in the case of different species, but polymorphism was also observed. Heteroduplex analysis separated the homoduplexes and generated heteroduplexes according to their mobility in the polyacrylamide gel. We found that some isolates belonging to the same DGGE cluster resulted in different patterns by heteroduplex analysis. Based on our results, heteroduplex analysis proved to be more powerful in the discrimination of *Campylobacter* isolates than DGGE.

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VIRUS TAXONOMY IN THE NEW MILLENNIUM

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The practical needs of virus classification have largely been satisfied by the use of four officially recognized hierarchical taxon levels (genus, subfamily, family and order) for more than three decades. The concept of virus species as the lowest viral taxon was formalized for the first time in the 7th ICTV Report published in 2000. By definition, a virus species is "a polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche". Polythetic class here means that its members are defined collectively by a consensus set of properties, however, none of the properties is necessarily shared by all members, and none of the members should possess every demarcation property, either. Consistent assignment of viruses into taxa requires the specification of the demarcation criteria. The following characters are useful for discriminating between virus species within the same genus: natural host range, cell and tissue tropism, pathogenicity and cytopathology, mode of transmission, physicochemical properties of virions, antigenic properties of viral proteins. In addition, sequence comparisons are an increasingly dominant criterion because they provide a quantitative measure of divergence. In optimal cases, phylogenetic relationships overlap with and confirm the results of former classification methods including serology and phenotypic characterisation. The 7th Report of ICTV contained the description of approximately 3000 viruses belonging to more than 1550 species, 233 genera, 9 subfamilies and 56 families. In the 8th Report, published in 2005, more than 5450 viruses and more than 1950 species are listed. There are 73 virus families, 9 subfamilies and 287 genera approved by ICTV. At the same time, only three viral orders and no higher taxa exist. While one can be confident that members of a virus genus or family share common ancestors, the evolutionary relationships between families as well as the origins of viruses are very uncertain. Therefore, it cannot be anticipated that all virus families could be integrated into a single phylogenetic tree with a corresponding global taxonomy. However, as the power and sensitivity of detection methods and the abundance of available viral sequence information increase, more distant phylogenetic relationships might become evident in the future. Since 1991, the ICTV has also been working for the establishment of a comprehensive and universal database containing virus isolate data with reliable links to the agreed taxonomy and to genome sequences. The aim of ICTVdb is to provide the research community and general public with online tools for precise identification of viruses at the isolate level (http://phene.cpmc.columbia.edu/).

EFFECT OF PHARMACEUTICAL WASTE ON THE MICROBIAL COMMUNITY STRUCTURE OF A MESOPHILIC SLUDGE DIGESTER

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Increasing energy demand and the depletion of fossil energy forced the scientific and economic communities to focus their attention on renewable forms of energy, such as biogas. Not only ordinary organic waste compounds (e. g. communal sludge) but also xenobiotic waste can be degraded under anaerobic conditions. Ecological observations suggest that sulphate-reducing and methanogenic bacteria might metabolize xenobiotic chemicals.
Pharmaceutical industry waste was added to a mesophilic pilot-scale biogas digester for a ten-day period and sludge samples were collected during the experiment. Genomic DNA was isolated from sludge samples and a section of the 16S rDNA was amplified with Bacteria and Archaea specific primers. Terminal Restriction Fragment Length Polymorphism (T-RFLP) was used to monitor the diversity, structure, and dynamics of microbial communities. The TRFs were identified by comparison with Bacteria and Archaea clone libraries of the same community.
Methane production broke off at the end of the experiment, while pH decreased from 7.4 to 6.4 and redox potential increased from -518 mV to -490 mV. Among the archaeal community, the methanogen *Methanosaeta concilii* ceased to be the dominant species but this place was gradually filled in by *Methanoculleus bourgensis*. The bacterial species diversity changed drastically following the addition of the waste containing xenobiotics, but most of the clones showed similarity to uncultured species. To summarize our results, we can state that the microbial community showed notable changes as an effect of xenobiotic addition.
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ADAPTATION OF *GLOMUS MOSSEAE* STRAINS OF DIFFERENT ORIGIN TO SOIL HEAVY METAL LOADING

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The use of plants to remediate contaminated sites is regarded as a low cost and environmentally friendly remediation technique. The efficiency of phytoremediation in heavy metal polluted soils can be enhanced using adequately selected, metal-tolerant inocula of root symbionts such as arbuscular mycorrhizal fungi (AMF). AMF form symbiotic association with more than 80% of terrestrial plants. The effectiveness of different AMF species–host associations may differ. Changes of infectivity of five *Glomus mosseae* strains of different origin and response of host plant (shoot Cd concentration, biomass production) to AMF inoculation were investigated in heavy metal contaminated calcareous chernozem soil (0, 50 and 100 mg total Cd kg-1 soil). The effects of five *Glomus mosseae* strains (BEG12, HS1, HS2, S and M) on their host plants (*Trifolium repens L.*) were compared with each other and the controls after 11 and 20 weeks, carried out in a pot experiment. The *G. mosseae*-BEG12 originated from the European Bank of Glomeromycota. Spores of HS1 and HS2 inocula were isolated from humous sandy soil, while *G. mosseae*-S originated from salt-affected soil and –M was isolated from calcareous loamy chernozem soil polluted with cadmium (90 mg Cd kg-1 soil).
Three *Glomus mosseae* strains (HS2, S, and M) could be emphasized from the point of view of being able to enhance the efficiency of phytoremediation in heavy metal contaminated soils. The arbuscularity (A%) of strains (G. mosseae-HS2, -S) isolated from non-polluted soils was high and increased with increasing soil Cd content, but values of A% significantly decreased after 20 weeks. At each rate of Cd application, mycorrhizal plants colonized with *G. mosseae*-HS2, and -S had

significantly higher biomass production and lower shoot Cd concentration compared to the controls and other mycorrhizal plants. The observed elevation of phytotoxicity can be explained by a dilution effect due to higher biomass production of these mycorrhizal plants. *G. mosseae*-HS2 and –S strains isolated from non-polluted soils would be suited to considerably enhance the efficiency of phytoremediation. The *G. mosseae*-M strain isolated from Cd-polluted soil can be regarded as a Cd-adapted strain. Its arbuscularity was also high and was affected neither by increasing soil Cd loading nor by harvesting time. Its origin had importance in maintaining its high infectivity in polluted soils, but the beneficial effect of this strain on the biomass production and shoot Cd concentration of their host appeared only after 20 weeks at 100 mg Cd kg-1 soil pollution.
During the 20 weeks of the experiment, the five *Glomus mosseae* strains of different origin showed great differences in infectivity in response to soil Cd loading and had various effects on their hosts. According to our results, the probably high intraspecific variability of AMF could also be utilized in phytoremediation. Otherwise, greater attention should be paid for the selection of sampling times because the behaviour of AMF strains could not be interpreted properly within a short time.

BACTERIOLOGICAL DIVERSITY OF ULTRA PURE WATER EVALUATED BY CULTIVATION AND CULTIVATION INDEPENDENT METHODS

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It is known that ultra pure water (UPW), used as cooling water in power industry (e.g. a nuclear power plant), can suffer microbial contamination, which poses a threat of corrosion. These oligotrophic waters are characterised by extremely low salt and nutrient concentrations. Scanning electron microscopic (SEM) examination was carried out on the “biofilms” and water samples were taken from feed (U) and product (R) pipelines of ion exchange units.
Oligotrophic media were used for the enrichment procedure and cultivation. After random isolation, bacteria were grouped based on their fatty acid profile. Phenon representatives and ungrouped strains were subjected to 16S rDNA sequencing. Simultaneously, 14-14 litres of water samples were filtered and, following direct DNA isolation, T-RFLP analysis was carried out.
According to the SEM examination, diverse microbial communities were found in the biofilms, among them budding forms of *Hyphomicrobium*. Results from cultivation showed that aerobic, chemoorganotrophic β-proteobacteria (*Delftia acidovorans*, *Chromobacterium violaceum*, *Ralstonia insidiosa)* dominated the water samples. They may also have an important role in the degradation of e.g. ion exchange resins originating aromatic compounds, moreover some of them are H_2 autotrophs. Nevertheless, members of γ-proteobacteria (*Enterobacter aerogenes*, *Serratia marcescens, Stenotrophomonas maltophilia*) and members of the genus *Bacillus* could be detected. The culture independent T-RFLP method also indicated a complex microbial community structure. The profile of the two water samples were similar, differences could mainly be detected in the ratio of TRFs. The biofilms of the feeding and product pipes showed different profile from each other. The water and biofilm samples showed characteristic differences. According to results of PAT analysis of TRF patterns, the β-proteobacteria dominated all samples as well as in case of cultivation. In addition, the presence of other bacteria (α-proteobacteria, other non culturable Gram- negative bacteria, Cyanobacteria) could also be detected.
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OCCURRENCE OF ANTIBODIES AGAINST *BORRELIA BURGDORFERI* IN FORESTRY WORKERS EXPOSED TO INCREASED RISK OF TICK BITES

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Lyme disease is the most common arthropod-borne human infection in North America and Europe. The disease is a multisystem infection, caused by spirochetes of a genospecies complex *Borrelia burgdorferi* sensu lato. In Europe, at least three species are known as causative agents of the disease: *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii*. The principal vectors of these spirochetes are infected ticks of the *Ixodes ricinus* species. The diagnosis of Lyme disease is recently based on clinical symptoms, objective physical findings (such as erythema migrans (EM), arthritis, or facial palsy), and a history of possible exposure to infected ticks. Supporting the diagnosis, the recommended tests detect *B. burgdorferi* specific antibodies. The prevalence of the specific antibodies could be considered as a marker of previous exposure to the pathogen, and allows the identification of endemic regions. Because positivity itself does not necessarily mean an active illness, the high rate of seroprevalence in populations living in endemic areas or occupationally exposed to tick bite makes it difficult to interpret the laboratory result. The aim of our study was to evaluate the prevalence of anti-*B. burgdorferi* antibodies in forestry workers, and compare the data with those of healthy blood donors.

Between January 2005 and July 2006, we collected samples from outdoor workers (hunters and foresters; n = 93). According to the CDC guidelines, we applied a two-step protocol: in the first step, as a screening test, we used sensitive ELISA (Mikrogen *recom*Well Borrelia, IgG/IgM). In case of positive or indeterminate ELISA result, as a confirmatory assay, we used a Western blot (Virotech Borrelia afzelii EcoBlot or Mikrogen recomBlotNB Borrelia IgG/IgM).

Among forestry workers, 43 samples gave a positive result with the ELISA test (IgG: 46.23%). When we confirmed these results to the Western blot, we found 29 positive and 8 indeterminate samples and the result of the screening test could not be confirmed in 6 cases. The overall IgG positivity in the collected samples was 31.18%. The results were compared to sera from a seroepidemiological survey carried out in 2000 in Hungary (n = 180). We found 29 positive samples (IgG: 16%) in this group with the ELISA test. In the confirmatory assay, we verified the results in 5 cases as positive, in 3 cases as indeterminate, and in 21 cases as negative. The overall IgG positivity in the tested blood donor samples was merely 4%.

Our data show that there is high rate of prevalence of specific *B. burgdorferi* antibodies in the risk population of forestry workers; according our data, nearly every third person has been exposed to the pathogen. The high rate of positivity influences the predictive value of laboratory tests. One should be cautious when interpreting Lyme serological results in high-risk populations occupationally exposed to the habitat of ticks.

PROTEOMIC ANALYSIS OF COPPER DEPENDENT REGULATION OF METHANE MONOOXIGENASES IN *METHYLOCOCCUS CAPSULATUS* (BATH)

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Methylococcus capsulatus (Bath) is a gram-negative, coccoid bacterium. It is able to express two methane monooxigenases (MMO): in the presence of copper ions, the particulate MMO (pMMO), its helper proteins and transport proteins responsible for copper uptake are expressed changing morphology of the cells (extended membrane systems can be seen), while, in the absence of copper,

soluble MMO (sMMO) and its accessory proteins are expressed. sMMO can oxidize a wide range of compounds from alkenes, ethers and haloalkenes to aromatic and even heterocyclic hydrocarbons. Many biodegradation and biotransformation applications for sMMO are currently being investigated.
Unfortunately, the sMMO has not been expressed yet in other bacteria in a working form. To reach this, it is essential to know how the gene of the enzyme is expressed and how the protein matures; what kind of accessory proteins help its maturation, what transcriptional factors are activated or inactivated by copper. My project is to compare the proteomes of two different cell cultures: grown in copper containing medium, and grown in copper free medium. The protein expression pattern of the different cultures can be compared on 2-D gels and the proteins that are expressed at different levels are to be identified with MALDI-TOF MS (matrix assisted laser desorption ionization time of flight mass spectrometry).
The presence of copper ions is sensed by a two component signal transduction system. To find out what transcription factors are activated under different circumstances (copper presence vs. copper absence), the phosphoproteomes of the two cultures were analysed. After selective enrichment of phosphoproteins from the two cultures, the phosphoproteomes were analysed using proteomic tools (2-D gels, MS) and we hoped to find out which proteins get phosphorylated or non phosphorylated discounting changes in copper concentration.

CAROTENOID PRODUCTION OF DIFFERENT ZYGOMYCETES FUNGI

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Carotenoids recently attracted great attention due to their beneficial effects on human and animal health (for example their antioxidant property linked with a preventive action on different types of cancer and the enhancement of the immune system). However, the average intake is restricted to only a few carotenoids. Some important dietary carotenoids are not abundant in our food and cannot be taken as supplements (for example zeaxanthin, which is an essential component of the macular pigment of the eye). Most of the carotenoid production is performed by chemical synthesis and only a few natural compounds are obtained from cheap plant sources. Currently, there is an increasing interest in sources of carotenoids from microbial origin, especially in cases of beta-carotene and its oxygenated derivatives.
Traditionally, three closely related Zygomycetes fungi, *Blakeslea trispora*, *Phycomyces blakesleanus* and *Mucor circinelloides*, have been involved in the study of carotene biosynthesis. The main industrial carotenoid producing fungus is *B. trispora*, but it has probably achieved the maximum production level by the selection of mutants and growth conditions. On the basis of morphological observations, a number of related species seem to be promising producers, but their carotenoid content has never been analysed. The aim of the present study was to reveal the carotenoid spectra and to characterize the carotenoid production of several Mucoralean fungi in order to determine new producer strains potentially applicable in further molecular and biotechnological analyses.
Nineteen isolates representing eight species, namely *M. circinelloides*, *M. mucedo*, *M. rouxii*, *M. albo-ater*, *M. bainieri*, *M. hiemalis*, *Backusella lamprospora* and *Gilbertella persicaria*, were involved in the study. For carotenoid extraction, strains were cultured on solid medium for 4 days under continuous light. The carotenoid composition of the strains and the amount of carotenoid compounds were determined by HPLC analysis; total carotenoid content was measured by spectrophotometer. Some *Mucor* species produced the valuable hydroxylated derivatives of beta-carotene (e.g. beta-cryptoxanthin and zeaxanthin) in considerable quantities. The most promising strains belonged to the species *M. bainieri* and *M. hiemalis,* producing two times more carotenoids than *M. circinelloides* and the wild-type *B. trispora* model organisms of the carotenogenic studies. *G. persicaria* produced a significant amount of pigments only when it was plated as a mixture of two

strains with opposite mating types. Carotenoid profile of this fungus was unique among the tested organisms: it produced astaxanthin and other keto-carotenoids in detectable amount. The effect of different growth conditions (e.g. duration of cultivation, light and temperature) were also examined.
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APPLICATION OF ALKALIPHILIC BACTERIUM SPECIES FOR COMPOST PRODUCTION ENRICHED WITH CALCIUM-MUD

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Mixing of alkaline, inorganic waste found in large quantities in the Pécs region, with organic waste and composting of the mixture is only possible if the mixture is inoculated with bacterium species fulfilling the parameters of compost production, thus enhancing and economizing the process of aerobic compost production. *Bacillus* species are required that fulfil the physical parameters of compost-making. In theory, such bacterium species can occur in compost, however, their enrichment in the compost prism containing calcium-mud would prolong the duration of compost production, therefore its economic efficiency would suffer.
For the experiments, bacterium species have been isolated from the sediments of Lake Fertő, Lake Velence, Bödi-szék and Kelemen-szék. Isolates represented six *Bacillus* species (*B. halmapalus, B. firmus, B. pseudofirmus, B. cohnii, B. alcalophilus*). Two of them were selected for further experiments: *B. pseudofirmus* grew at 50°C, and pH 13, in the presence of 12% KCl, and its cellulose degrading activity proved to be the highest (0,246 ± 0,015 mg/ml). The second, *Bacillus* sp. No. 362 (DSMZ No.: 2522) grew at 45°C, and pH 13, in the presence of 12% KCl, and its cellulose degrading activity was 0,250 ± 0,01 mg/ml. In solid stage fermentation experiments it has been found that the two chosen bacteria added to organic bases outgrew the natural bacterium consortia in the presence of calcium-mud. On the basis of the preliminary results, we found that the compost designated BK can be blended with 50% of calcium-mud in the presence of *Bacillus* isolates. The above experiments proved that the examined alkalohalophilic strains are able to reproduce even at an extreme pH of 13, and they regulate their environmental pH to pH 9 by metabolic products. The strains will be used in the practice, too.

RAPID DISSEMINATION OF A CTX-M-15 PRODUCING *KLEBSIELLA PNEUMONIAE* CLONE IN HUNGARY

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The aim of our studies was to monitor the occurrence of the CTX-M-15 type ESBL producing *Klebsiella pneumoniae* clone in Hungary. This clone, called the Hungarian Epidemic Clone (HEC),

was first detected in 2003 causing nosocomial infections.
127 phenotypically ESBL producing *Klebsiella pneumoniae* strains sent to the National Reference Laboratory of the National Center for Epidemiology (NCE) between 2004 and 2005 were tested by PCR for the presence of *bla*SHV, *bla*TEM and *bla*CTX-M. The detected β-lactamase genes were sequenced. The genetic relatedness of the CTX-M-15 producing isolates was investigated by pulsed-field gel electrophoresis. In addition, the plasmid content of all strains was analysed and the transferability of the resistance determinants was tested.
In 2004, the incidence of HEC was low: only three HEC strains were sent to the NCE. However, in April 2005, an abrupt rise in the number of HEC strains was observed: 124 HEC isolates were detected in 24 hospitals throughout the country. HEC isolates proved most prevalent in the following wards: intensive care, surgery, traumatology and urology. Apart from cephalosporins, HEC isolates showed resistance to aminoglycosides, ciprofloxacin and tetracycline. All HEC strains carried both *bla*CTX-M and *bla*SHV genes. By sequencing, the CTX-M type enzymes turned out to be CTX-M-15, and the SHV enzymes SHV-28.
Transmission studies demonstrated that the *bla*CTX-M genes were located on either a 140 kb or a 90 kb plasmid. *Bla*TEM could not be detected in the HECisolates.
Klebsiella pneumoniae isolates belonging to HEC and producing CTX-M-15 and SHV-28 enzymes rapidly spread in 2005 to various Hungarian hospitals causing multiple nosocomial outbreaks.

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF PHYLLOSPHERIC MICROBIAL POPULATION IN RAGWEED (*AMBROSIA ELATIOR* L.) GROWN IN CONTAMINATED AREAS

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Ragweed (*Ambrosia elatior* L.) is one of the most common problem weed in Hungary and clinically is one of the most important sources of seasonal aeroallergens. Toxic metal accumulation (cadmium, copper, nickel and zinc) in the soil-plant ecosystem of ruderal sites may occur in the organs of ragweed plants in quantities exceeding the physiological requirements of the plant. During the growing and flowering seasons, comparisons were made of the leaf surface (upper and lower) microflora of the ragweed plants grown in three sites: i) a relatively uncontaminated site (Control), ii) communal landfill and other iii) a complex heavy metal contaminated site. Corresponding to metal contents in the ragweed plants grown in different sites of heavy metal contamination, aerobic heterotrophic bacteria, filamentous fungi and yeasts were enumerated of the ragweed phyllosphere as well as from the atmospheric area surrounding the plant. The microorganisms were easily detachable from the upper and lower leaf surfaces, which were isolated by touch contact on different culture media. Air-borne microorganisms were determined with the impact method (OMEGA airTEST LCB, France) at the highest vegetation of ragweed inflorescence.
The results indicated that the densities of phyllosphere microbial communities were qualitatively and quantitatively related to the heavy metal content of the plant leaf and leaf surfaces and to the heavy metals contaminating the ecosystem where the plants were grown. The results showed that there was a high positive correlation between the densities of phyllosphere microbial communities and the Cd, Ni, and Zn content in the ragweed leaves, while the correlation was low in the presence of Cu. Also, the correlation was the highest in the case of aerobic, heterotrophic bacterial population densities, higher than that of yeasts and filamentous fungi.
During the five-year (2000 to 2004) investigation, the community density and diversity of Gram-negative aerobic heterotrophic bacteria increased progressively more pronounced than that of Gram-

positive inhabitants of the ragweed phylloplane. The most common members of aerobic heterotrophic bacterial populations were related to *Enterobacter agglomerans*, *Pseudomonas syringae*, *P. putida*, *Bacillus cereus*, *Corynebacterium striatum*. Yeasts prevalent in the ragweed phylloplane were of the genus *Cryptococcus*, while the most commonly isolated fungal genus was *Alternaria*. The results demonstrated that highly significant variations occurred in the population density and diversity of epiphytic aerobic heterotrophic bacterial, filamentous fungal, yeast and total microbial populations in the upper and lower surfaces of the leaves, different in the metal content. This outcome is crucial in terms of allergology.

THE RELATIONSHIP BETWEEN THE DIRECT ENVIRONMENT AND THE MICROORGANISMS OF POLLEN SURFACE OF RAGWEED ON RUDERAL SITES

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Studies focused on „total" cadmium, copper, nickel and zinc content of the soil in three ecologically distinct areas – species composition, metal content and formation – as well as the corresponding metal contents in ragweed plants in the same areas, and the microorganisms in the pollen in the years 2000–2003. With regards to metal content related to average dry weight, the soil of Investigation Site I. (control site) and Site II. (communal landfill site) were not contaminated with metals, whereas the soil of Site III. (industrial galvanic-sludge disposal site) could invariably be characterized as contaminated with metals. At the communal landfill site and at the industrial galvanic-sludge disposal site, the following average contents were measured in *Ambrosia elatior*: cadmium (0,25 – 0,43 mg/kg), copper (8,68 – 11,50 mg/kg), nickel (2,18 – 9,10 mg/kg) and zinc (37,00 – 133,45 mg/kg). The zinc content of staminate inflorescence at Site II. and the cadmium, copper, nickel and zinc of staminate inflorescence at Site III. were significantly increased as opposed to that of the non-ruderal site (Site I.) considered as a control site.

Parallel with the increase in the cadmium, copper, nickel, and zinc contents of the staminate inflorescence, the number of microorganisms that were easily detachable from pollen surfaces and be cultured rose. The number of microorganisms increased at the ruderal sites (II., III.), as opposed to that at non-ruderal site (I.). we could prove that the pollen surface of ragweed was dominantly colonized by the species of the genera *Pseudomonas*, *Enterobacter* and *Alternaria*. The increasing amount of metals in the staminate inflorescence has given way to the emergence of different microbial communities on the pollen surface in areas with distinct metal contents. The species diversity of colony-forming-microorganisms has reflected a decreasing tendency in the sequence of investigations sites I.>II.>III.

Deformed pollen increased on ragweed of the ruderal sites. There was a positive correlation between the number of microorganisms on the pollen and the amount of deformed pollen. There were no significant differences in the species diversity of microorganisms at the investigation sites that were collected from the pollen of the ragweed as well as from the surrounding air. The microorganisms of the pollen surface that could be cultured were in larger numbers at the ruderal sites than in the uncontaminated ecosystems. The metal contents of all investigated metals of ragweed at the industrial galvanic-sludge disposal site were significantly larger than at the non-ruderal sites. Here, the number of deformed pollen was larger and also the number of bacteria and fungi that colonize the pollen surfaces was significantly larger.

THE ROLE OF THE HYNH PROTEIN IN THE BIOSYNTHESIS OF THE HYN HYDROGENASE IN *THIOCAPSA ROSEOPERSICINA* BBS

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Hydrogenases are metalloenzymes catalysing the reversible oxidation of molecular hydrogen. The [NiFe] hydrogenases consist of a large and a small subunit. A special catalytic metallocenter is complexed in the large subunit, while the small subunit has few iron-sulphur clusters transporting the electrons between the active center and the surface of the enzyme. *Thiocapsa roseopersicina* BBS is an anaerobic purple sulphur phototrophic bacterium possessing at least three [NiFe] hydrogenases. Two of them are membrane-bound (HynSL and HupSL), while the third hydrogenase, HoxYH is a soluble enzyme. NiFe hydrogenases require a set of accessory enzymes for their biosynthesis, which includes a special complex posttranslational maturation process.

So far, the *hyn* operon coding for the HynSL hydrogenase was believed to consist of four genes (*hynS isp1 isp2 hynL*). However, upstream of the *hynS* gene there is an additional open reading frame coding for a putative protein – named as HynH - similar to the HupH/HoxQ proteins of various bacteria. It is to note that in *T. roseopersicina,* the *hupH* gene codes for a homologous protein probably with analogous function to the HynH. The similarity among HynH/HupH/HoxQ proteins is around 50-60% (35-45% identity) and few conserved motifs/residues can be recognized. The proteins seem to be localized in the cytoplasm and studies on several enzymes in other bacteria revealed that this protein was not required for the posttranslational maturation of the large subunit but might be involved in the transport of the membrane-bound hydrogenases to the periplasm. It was demonstrated with *Rhizobium leguminosarum* that the HupH protein interacted with the pre-small subunit, which interaction might have stimulated the assembly of the mature large and small subunits.

In this work, the *hynH* gene is inactivated by *in frame* deletion mutagenesis in the wild type and the HynSL hydrogenase-only *Thiocapsa roseopersicina* strains. In the mutant strain, the effect of the mutation on the *in vivo* and *in vitro* hydrogenase activities as well as on the maturation/transport of the various hydrogenases is examined. The deletion mutants are complemented using a construct containing an expression cassette producing the HynH protein C-terminally fused to FLAG-STREP II tag. Beside the complementation, this approach allows us to fish out protein partners interacting with HynH during the hydrogenase assembly.

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DETERMINATION OF *CHLAMYDIA TRACHOMATIS* INFECTIONS IN PREGNANT WOMEN BY MEANS OF COBAS TAQMAN PCR

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Chlamydia trachomatis (*C. trachomatis*) infection is the most common bacterial STD. The incidence of asymptomatic infections is currently increasing in Europe. The advantages and disadvantages of the new methods, APTMA Combo 2 and the use of urine and pooled urine samples, were recently described. In accordance with the recommendations of the U.S. Preventive Services Task Force according to which young pregnant women should be screened with nucleic acid amplification tests, a real-time qualitative method, the COBAS TaqMan PCR was recently introduced in our laboratory.

107 pregnant women were screened for *C. trachomatis*. 78 participated in regular obstetric examinations at the Outpatient Clinic for Pregnant Women and 29 at the Department of Obstetrics and Gynecology in Szeged. Urine samples taken from the women were kept at +4ºC overnight, and the examinations were performed the next day. A short questionnaire was filled out by the patients with the help of specially trained nurses. Urine samples were pelleted, and treated with detergent and a lysis buffer before amplification. Target and internal control amplification were performed simultaneously. The *C. trachomatis* internal control is included in the COBAS TaqMan *C. trachomatis* test; it is designed to ensure that specimens do not contain inhibitors. The test utilizes real-time PCR technology. The use of dual-labelled fluorescent probes allows the real-time detection of PCR product accumulation by monitoring the intensity of emission of fluorescent reporter dyes released during the amplification process. The amplification of *C. trachomatis* DNA and of the internal control DNA are measured independently at different wavelengths.
In 1995, a large-scale *C. trachomatis* study was performed on asymptomatic pregnant women (n = 6156) in Hungary. The overall average infection rate was 5.9% (range 1.3-9.8%) in different towns in Hungary. The prevalence in Szeged was 3.21%. Some recently reported data concerning the prevalence of *C. trachomatis*: 2.1%, in the Czech Republic (Prague); 4.7% in Russia (Novosibirsk); and 4.3% of pregnant women who intended to deliver and 7.5% of those who decided to terminate their pregnancies in Russia (St. Petersburg). Between the end of December 2005 and January 13, 2006, 107 pregnant women were screened for *C. trachomatis* at the Gynecological Outpatient Clinic and the University Clinic in Szeged. The prevalence of infection was found to be 4.7%. Two of the five women were asymptomatic for *C. trachomatis* infection; one is undergoing treatment with a diagnosis of foetal hydrops in the pregnant pathology unit. Missed abortion was observed in two cases. The incidence of *C. trachomatis* 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. The COBAS TaqMan real-time *C. trachomatis* PCR is a useful and reliable method.

HUMAN PAPILLOMAVIRUS SCREENING - A PROGRAM BEFORE THE VACCINATION ERA

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Cervical carcinoma is the tenth leading cause of mortality among women in Hungary. The annual number of deaths from 1999 to 2003 varied between 465 and 539. Human papillomavirus (HPV) infections are responsible for cervical carcinomas. A multicenter study was performed by means of cytology and a nucleic acid hybridization probe (Digene) in 1997. The average HPV prevalence in those women who participated in positive or negative family planning programs was 17.6%. In another survey, the prevalence of HPV among those selected group of women who participated in a carcinoma screening program was 43.4%. In order to achieve a better comparison of the Hungarian data with those of other countries, a new method, HPV PCR, was introduced for the detection of HPV in 2006 (Roche). In the positive cases, the HPV types were determined by linear array (Roche). Parallel cytological examinations were performed with HPV diagnostic methods.
Results of comparative examinations of HPV PCR and cytology are tabulated. A higher prevalence of HPV was detected by PCR in comparison with the results of an earlier survey where the nucleic acid hybridization was used. The higher sensitivity of the amplified method can explain the higher prevalence. On the other hand, while the PCR can detect 37 types, the hybridization method can detect only 18 HPV types. Many carcinoma prevention screening programs in Hungary are free. However, the statistical survey demonstrated that screening programs among women were not

effective. A compulsory vaccination program against HPV infection could possibly decrease the mortality of cervical carcinoma among Hungarian women.

	HPV PCR-positive N (%)	HPV PCR-negative N (%)	Total N (%)
Cytology-positive	15 (16.6)	2 (2.2)	17 (18.9)
Cytology-negative	10 (11.1)	63 (70.0)	73 (81.1)
Total	25 (27.7)	65 (72.2)	90 (100.0)

ENDEMIC CANINE DISTEMPER INFECTION AT A DOG SHELTER: EPIZOOTOLOGY AND VIRAL VARIABILITY

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Canine distemper (CD) is a highly contagious viral infection of carnivores belonging to different animal families, such as *Canidae, Mustelidae, Procyoidae, Felidae* and several others. It is caused by the canine distemper virus (CDV), which belongs to the *Morbillivirus* genus of the *Paramyxoviridae* virus family. The clinical symptoms of the disease include severe respiratory, digestive and neurological signs, often with a fatal outcome. The infection represents a great risk for all receptive carnivore populations, especially for those which are “open” (i.e.: new animals are introduced frequently and regularly) and hence large numbers of animals with unknown vaccination histories live together, such as those living in dog shelters.
The authors have conducted a diagnostic study for the detection of CDV at the Dog Shelter of the Local Government of Budapest, within the framework of a monitoring program started in spring 2005. The investigations demonstrated that there was a high incidence of CD at the establishment: from the 99 clinically ill dogs examined during a 16-month period, 25 turned out to be positive for this disease. The research also revealed that in case of large populations, such as those living at dog shelters, nasal swab samples do not provide reliable results and therefore cannot be used for diagnostic purposes, since a large number of animals that are positive for CDV, according to the analysis of the nasal swab samples, turn out to be negative at the repeated examination of the more relevant blood samples. The analysis of the nucleotide sequence of the viral strains proved that the infection at the establishment was caused by an endemic strain of CDV. The investigations also demonstrated that the viral strains had suffered a homologous amino acid change between the first and second examination periods, so the new, mutant strain replaced the earlier virus at the establishment. Based on the analysis of the nucleotide sequences of the products amplified by the applied PCR method, the wild-type virus strains could be reliably differentiated from the strains currently used in vaccines. The efficacy and necessity of the vaccination program launched in 2005 is proven by the rising number of dogs that carry the virus in the nasal cavity without becoming viremic.

ANALYSIS OF HEPATITIS C GENOMES FROM HAEMODIALYSED PATIENTS

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Hepatitis C infection is known to be frequent in patients undergoing haemodialysis. Serum samples of 26 patients of a dialysis centre in Budapest were tested for the presence of anti-HCV IgG and hepatitis C RNA. 11 of the patients were serologically positive, and the result was indeterminate in

two cases. HCV RNA could be detected in 12 of the patients by screening PCR using primers located on the conserved 5' untranslated region of the genome. Another PCR was performed with primers that amplified a region within the variable E2 gene. This assay was less sensitive and only 8 samples gave positive results. All PCR products were directly sequenced. The twelve sequences obtained from the 5'UTR were found to be identical, the eight E2 sequences however showed differences in several nucleotide and amino acid positions.
The genetic relationship of the detected viruses based on the sequenced E2 segment was determined by phylogenetic analysis. A phylogenetic tree was also constructed including representative isolates of different published HCV genotypes. All sequenced viruses belonged to genotype 1b, their genetic distances however indicated that a common source of infection was unlikely.

SEROTYPING OF *STREPTOCOCCUS PNEUMONIAE* BY PCR – APPLICATION OF THE METHOD FOR THE HUNGARIAN SITUATION

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Streptococcus pneumoniae can express 90 different serotypes, based on the capsular polysaccharide antigens. As certain serotypes are linked to resistance or virulence or different diseases, it is essential for the epidemiological studies and surveys to determine the serotype of the isolates. The conventional method uses antisera, but it is time-consuming, expensive and difficult to evaluate. Therefore, there are a few publications on serotyping by PCR. We have adapted this methodology for the Hungarian situation.
Conventional serotyping was performed with MAST antisera on microscope slides. For the PCR, we used several primers for the individual serotypes published by Brito *et al.* (1, 3, 4, 6, 14, 18C, 19F, 19A, 23F), and have designed a set of new primers to other serotypes that are also relatively common in Hungary (9V, 6A, 6B, 7F, 11A, 15A, 15B). The PCR was done in two steps. The first was a multiplex PCR reaction, which divides the strains into 6 different groups, identified according to the gel pattern. Then another multiplex PCR reaction followed, which included the primers for the individual serotypes, producing PCR products of different size. We first tested isolates of known serotypes with the adequate individual primers in single PCR reactions. Then we tried the grouping reaction with these strains. The multiplex PCR worked best with *Tth* polymerase, but with *Taq* buffer. We had to change the annealing temperature and other parameters as well, e.g. nucleotide concentrations. Adjusting the amount of certain primers in the second step was very important. After optimizing the method, we tested a few invasive isolates of unknown serotypes, and compared the results with the conventional method. In all cases, the two methods provided the same results.
The new 7-valent conjugate vaccine was introduced in Hungary very recently, so especially the examination of invasive strains is of great importance. Serotyping of great numbers of isolates by the conventional method requires skilled stuff, financial support and time; however, the ability of performing it by a PCR method makes it much simpler. We have extended the method of Brito *et al.* for the Hungarian situation by designing primers for new serotypes.

PHYLOGENETIC ANALYSIS OF THE DNA POLYMERASE GENE OF A FISH HERPES VIRUS ISOLATED FROM WHITE STURGEON (*ACIPENSER TRANSMONTANUS*)

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Although *Herpesviridae* is one of the best-studied virus families, herpes-like viruses of oysters, fish and frogs could not be assigned into any of the existing three subfamilies due to lack of obviously homologous gene blocks. In the newest taxonomy report, more than fifty herpes viruses (HV) are listed as "unassigned viruses within the family". Until recently, full genome sequence has only been available on one fish HV from the channel catfish (*Ictalurus punctatus*). Partial HV sequences have been determined from additional fish species (rainbow trout, eel, carp and salmon) as well as frogs. Highly pathogenic koi HVs have also been sequenced but not yet released.
We started to study a HV isolate (SRWSHV) originating from a free-living white sturgeon caught in the Snake River (Idaho, USA). The viral genome was randomly cloned with *Hin*dIII and *Pst*I enzymes. Cycle sequencing, in combination with subcloning and primer walking strategy, was applied. The sequences were identified with BLAST. A putative genome map of SRWSHV was constructed assuming that its size and organization were comparable with those of the ictalurid herpesvirus 1 (IcHV-1). Acquisition of missing genome parts between cloned fragments was attempted by PCR with custom-designed specific primers. Based on multiple alignments, a highly degenerate, consensus primer pair for a 1700 bp fragment of the viral DNA polymerase (pol) gene was designed and used successfully. The DNA sequences were assembled into large contingents with the Gap4 program of the Staden package.
To date, the sequence of 8 genes were fully, and that of an additional 12 genes were partially determined from SRWSHV. In BLAST applications, these genes almost invariably showed the closest similarity to their counterparts in IcHV-1. The size, position and orientation of the genes seem to be similar or identical in the two viruses. However, most of the IcHV-1 genes are as yet putative and only a limited number of them have been assigned for a specific function (SFI helicase, serine protease, DNA polymerase, membrane glycoprotein, etc.). For phylogenetic calculations, we used a 450 bp fragment of the pol gene, because the sequence of this fragment was available from the largest number of amphibian and fish HVs, including the recently published sequences of three different types of acipenserid HVs. SRWSHV clustered with WSHV-2, whereas WSHV-3 seemed to be closer related to IcHV-1. The branch of cyprinid and anguillid HVs was farther than the ranid HVs. The pol sequence is frequently used for the comparison of large dsDNA viruses even from different families. In our phylogenetic tree, constructed on full pol alignments, the African swine fever virus appeared between the branches of members of the official *Herpesviridae* family and the fish and frog HVs. This result suggests that fish and frog HVs are very distant from the reptilian, avian and mammalian HVs and they should be assigned to a separate family.
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BIOTECHNOLOGY INCUBATOR HOUSE IN THE UNIVERSITY OF DEBRECEN: TRANSFER OF EXPERTISE, INNOVATION, SPIN-OFF COMPANIES

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In the past few decades, universities all around the world, and thus also in Hungary, have been expected to transform themselves into *de facto* centres of knowledge and expertise, which are accessible for society and the economic sector in particular. What do these expectations mean in practical terms? A university worthy of its name should certainly retain its excellence in basic science and technology, but should also be able to understand and – at least to a certain degree – satisfy the needs of industrial, agricultural and service enterprises and not-for-profit organisations utilizing their knowledge accumulated for many years. In Hungary, this worldwide process is strengthened (some

may say forced) by the new national system of grant distribution. The University of Debrecen was among the first ones to realize and internalize the strategic importance of these processes, and – as a consequence – major efforts were undertaken to establish and sustain those professional centres that may be the engines of innovation and knowledge transfer between the university and the „outside" world. This lecture will focus on the innovation-related activities currently ongoing at the Faculty of Science of the University of Debrecen. We will analyze the already existing projects, with an emphasis on the grants and industrial co-operations that support them. Perspectives will also be discussed in detail.

DETECTION OF POTENTIAL FOOD-BORNE PATHOGENS FROM STOOL SAMPLES OF BROILER CHICKENS

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Food-borne diseases pose a considerable threat to human health and the economy, therefore serious efforts are taken to monitor and control the presence of zoonotic pathogens in the food chain, "from the farm to the fork". In the framework of a food safety project, broiler chicken flocks were investigated to evaluate their level of contamination by potentially pathogenic bacteria.
Composite faeces samples were collected from broiler producing farms according to the current regulations of the European Union/Ministry of Agriculture. A commercially available kit was used for the extraction of DNA from the stool specimens, and polymerase chain reaction (PCR)-based methods were applied for the detection of potential pathogens. Results of different PCR- and culture-based assays were compared where it was possible.
Thermotolerant *Campylobacter* species (*C. jejuni* and *C. coli*) and *Salmonella* serovars (sv. Enteritidis, sv. Typhimurium, sv. Infantis) are considered as primary causative agents of bacterial food-borne infections. *Listeria monocytogenes* may cause serious, life-threatening infection and the *Arcobacter* species - which were formerly classified in the *Campylobacter* genus - are considered as emerging food-borne pathogens.
Samples collected from eight flocks of broiler chickens were tested for the presence of *Salmonella ssp*., thermotolerant *Campylobacter ssp.*, *Arcobacter ssp.* and *Listeria monocytogenes.* The highest frequency of occurrence was observed in the case of *Campylobacter ssp.*, where 59 out of 93 samples were found positive in the PCR specific for the ceuE/cadF regions (Cloak et Fratamico, 2002). Real-time PCR (iQ-Check™ *Listeria monocytogenes/Salmonella* kits, Bio-Rad) results showed that 53/93 samples contained DNA from *Salmonella*, while *Listeria monocytogenes* was not detectable in the faecal specimens (0/70). According to the data of a multiplex PCR (Houf et al. 2000) *Arcobacter ssp.* were present in the poultry population at a relatively low level, amplification products were found in 7 out of 93 samples. Comparison showed that the number of *Salmonella* positive samples was not significantly different in real-time (53/93) than in traditional (49/53) PCR, while it was considerably lower in culture-based methods (36/93). Our data suggest that i. beside *Campylobacter* and *Salmonella* species/serovars, *Arcobacter* species are also present in the domestic poultry flocks, and they should be taken into consideration as emerging food-borne pathogens in Hungary; ii. PCR technology can complement culture-based assays, and the application of molecular biology methods is recommended in monitoring/surveillance programs.
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PESTIVIRUS CONTAMINATION IN VETERINARY VACCINES USED IN HUNGARY BETWEEN 1992-2006

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Safety of marketed veterinary vaccines has a paramount importance. There are critical steps during the manufacturing of vaccines, which involve potential risk. Using contaminated foetal calf serum (FCS) in cell cultures is a crucial risk factor resulting in the presence of Pestiviruses in live viral vaccines for both veterinary and public health use. Members of Pestiviruses, bovine viral diarrhoea virus (BVDV), border disease virus (BDV) and classical swine fever virus (CSFV) cause economically important diseases in pig, cattle and sheep. In our study, 33 vaccines against different diseases produced by different companies were chosen randomly from the period of 1992-2006 and subjected to Pestivirus detection by RT-PCR. 5' non-coding region (5'NCR) was amplified. Beside RT-PCR, cell culture test and animal experiments were carried out.

The tested vaccines proved to be free of Pestivirus except for one case, when the vaccine was found positive by RT-PCR, however, neither the cell culture test nor vaccination of susceptible animals with contaminated vaccine were able to confirm the result obtained by RT-PCR. There is an ongoing debate about the evaluation of positive results obtained by RT-PCR; however, Laamen et al. reported a case when BVDV detected in a vaccine by RT-PCR was cultivatable on cells. Regardless of the potential infectivity of the contaminant, the present regulation of vaccine quality considers RT-PCR positivity as a sign of contamination, thus, further tests for Pestivirus contamination are needed.

ONCOGENIC HUMAN PAPILLOMAVIRUS AND *CHLAMYDIA TRACHOMATIS* CO-INFECTIONS IN CYTOLOGIC ATYPIA OF THE UTERINE CERVIX

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Oncogenic human papillomavirus (HPV) types are the most frequent infectious causes of human malignancies. Persistent infection with these HPV types is essential for the progression to high-grade *cervical intraepithelial neoplasias* (CIN) and cervical cancer. HPV is a typical sexually transmitted infection and this type of transmission is determined mainly by behavioural factors of the infected persons and their sexual partners, which factors increase the risk of other STD infections, too. Beside the major etiologic factor, the HPV, there are several cofactors promoting the development of CIN.

Chlamydia trachomatis infection is a leading cause of sexually transmitted diseases. Hungary is a low prevalence area in Eastern Central Europe. A history of having substantial *C. trachomatis* exposure, as detected by serum antibodies, is a cofactor of HPV mediated cervical carcinogenesis.

In this study, we examined the concurrent *C. trachomatis* infections in cytologic atypia of the uterine cervix in order to evaluate the impact of *C. trachomatis* infection in patients with a high risk for cervical intraepithelial neoplasia. Cervical scrapes form 707 patients were subjected to PCR amplification with primer sets for HPV and *C. trachomatis*. Based on negative beta-globin results, 10 specimens were not eligible for further analysis. Oncogenic HPV types were detected in 278 specimens (39,8%). *C. trachomatis* was found only in six specimens (0.9%). Thus concurrent *C. trachomatis* infection was uncommon and it was an improbable risk factor in cytologic atypia.

MUTAROTATION OF D-GALACTOSE ANOMERS PLAYS A SIGNIFICANT ROLE IN THE INDUCTION OF CELLULASES BY LACTOSE IN THE FUNGUS *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 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 lactose disaccharide itself, since an equimolar mixture of D-glucose and D-galactose yields a similar induction as seen on D-galactose alone. Obviously, *T. reesei* recognizes the presence of the 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 β-anomer, whereas D-galactose is (because of mutarotation) a mixture of the α- and β-anomers. Consequently, β-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 behind the constitutive *T. reesei* promoter *pyr4*. This construction was transformed into *T. reesei*, yielding an integration of 3-5 copies, as confirmed by Southern blotting. According to a Northern blot 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 transported lactose considerably faster than the re-transformed control strain, but there were no differences in the uptake rate of any of the 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 β-D-galactose into α-D-galactose indeed heavily contributes to the induction of cellulase genes on lactose.

GENETIC ANALYSIS OF PICOCYANOBACTERIAL ISOLATES OUTLINES A NEW GROUP OF *SYNECHOCOCCUS* WITHIN THE PICOPHYTO-PLANKTON CLADE (SENSU URBACH ET AL., 1998)

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The *Synechococcus* genus is a provisional assemblage that can be loosely defined as unicellular, coccoid to rod-shaped cyanobacteria that divide by binary fission in a single plane. The cells are less than 3 μm in diameter, contain photosynthetic thylacoids located peripherally, lacking structured sheaths. Phylogenetic analyses show that these organisms are polyphyletic, and most strains isolated from freshwater as well as from marine samples belong to one phylogenetic lineage, referred as the picophytoplankton clade *sensu* Urbach *et al.* (1998). Furthermore, *Synechococcus*-type cyanobacteria are known as the most abundant members of freshwater prokaryotic picoplankton.
We characterised several *Synechococcus* strains isolated from Lake Balaton and from Hungarian saline lakes based on two regions of the genomic DNA, the 16S rDNA and a part of the phycocyanin operon (*cpcBA*-IGS). 16S rDNA is a widely used marker molecule in bacterial phylogenetic analyses, while *cpcBA*-IGS provides higher sequence variability due to the protein-coding and non-coding regions. Most strains from Lake Balaton are closely related to group A (*Cyanobium gracile* cluster), the others are related to group B (subalpine cluster I) and group F Synechococci. Strains isolated from saline lakes form a distinct group within the picophytoplankton clade based on the sequence analysis of both DNA fragments. This group previously contained only environmental clones. Phylogenetic analysis of the *cpcBA*-IGS region was largely consistent with that obtained from 16S rDNA sequences, but gave a higher resolution among closely related isolates.
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ENHANCED MANIFESTATION OF TICK-BORNE ENCEPHALITIS IN A PATIENT PREVIOUSLY INFECTED BY WEST NILE VIRUS

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Viral diagnostics of the suspected tick-borne encephalitis (TBE) patients started in the early 1950s, and the systematic search for natural foci of arboviruses performed between 1966 and 1986 revealed endemic areas of TBE in Hungary. Our laboratory could detect the first few indigenous human West Nile virus (WNV) infections in 2003 through TBE diagnostics. Hospitalized patients with both virus infections characteristically suffered from meningitis or encephalitis.
Consequences of TBEV infection in Hungary are the same as everywhere else in Europe. Most recently, researchers published a study on a group of 477 hospitalized TBE patients in Poland. By analyzing the data, they found the following: persistent neurological sequelae were noted in 10.9% of patients: paresis or plegia in 5.66%, spinal dysfunction with paraparesis of the lower limbs in 1.68%, significant paresis of the upper extremities in 0.63%, brachial plexus paresis in 2.31%. Cranial nerve involvement was present in 2.93%, including uni- or bilateral facial nerve palsy in 2.73% and trigeminal nerve involvement in 0.21%. Cerebellar dysfunction was present in 2,09% of patients. No fatal cases of TBE occurred. Consecutive infections/immunizations by two flaviviruses may lead to the mitigation of the disease, or to a more severe course of the disease following the second virus infection. We describe a tick-borne encephalitis patient who, according to the results of virus serology, had previously experienced West Nile fever, another flavivirus infection endemic in Hungary. The shortened incubation period, the rapid deterioration of the patient's conditions, the paralysis of both upper limbs developing at two different time points and the irrevocable muscle

atrophy raised the possibility of antibody-dependent enhancement.

INVESTIGATION OF CHRONIC BEE PARALYSIS VIRUS DETECTED IN HUNGARY

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Chronic Bee Paralysis Virus (CBPV) is a virus specific to the honeybee (*Apis mellifera* L.). The symptoms caused by the virus infection usually appear in two main forms: worker bees show central nervous symptoms (trembling and crawling) and become black coloured because of the loss of hair. The disease usually emerges in the spring, and may cause economic losses in beekeeping.
In this study, the occurrence of CBPV was investigated in a total of 356 honeybee samples. Bees were collected in Hungarian apiaries between 1999 and 2006 and they were tested with reverse transcription polymerase chain reaction (RT-PCR) method. One of the samples was found to be positive, which was sent from an apiary in Keszthely in the spring of 2005, after the sudden collapse of the colony. The specificity of the reaction was confirmed by the direct sequencing of the amplification product. The partial nucleotide sequence of a CBPV obtained from a bee sample collected in Austria was also determined.
Phylogenetic analysis was performed involving the corresponding nucleotide sequences (partial RNA dependent RNA polymerase gene) of the Hungarian and the Austrian viruses, as well as CBPV sequences from France (nine sequences) and Uruguay (one sequence) deposited in GenBank. The nucleotide sequence of the Hungarian virus showed higher similarity to the Austrian (94%) and the Uruguayan (90%) CBPV, and lower similarity to the French sequences (88%). Phylogenetic tree demonstrates unambiguous clustering of the sequences: three branches are seen, one is the "Central European" viruses close to the branch of the Uruguayan CBPV; the French viruses form a distinct group within the tree. The comparison of the putative amino acid sequence showed that the Hungarian, the Austrian and the Uruguayan sequences were 99% identical, and shared 98 to 97 % similarities with the French viruses. However, the presence of CBPV was already suspected on the basis of the symptoms; to our knowledge, this is the first detection and molecular identification of the virus in Hungary. Because the investigations were focused on the RNA dependent RNA polymerase coding region, which is usually a relatively conserved section of the virus genomes, the reliability of statistical analysis was lower. Further studies involving other genomic regions and more sequences might provide a more detailed picture on the diversity and phylogenetic clustering of CBPV.
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MOLECULAR ANALYSIS OF PNEUMOCOCCI ISOLATED FROM INVASIVE INFECTIONS OF CHILDREN IN HUNGARY

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The objective of the study was to determine the serotype coverage of the 7-valent pneumococcal conjugate vaccine (PCV7) and to investigate the epidemiology of *Streptococcus pneumoniae* in invasive infections of children under 5 in Hungary. Isolates were collected between November 2002

and October 2004. Results showed that the 7-valent vaccine covered 60.6 % of the strains. The partial coverage rate was 62.1 %. About one-third of the isolates belonged to or was closely related to one of two large international clones (Spain9V-3, England14-9).
The rate for penicillin and erythromycin resistance proved high - 16.7 % and 47 %, respectively - to which some of the prominent serogroups/serotypes (14, 6B, 19A) contributed significantly. All erythromycin resistant strains related to the Spain9V-3 clone carried *erm*B genes, while most macrolide resistant isolates linked to the England14-9 clone harboured the *mef*A gene. Our findings suggest that the heptavalent conjugate vaccine could substantially reduce not only the incidence of invasive *S. pneumoniae* infection in children, but also the rate for penicillin and macrolide resistance in this patient group in Hungary.

COMBINATION EFFECT OF STATINS WITH *PENICLILLIUM CHRYSOGENUM* ANTIFUNGAL PROTEIN (PAF) ON SPORANGIOSPORE GERMINATION OF DIFFERENT ZYGOMYCETES

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The number of zygomycosis (caused by members of Zygomycetes) has increased over the past years. Most of the antifungal agents are ineffective against these fungi, furthermore, treatments have serious side effects and could not be applied without damage of the host.
Penicillium chrysogenum secretes an antifungal compound, the *Penicillium* antifungal protein (PAF). PAF is a small, highly basic cystein–rich protein, which inhibits the growth of several filamentous fungi. This inhibition has an effect on the conidium formation, the germination of conidia and/or the hyphal extension. Statins are known to be competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol and some isoprenoid compounds having important role in cell signalling.
The combination effect of statins with PAF were investigated on four isolates of Zygomycetes (*Rhizopus stolonifer*, *Mortierella wolfii*, *Syncephalastrum racemosum* and *Mycotypha africana*), which represented the combination of lovastatin sensitive/insensitive and PAF sensitive/insensitive properties based on preliminary experiments. Efficiency to inhibit sporangiospore germination was studied with 4 statins (lovastatin, simvastatin, rosuvastatin and atorvastatin) at different concentrations with a constant concentration of PAF. The in vitro antifungal activity of statins, PAF and the combinations of these was determined with microtiter plate bioassay by measuring the absorbance at 620 nm. Abbot formula was used to reveal the nature of interactions between the two compounds.Most of the tested statins had antifungal effects, but in some cases the antifungal activity increased in the presence of PAF. No interactions were detected between the 4 statins and PAF for *R. stolonifer* which was sensitive to both. In case of *M. wolfii* (lovastatin insensitive and PAF sensitive species), additive effects were observed. Synergistic and additive effects were observed for S. *racemosum* (except for lovastatin). Statins and PAF acted synergistically or additively on *M. africana* (sensitive to both).

MICROBIAL COMMUNITY ANALYSIS OF ACTIVATED SLUDGE TREATING COKE-OVEN WASTEWATER

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Coke plant wastewater is generated in iron and steel plants at coke-oven gas-cleaning operations. The main organic contaminants of the produced wastewater are phenols and other aromatic cyclic compounds, the inorganic pollutants are thiocyanate, nitrate and ammonium. These toxic compounds can cause severe pollution to the environment, therefore wastewater is subjected to chemical and biological (activated sludge process) treatment. The biodegradation rate of phenols and thiocyanate usually exceeds 95-99%, but shows periodic fluctuations. Nitrate removal is efficient enough; though the biological treatment results in an increased ammonium concentration. The aim of the present work was to identify the members of the bacterial community existing in this very special environment and to compare the community structure and activity of samples originating from periods characterized by different biodegradation capacities.

DNA and RNA based molecular methods were used to investigate the communities. Samples were analyzed by terminal restriction fragment length polymorphism (T-RFLP) of 16S rDNA to determinate the relative abundance of members of the bacterial community, their activity and temporal changes. 16S rDNA clone libraries were created and the clones were grouped also by T-RFLP. Partial sequencing was used for phylogenetic identification of the representatives.

Community fingerprints and phylogenetic analysis of cloned 16S rDNA show that most of the clones belonged to Proteobacteria and the community was dominated by a strain closely related to the phenol degrading *Comamonas badia*. Members of genera *Pseudomonas*, *Thiobacillus*, *Rhodanobacter*, *Sphingomonas*, *Alcaligenes*, *Nitrobacter* and Bacteroidetes were also detected in the samples. *Pseudomonas* spp. can also degrade phenol; *Thiobacillus* can denitrify and oxidize various sulphur compounds and utilize thiocyanate. The relative abundance and activity of these groups showed differences in samples characterized with different efficiency of biodegradation. In the sample with decreased thiocyanate removal, *Comamonas badia* was partly substituted by *Pseudomonas* spp. The proportion of the *Thiobacillus* group compared to other members of the community changed with the varying efficiency of thiocyanate removal.

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DETECTION OF VANCOMYCIN RESISTANT GRAM POSITIVES FROM ENVIRONMENTAL SAMPLES

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The authors examined the presence of vancomycin resistance in environmental samples („clear” soil, litter, manure enriched soil and commercial compost). The environmental impact of antibiotic and disinfectant use is reflected in the quantity and type of resistance factors in the normal flora of the examined niche. Resistant microorganisms may serve as indicators of previous antimicrobial load. Vancomycin producing *Streptomyces venezuelae* strains are absent in Europe, so the resistance represents the impact of human activities.

The proportions of vancomycin-resistant Gram positives were examined in environmental samples („clear” soil, litter, manure enriched soil and commercial compost), following pasteurization. Results were given as percentages of resistant colony counts grown on agar plates containing vancomycin (4 mg/l), compared with counts of the same sample grown on antibiotic-free agar, as total (100%).

Clear soil samples contained 5-200 CFU/g vancomycin resistant Gram positive bacteria, their average rate compared with total heat resistant CFUs was 0,02%. Other samples contained 100-5000 CFU/g vancomycin resistant Gram positives, with an average rate of 0,07 % (litter), 0,16 % (soils with manure) and 0,38 % (compost) as compared with total heat resistant CFUs. Vancomycin resistant bacteria (all aerobic spore formers) were detectable widespread in soil samples, forming small fractions of the environmental flora. Enterococci were not detected.
Colony count technique applied on antibiotic containing agar plates is available for the detection of hidden pools of resistant bacteria within a microbial community. Complementary to classic monitoring methods, it may help us learn more about the dynamics of the resistance reinfection chain. Antimicrobials released to the environment will impair the biodiversity and functions of water and soil microbiota. These microbial communities form the base of life on Earth as a renewing environmental source; their mass and diversity are essential for the integrity of ecological systems. Regulation of antimicrobial use under complex chemical safety precautions is needed, followed by consequent and effective biological waste handling. Normal biota of our inner and outer environment should be regenerated, and antibiotics kept to be used in those cases, where they are really needed.

ELECTRON TRANSFER SUBUNITS OF THE HYN HYDROGENASE IN THE PURPLE SULPHUR PHOTOTROPHIC BACTERIUM, *THIOCAPSA ROSEOPERSICINA* BBS

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Hydrogenases are metalloenzymes catalyzing the following simple reaction: $2H^+ + 2e^- \leftrightarrow H_2$. The structural component of a [NiFe] hydrogenase consists of a large and a small subunit. The large subunit contains a special binuclear metallocenter, which is the active site of the enzyme. The small subunit carries iron-sulphur clusters responsible for the electron transport between the active center and the surface of the hydrogenase. The assembly of the metallocenter and the biosynthesis of a NiFe hydrogenase requires the concerted action of many accessory proteins.
Thiocapsa roseopersicina BBS is an anaerobic purple sulphur phototrophic bacterium isolated from the cold water of the North Sea. For photosynthetic growth, it requires reduced sulphur compounds (sulphide, thiosulphide or elementary sulphur), but the cells can also be cultivated microaerobically on organic compounds (sugar and acetate) in the dark. There are at least two membrane-associated (HynSL and HupSL) and one soluble (HoxEFUYH) [NiFe] hydrogenases in the cells.
HynSL shows extraordinary heat stability and is remarkably resistant to oxygen inactivation. The arrangement of the structural genes coding for this enzyme is unusual since the genes of the small and large subunits are interrupted by two *orf*s: *isp1* and *isp2*. It was shown by *in silico* analysis that Isp1 is a cytochrome b like protein containing five transmembrane helices, while Isp2 seems to be a cytoplasmic protein belonging to the heterodisulfide reductase family. The genomic context of the *hynS-isp1-isp2-hynL* genes suggests that the gene products have related function. Transcriptional analysis showed that the four genes are located on the same transcript. Furthermore, in a T7 expression assay, both the Isp1 and Isp2 proteins could be successfully expressed. The deletion of the *isp* genes had no effect on the hydrogenase activity *in vitro*, although the hydrogenase activity was abolished *in vivo*. This indicates an electron transfer role for these proteins. In the Isp2 protein, many highly conserved amino acids could be recognized. *In vitro* mutagenesis is performed to disclose the molecular mechanism of the electron flow in the HynSL-Isp1,2 hydrogenase *in vivo*.
Acknowledgement: The work has been supported by EU 5th and 6th Framework Programme projects (QLK5-1999-01267, STREP SOLAR-H, HYVOLUTION) and by domestic sources (NKFP, DEÁK-KKK, KN-RET, ASBÓTH, BAROSS). International collaboration within the EU network COST Action 841 is greatly appreciated.

MONITORING THE OCCURRENCE OF *TRICHODERMA* SPECIES DURING COMPOST PRODUCTION AND CULTIVATION OF *AGARICUS BISPORUS* IN HUNGARY

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Certain species of the filamentous fungal genus *Trichoderma* are known as the causative agents of green mould epidemics that are responsible for serious losses in *Agaricus* production world wide. The most aggressive strains were originally identified as *T. harzianum* Th2 and Th4 in Ireland and North-America, respectively, but subsequently recognized as the separate species *T. aggressivum* f. *europaeum* and f. *aggressivum*. The aim of this work was to monitor the occurrence of *Trichoderma* species during compost production and subsequent cultivation of *Agaricus bisporus* in Hungary.
A large number of *Trichoderma* strains have been isolated from *Agaricus* compost at two compost producing companies in Hungary. A previously designed PCR-based test for the specific identification of *T. aggressivum* was performed. Sequence analysis of the ITS region was carried out in order to identify the putative *T. aggressivum* isolates and to study the genetic diversity of *Trichoderma* strains occurring in mushroom compost in Hungary. The results showed that - beside *T. aggressivum* - further *Trichoderma* species including *T. harzianum*, *T. atroviride*, *T. asperellum*, *T. longibrachiatum* and *T. ghanense* were also present in the examined compost samples. The mtDNA RFLP patterns of the strains from this study were compared with those of the *T. aggressivum* f. *europaeum* isolates collected during the onset of the epidemic in Ireland and England in the late eighties. The comparison showed that both populations shared exactly the same RFLP pattern, which was clearly different from that of *T. aggressivum* f. *aggressivum*, indicating that the green mould outbreak observed in Hungary resulted from the spread of the Western-European epidemic.
A large-scale sampling program has been initiated in order to identify the possible sources of the green mould infection. The closest environment of mushroom farms affected by green mould has been examined, which included the sampling of the covering soil, the walls of mushroom growing cellars as well as the clothes and shoes of workers. The most frequently occurring species in these samples were *T. harzianum* and *T. atroviride*, however, the presence of other species - including the yet undescribed phylogenetic species *Trichoderma* sp. MA 3642 from section *Longibrachiatum* - could also be detected. Sampling of the individual ingredients used for compost production (wheat straw, chicken manure, horse manure, soya meal) as well as the spawn has been performed. Progress of the project will be presented and discussed.
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PCR-BASED DETECTION OF SELECTED PATHOGENIC BACTERIA IN THE DRINKING WATER SYSTEM OF A HOSPITAL

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Diseases and epidemics associated with drinking water pose a significant risk for public health in

developing countries, and the countries of the European Union even today. Although the number of these diseases have decreased in Hungary in parallel with the growth of the living standard, there are still people who are more sensitive to such infections. Microbes can attach to the inner surface of the drinking water distribution system, and this biofilm can be a substrate for obligate and facultative pathogens, in which they can proliferate. Biofilms can serve as first bases of infections of hospital origin (nosocomial) infections, which can be easily offended by a healthy person, but poses a serious risk to a weak immune system.
Our samples derived from five different points of a water system in a Hungarian hospital. Approximately one litre of water was filtered from each sampling point and total genomic DNA was extracted. Taxon-specific PCRs were carried out to detect pathogenic bacteria, and denaturing gradient gel electrophoresis (DGGE) analyses were performed to identify and compare the members of the microbial community. Culture-based methods were also used beside the molecular techniques, which allow the comparison of the two methods.
The primers used were suitable for the detection of the members of the genus *Legionella* and *Pseudomonas*, and the PCR-based technique seemed more sensitive than the culture-based method performed parallel. Similarities of DGGE patterns correlated with the distance of the sampling points within the water pipe network. Sequence analysis of the DNA fragments derived from the DGGE bands confirmed the presence of the species of the genus *Planococcus* (non-pathogenic bacteria that usually occur in water) and the genus *Burkholderia* (which contains facultative pathogenic members).
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INDUSTRIAL SCALE L-THREONINE PRODUCTION WITH *ESCHERICHIA COLI*

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World market of L-threonine was around 70.000 t/year in 2005 and is steadily increasing. Degussa is one of the two biggest producers of L-threonine in the world, using it as a feed additive in animal nutrition. In 2007, we will put the world's largest L-threonine production site located at Kaba into operation. Since 2005, we are already producing ca. 10.000 tons per year on our site. Thus, our plant is one of the biggest biotechnological production sites in Hungary in terms of quantity and annual turnover. This presentation will focus on the necessity to set up a state-of-the-art production site using *Escherichia coli* strains. It will focus particularly on recent advances in strain and process development and the unobvious connections every development has for the other field. Examples from model production strains will be given and discussed.

STUDY OF HSV2 SEROPREVALENCE IN HUNGARY; THE COMPARISON OF HSV2 SEROPREVALENCE OF PREGNANT AND INFERTILE WOMEN

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In the first part of our research, the sera of the seroepidemiological screening in 2000 were examined and anti Herpes simplex 2 (HSV2) IgG antibody was detected with ELISA method. More than 3600 sera from the entire country – from each county and Budapest - were examined. The examined people were born between 1920 and 1994. There has never been such a thorough research about HSV2 seropositivity in Hungary before. During the analysis, it has been found that the seropositivity rates are higher among women and seroprevalence is growing with age – as could be predicted based on

literature. It is an interesting fact that the seropositivity is higher in Budapest and Pest County compared to the country average. In the second part of our research, the seroprevalence of pregnant and infertile women (more than 500-500 sera from both groups) were compared. A difference was found between the two groups, seropositivity among infertile women was three times higher than that among pregnant women. Among pregnant women, seroprevalence was similar to the results of the seroepidemiological screening. In both groups, seroprevalence grew with age.

E266K CARD4/NOD1 AND TOLL-LIKE RECEPTOR 4 GENE POLYMORPHISMS IN HELICOBACTER PYLORI-INFECTED PATIENTS WITH DUODENAL ULCER OR GASTRITIS

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Helicobacter pylori (HP) can be recognized in epithelial cells by the intracellular pathogen receptor NOD1 or extracellular LPS detecting toll-like receptor 4 (TLR4). The aim was to evaluate the frequency of the NOD1 and TLR4 gene polymorphisms in HP-infected patients with duodenal ulcer (DU) and gastritis.
131 HP-positive patients with dyspeptic symptoms were examined by gastroduodenoscopy. HP positivity was detected by 13C-UBT and histopathology. DU was found in 58 and gastritis in 73 patients. E266K CARD4/NOD1 (G to A) was determined by RFLP, and the TLR4 (ASP/299/Gly and Thr/399/Ile) gene polymorphism was determined by melting point analysis with a real-time PCR method. Statistical analysis was performed by using the Fisher exact test or $\chi 2$ test as appropriate.
AA homozygote mutant variants of NOD1 were detected in 12 out of 58 HP-positive patients with DU (20%) vs. 5 out of 73 HP-positive patients with gastritis (6.8%); this difference is significant ($p = 0.034$, OR: 3.42, 95% CI = 1.184-2.519). Conversely, the G allele was significantly more frequent in patients with gastritis (76%) than in DU patients (62%) ($p = 0.014$, OR: 2.992, 95% CI = 1.574-5.789). However, no significant correlation in the frequency of the TLR4 gene polymorphism could be revealed between these two groups. The genotype frequency of AG heterozygotes concerning gene polymorphism ASP/299/Gly was 13.8% in patients with gastritis vs. 8.6 % in DU patients ($p = 0.490$). Similarly, there was a 13.2% frequency of CT heterozygotes concerning the Thr/399/Ile gene polymorphism in patients with gastritis vs. 8.66% in DU patients ($p = 0.568$). E266K CARD4/NOD1, but not the TLR4 gene polymorphism increases the risk of peptic ulceration in HP-positive patients. Host factors, including intracellular pathogen receptors, play an important role in the severity of the HP-induced gastric mucosal damage.

GENETIC POLYMORPHISM OF NOD1 AND IL-8, BUT NOT OF TLR4 GENES, ARE ASSOCIATED WITH *HELICOBACTER PYLORI*-INDUCED DUODENAL ULCER AND GASTRITIS

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Intracellular pathogen receptor Nod1 is involved in the epithelial cell sensing of *Helicobacter pylori* (*HP*), which results in a considerable IL-8 production. The aim was to evaluate the relationship between Nod1 and IL-8 genetic polymorphisms and the development of *H. pylori*–induced gastritis and duodenal ulcer (DU), compared with TLR4 polymorphisms.
85 patients with DU and 135 patients with gastritis were enrolled in the study. Seventy five *HP*-positive subjects without gastric or duodenal disease served as controls. The G796A (E266K) Nod1 polymorphism was determined by RFLP, and the – 251 IL-8 polymorphism by ARMS method. The TLR4 (ASP/299/Gly and Thr/399/Ile) gene polymorphisms were examined by melting point analysis.
AA homozygote mutant variants of NOD1 were detected in 20 % of the *HP*-positive patients with DU vs. 7 % of *HP*-positive patients with gastritis and 6 % of the *HP* positive healthy controls. The IL-8 heterozygote mutant variant was detected with a significantly higher frequency among DU patients and those with gastritis than among the *HP* - positive healthy subjects. However, no significant correlation concerning the frequency of the TLR4 gene polymorphism could be revealed between either group of patients and the controls.
E266K CARD4/NOD1, but not the TLR4 gene polymorphism increases the risk of peptic ulceration in *HP*-positive patients. The -251 IL-8 polymorphism was associated significantly with either gastritis or DU in *HP* infected subjects. Host factors, including intracellular pathogen receptors and IL-8 production play an important role in *HP*-induced gastric mucosal damage.

BACTERIAL COMMUNITY OF *EUDIAPTOMUS GRACILIS* (CRUSTACEA: COPEPODA) STUDIED BY CULTIVATION AND MOLECULAR CLONING

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Eudiaptomus gracilis (Crustacea: Copepoda) is the most abundant member of the zooplankton and plays a key role in the food net of Lake Balaton.
Individuals of this species were collected with plankton net in Lake Balaton at Tihany and washed 5 times with sterile Balaton-water. 30 crustaceans were homogenized (total body sample) and guts were dissected from other 50 – 50 individuals (gut samples). Homogenized samples were diluted and spread onto the following media: King B, chitin, peptone-meat extract agar, *Cytophaga* agar medium, Kidney disease medium. After random isolation of bacteria, representative strains were selected: 54 from total body sample and 45 from gut samples. Fatty acids of all strains were isolated and analysed by GC (HP 5890, HP1 capillary column), then strains were grouped based on their fatty acid profile using the SYNTAX 2000 programme package. DNA was isolated from strains representing each phenon as well as ungrouped bacteria, and their 16S rRNA genes were sequenced (BigDye™ Terminator v3.1, ABI 310 Genetic Analyzer). Simultaneously, total DNA was isolated (QIAGEN DNeasy Tissue kit) from the gut sample and bacterial 16S rDNA clone library was constructed (pGEM-T Easy Vector System). The 108 clones were grouped using ARDRA (*Alu*I, *Hin*6I restriction endonucleases) into 26 groups. One clone of each group was sequenced.
Results indicate diverse bacterial communities in *Eudiaptomus gracilis*. Some of the identified bacteria have already been isolated from different parts of Lake Balaton (e.g. *Aeromonas, Pseudomonas, Bacillus* genera), others are unknown in this habitat. *Exiguobacterium* species (*E. acetylicum, E. aurantiacum, E. undae*) were detected as dominant bacteria with both methods. *Staphylococcus* spp., *Chryseobacterium* spp., *Micrococcus luteus* strains were isolated from total

body as well as gut sample. *Bacillus* spp. and *Pseudomonas* spp. were also detected with cloning.
Several actino- and proteobacteria were identified by cultivation and/or cloning. Actinobacteria: *Arthrobacter nitroguajacolicus* (gut sample), *Promicromonospora sukumoe* (total body sample) could be cultivated, *Agromyces fucosus*, *Nocardioides sp.*, *Microbacterium sp.,* were identified by cloning (gut). Proteobacteria: *Sphingomonas sp.* (body), *Moraxella osloensis, Acinetobacter lwoffii* and *Aeromonas veronii* bv. *sobria* (gut sample) were cultivated, *Serratia marcescens, Camylobacter concisus, Halomonas venusta* and *Schineria sp.* were cloned from gut sample.
Results of cloning and cultivation showed several overlapping, which indicates that the methods used in this study can be well applied in the investigation of bacterial communities of aquatic invertebrates.

DIFFERENCES IN ANTIBIOTIC RESISTANCE OF MRSA AND MSSA STRAINS ISOLATED FROM CLINICAL SAMPLES OF HOSPITALIZED PATIENTS TREATED AT THE CLINICS OF THE SEMMELWEIS UNIVERSITY

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Significant differences in the resistance to antistaphylococcal drugs were found in MRSA and MSSA isolates supporting our previous opinion that MRSA strains may form a subspecies of *S. aureus*. In case of the *mecA* gene positive strains, determination of the MIC of vancomycin, teicoplanin and amikacin is only reasonable. The resistance of MRSA strains to a variety of antimicrobials was significantly higher than that of the MSSA strains. The MRSA strains proved to be more resistant to macrolide, ketolide and lincosamide antibiotics: 26% of the MSSA and 96% of the MRSA strains were resistant to clarithromycin, 7.3% and 95.7% to telithromycin, and 70% of the MSSA and 100% of the MRSA strains were resistant to clindamycin. The MSSA strains were also more sensitive to aminoglycosides than the MRSA strains: 3.25% of the MSSA and 81.2% of the MRSA strains were resistant to gentamicin, while 5.7% of the MSSA and 18.2% of the MRSA strains were resistant to amikacin. All strains were sensitive to vancomycin, but 2.4% of the MRSA strains were resistant to teicoplanin, while all MSSA strain proved to be sensitive. Fluoroquinolones are useful antimicrobials against MSSA strains: to ciprofloxacin, levofloxacin and moxifloxacin 87.8%, 68.3% and 78.8% of the strains were sensitive, respectively. In contrast, they cannot be recommended in MRSA infections, since 94.5%, 97.6% and 90.9% of the strains were resistant to them. The identification of the *S. aureus* strains was preformed by traditional and molecular methods (catalase, coagulase, *nucA*). The PCR method was used for detecting the *mecA* gene. The minimum inhibitory concentrations (MIC) of the antimicrobial agents were tested by broth microdilution method according to the NCCLS/CLSI recommendations. The aim of this study was to reveal any significant difference in the resistance to antistaphylococcal agents of methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains of *Staphylococcus aureus*. The antimicrobial susceptibility of 121 MSSA and 165 MRSA strains was examined. The samples were isolated from clinical specimens of hospitalized patients (mostly from invasive infections) between January 1st 2001 and January 10th 2006.
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CHARACTERIZATION OF A CAROTENOID DEFICIENT MUTANT STRAIN OF *PHAFFIA RHODOZYMA*

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The astaxanthin producing yeast *Phaffia rhodozyma* is used in industrial fish production. In addition, astaxanthin disposes high efficiency of antioxidant property e.g., protects against the effect of free radicals in cell injury and DNA-damage. In our study, a white strain blocked in carotenoid biosynthesis and its pink coloured parental strain has been investigated. The mutant strain showed resistance to oxidative- and heavy metal stresses. The two strains were phenetically characterised and their carotenoid composition was determined by HPLC.
The mutant strain showed lack of carotenoids. Although the generation time was similar in the two strains, the cell size of the mutant was almost three times smaller than its parental strain. The carotenoid deficient mutant was sensitive to hydrogen peroxide and miconazole, but it was resistant to zinc, hexavalent chromium and lipid peroxide (t-BOOH). The concentration of superoxide-radical in the mutant strain proved to be nine times lower, however, the intracellular peroxide concentration was significantly higher. The Cr(VI) reduction capacity was smaller in the mutant, although there was no difference between the concentration of hydroxyl-radicals. The specific enzyme activity of catalase proved to be 14 times smaller in the mutant strain, whereas the specific activity of glutathione reductase enzyme did not change. The concentration of the reduced glutathione became significantly smaller, however, the concentration of oxidated glutathione did not change. Due to decreased catalase activity in the mutant strain, peroxide-ion concentration increased, thus the sensitivity to hydrogen peroxide increased as well.
Based on our results, we concluded that the change in the oxido-reduction equilibrium was due to the decrease of GSH concentration, which caused the decrease of both chromium-(VI), induced chromium-(V) and hydroxyl-radical concentration. Resistance of the carotenoid deficient mutant strain against oxidative and heavy metal stresses is not the consequence of the lack of carotenoids, but it is rather influenced by the change of the oxido-reduction system equilibrium of the cells.

APPLICATION OF RAPID INSTRUMENTAL METHODS FOR MONITORING BACTERIOLOGICAL SPOILAGE OF MEAT

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Experimental batches of aerobically packed pork cutlets have been stored in subsequent experiments at 4, 8, and 12 °C, respectively. During storage, total aerobic plate counts and colony counts of pseudomonads – the latter are known as the dominant components of the spoilage biota – were estimated periodically. Simultaneously with the estimation of the viable cell counts, respective stomachered samples were inoculated into conductance cells of a Malthus Microbiological Analyzer in triplicates and conductance detection times were automatically recorded at 30 °C of incubation, using general impedance broth for the total viable cells, and selective impedance medium for the *Pseudomonas* spp., respectively. Head space volatiles formed in the stoppered glass containers of the refrigerated meat samples were analyzed by a chemosensor array (NST 3320 type electronic nose, Applied Sensor AG) at similar time intervals as for sampling for the plate counts, while sensory quality of the raw pork cutlets was estimated subjectively for faultiness of the odour and colour of the samples. Parameters of the bacterial growth curves were calculated as a function of the meat storage

temperatures by curve-fitting of the colony counts by the DMFit programme package of the ComBase international microbiological modelling software. Linear correlations were found between the respective logarithmic colony counts and the conductimetric detection times. According to these calibrations, the automatic conductimetric method at 30 °C incubation is able to assess within 8 hours whether a sample of aerobically stored pork cutlets contains greater or less than 10^7 CFU/g of the psychrotrophic spoilage bacteria. Regarding electronic nose measurements, partial least-squares (PLS) regression was used to define quantitative mathematical relationship between the sensor signal responses and the degree of the bacteriological spoilage, independently of storage temperature. Different sensor selection techniques were applied to reduce dimensionality. More robust calibration models were computed by determining few individual sensors having the smallest cross correlation and the highest correlation with the reference bacteriological data. By this means, construction of a cheaper and single-purpose instrument can be proposed for future use. Correlation between the predicted and observed bacteriological values were given on the cross validated data for both the data-reduced models and full calibrations using all the 23 sensor elements. These preliminary results indicated that both the conductimetric instrument and the electronic nose technique may be capable of detecting signals of bacteriological spoilage earlier, or, at the same time as olfactory quality-deterioration.

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TRANSMISSIBILITY OF VIRULENT STRAINS OF NEWCASTLE DISEASE VIRUS IN VACCINATED CHICKENS

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Newcastle disease virus (NDV) is an avian paramyxovirus that can elicit 100% mortality in susceptible poultry. It can be regarded as a typical emerging infection: in two-thirds of the countries it has become endemic but developing countries, from time to time, also experience introductions of exotic strains, and ensuing epizootics require costly eradication. At present, three genotypes (V., VI. and VII.) dominate in different regions of the world with strains in genotype VII being the most successful. The current situation is the most puzzling because ND-vaccines are cheap, efficacious, and easily applicable even in broader comparison.

Concerning the contradiction, two hypotheses have been put forward and examined in our experiments. Firstly, current methodology yields information only on the anti-disease efficacy but not on anti-infection immunity. Secondly, since the introduction of vaccination in the 1950's, strains capable of evading immunity were selected. Therefore, a challenge system has been applied to the evaluation and comparison of anti-infection immunity elicited by ND-vaccines. Direct infection of chickens with challenge virus, as in the traditional tests, has been replaced in our approach by contact infection simulating natural transmission.

In challenges, virulent strains isolated from different epizootics and belonging to different genotypes were used. Excretion and transmission between individual birds of the challenge virus were followed by daily oro-nasal swabbing and titration of the samples. Regardless of the virus, in susceptible (negative) populations within 2 weeks 100% of birds shed the virus to a high titre and most of them succumbed to the disease. In contrast, the dynamic of infectious chains in immunised (positive) birds were quite distinct. Surprisingly, however, the success of transmission was less dependent on the degree of immunity than on the origin of challenge virus. Namely, *old* NDV strains (genotype II and IV) deriving from epizootics *before* the introduction of vaccinations spread poorly and infected only a

fraction of chickens in the group. By contrast, viruses (genotypes V, VI and VII) from *recent* epizootics showed highly efficient spread even in multiply immunised chickens. Results of our experiments favoured the hypothesis that in the vaccination era virulent NDV strains were selected that were capable of breaking through immunity and spreading in populations of vaccinated birds.

ISOLATION OF CELLULOSE DEGRADING AEROBIC MICROORGANISMS FOR BIOREMEDIATION PURPOSES

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Lignocellulose is the building block of all plants and ubiquitous to most regions of our planet. Furthermore, tremendous amount of cellulose is produced as municipal and industrial waste, which contributes to pollution problems. The cellulose polymer consists of glucose monomers, which are valuable substrates for several biotechnological processes. Thus, the biodegradation of (ligno)cellulose wastes would provide enormous amount of substrates for various technologies. A variety of microorganisms and mechanisms are involved in the complete biodegradation of lignocellulose in the environment. However, the complex unsettled structure of lignocellulose composed of various polymers make its hydrolysis more difficult requiring chemical pre-treatment in many cases. Furthermore, the degradation rate of cellulose strongly depends on the environmental conditions. Cellulose can be degraded both aerobically and anaerobically and most of the strains studied so far decomposed cellulose anaerobically.
Several biotechnological processes are known in which straw is used to aerate the compact and fuel oil contaminated soils. The structure material of straw is lignocellulose composed of lignin, cellulose and hemicellulose. In our oil degradation experiments, straw was also applied as immobilizing carrier for the microorganisms. It would be beneficial if, beside aeration, straw could also be used as a carbon and energy source. In particular, the bacteria capable to degrade hydrocarbons synthesize surface active materials to enhance the bioavailability of oil derivatives. Some surfactants contain carbohydrate compounds as building blocks. In the presence of glucose, the cells can produce surface active materials, which result in the stimulation of the biodegradation of hydrocarbons present in their environment. Therefore, our final aim was to combine the oil degradation process with the degradation of the cellulolytic carrier. For this purpose, we isolated aerobic or microaerophilic microorganisms capable to degrade the cellulolytic materials of straw. We selected the best cellulose degraders from our microbial collection and they were combined with the oil degrader immobilized on straw for enhanced degradation of hydrocarbons. As an additional advantage, the carrier material is finally converted into an easily usable substrate for the environment.
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A STUDY OF THE UTILITY OF TRANSLATION ELONGATION FACTOR 1 AS A PHYLOGENETIC MARKER FOR *PHOMA* GENUS

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The cosmopolitan *Phoma* genus contains mainly phytopathogenic, opportunistic parasite, and saprophyte fungal species. Up to now, the characterization of *Phoma* species and other taxa of *Phoma* have so far been determined on the basis of morphology on standardized media, and gene sequence

analysis was only used as a confirmative or distinctive complement. In this study, we have tried to find a molecular marker that can be used as phylogenetic marker in the molecular based classification of the *Phoma* genus. The widely used molecular marker in phylogenetic studies, the ITS sequence, did not show significant nucleotide differences to infer relationships. Because of this, we have chosen an alternative molecular marker to accomplish this work. In this study, we employed the translation elongation factor 1 subunit alpha (EF-1α = tef1), as a potential genetic marker to infer phylogenetic relationships among different *Phoma* taxa using nucleotide characters obtained by sequencing a part of the EF-1α gene, containing both introns and exons. EF-1α is well suited for determining phylogenetic relationships due to its universal occurrence and typical single copy genome presence.
Total Genomic DNA from each isolate was extracted and a fraction of the *tef1* gene was amplified with the help of two primers (EF1-728F and EF1-986R). The phylogenetic analysis was conducted with PAUP*4.0b. Phylogenetic relationships of *Phoma* strains were inferred by the parsimony analysis of *tef1* sequences. Topological robustness in parsimony analysis was estimated using 1000 bootstrap replicates, and was rooted using an outgroup *Claviceps sorghi*.
Twelve different *Phoma* sequences were analysed together with the closely related *Ascochyta* sequences. The constructed phylogenetic tree does not support the traditional *Phoma* sections based on morphological characterization. However, we have managed to distinguish the *Phoma* strains from the *Ascochyta* species comparing their *tef1* sequences by parsimony analysis. We have proved that *tef1* could be a useful phylogenetic marker to resolve phylogenetic relationships at the species level within the *Phoma* genus. Further investigations would be necessary to clarify whether the *tef1* gene sequence as phylogenetic molecular marker is well suited for the classification of *Phoma* species.

FIRST DETECTION AND MOLECULAR CHARACTERIZATION OF TULA HANTAVIRUSES ISOLATED FROM *MICROTUS ARVALIS* SPECIES IN BARANYA COUNTY, HUNGARY

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Tula hantavirus belongs to the genus *Hantavirus*, family *Bunyaviridae* and carried by European common vole (*Microtus arvalis*) and Eastern vole (*M. rossiaemeridionalis*). The virus has been detected in many European countries. The aim of this study was to determine the prevalence of Tula hantaviruses among *Microtus arvalis* rodents in Baranya County, Hungary.
Microtus arvalis rodents were trapped in Görcsöny (15 km from Pécs) during the summer season of 2005. Rodents were dissected and lung tissues were used for Tula virus detection. The viral RNA was extracted from lung suspensions with TRIzol reagent following the manufacturer's recommendation. Viruses were detected by RT-PCR using a primer pair designed for the S-segment of the viral genome. Positive samples were sequenced and analyzed.
28 *Microtus arvalis* were tested during the study period for the presence of Tula hantaviruses. 12 out of these 28 animals were infected. Ten samples were sequenced and analyzed. Phylogenetic analysis showed that the Hungarian samples formed a separate group and could be divided to at least two different lineages. Our strains were closely related to those isolated in Western Slovakia and the Czech Republic. In this study, we described the presence of Tula hantaviruses in Hungary for the first time. Unlike other studies conducted in Europe, we found a higher proportion of Tula hantavirus-infected *Microtus arvalis* rodents. Because of the high proportion of Tulavirus-infected animals and unclear pathogenicity of the virus for humans, serological investigations are necessary in the area.

A HIGHLY SENSITIVE AND SPECIFIC MOLECULAR METHOD TO DETECT DOBRAVA HANTAVIRUSES

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Human hantavirus infections are accompanied by a short viremic period and usually low viral load, which makes early diagnosis based on virus detection difficult. The aim of the study was to develop a new, sensitive and specific molecular detection method for the rapid diagnosis of hantavirus infections. Part of the M segment coding nucleic acid of Dobrava hantavirus, isolated from *Apodemus agrarius* in Hungary was amplified and cloned into pGEM®-T plasmid. The plasmid was multiplied in JM109 competent cells and purified with a commercial preparation kit. The concentration of the plasmid was measured and the number of copies was calculated. Dilutions were made from 10^8 to 1 copies and the target sequence was amplified by standard nested-PCR or the combination of standard-PCR and SYBR Green-based nested real-time PCR, using newly designed virus specific primers.
The highest sensitivity and specificity were seen when standard PCR and nested real-time PCR techniques were combined. After the first standard PCR step, the amplicons were diluted and internal primers were used for the nested real-time PCR. Based on our results, as few as 1 copy of the plasmid could be detected. In this investigation, we described a new, highly sensitive and specific method to detect Dobrava hantaviruses. We believe that our method is reliable in environmental studies (for example animal reservoir studies) as well as in the case of individual clinical specimens.

DETECTION OF DOBRAVA AND PUUMALA HANTAVIRUS INFECTIONS IN RODENTS (*APODEMUS AGRARIUS*, *A. FLAVICOLLIS*, *CLETHRIONOMYS GLAREOLUS*) IN THE TRANSDANUBIAN REGION OF HUNGARY

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Dobrava and Puumala hantaviruses belong to the genus *Hantavirus*, family *Bunyaviridae* and carried by striped field (*Apodemus agrarius*) and yellow necked (*Apodemus flavicollis*) mice, while the main host of the Puumala virus is the bank vole (*Clethrionomys glareolus*). Dobrava hantavirus causes severe hemorrhagic fever with renal syndrome (HFRS), whereas Puumala virus mostly causes nephropathia epidemica (NE) in Europe. The aim of this pilot study was to determine the prevalence of Dobrava hantavirus in *A. flavicollis, A. agrarius, A. sylvaticus* and Puumala virus in *Clethrionomys glareolus* rodents in the Transdanubian region of Hungary.
The rodents were trapped in three different locations of the Transdanubian region of Hungary (the Gyékényes, Görcsöny and Kis-Balaton areas) during the summer and autumn seasons of 2005. The rodents were dissected and lung tissues were used for hantavirus detection. The viral RNA was extracted from lung suspensions with TRIZOL reagent according to the manufacturer's recommendations. Dobrava and Puumala hantaviruses were detected by SYBR Green-based real-time PCR, using newly designed virus specific primers. Positive samples were selected for sequence and phylogenetic analyses. During the study period, 18 *Apodemus sp.* (9 *A. agrarius*, 8 *A. flavicollis* and 1

A. sylvaticus) and 12 *Clethrionomys glareolus* were tested for the presence of hantaviruses. Out of the 18 *Apodemus* rodents, 2 *A. agrarius* were infected with Dobrava hantavirus; while 2 of the 12 *C. glareolus* were positive for Puumala virus. Phylogenetic analysis showed that the Hungarian Dobrava virus strains were most closely related to those isolated from *A. agrarius* mice in Slovakia. In this study, we reported the occurrence of Dobrava and Puumala hantaviruses in the Transdanubian region of Hungary. Based on the clinical experience and our recent data from the region, we concluded that extended reservoir studies as well as serological investigations might be important in the future.

THE EFFECT OF WATER ACTIVITY ON THE SURVIVAL OF *CAMPYLOBACTER JEJUNI*

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The effect of water activity on the destruction of *Campylobacter jejuni* was investigated at different water activities controlled by three substances: glycerol, glucose and NaCl. The aim of the study was to determine the combined effect of water activity and adjusting substances on cell survival. In addition, we had an assumption that NaCl had a more pronounced effect on cell destruction than the other two substances. We used a rapid redox-potential-based method for the evaluation of the experimental results. With the application of this new technique, the known disadvantages of plate spreading and MPN methods could be avoided.
The results of the experiments showed that *Campylobacter jejuni* was very sensitive to lower water activities, and the destruction of the cells began immediately after getting into the media of low aw. NaCl had a pronounced destructing effect as the bacteria lost their viability sooner and at higher water activity values compared to the effect of the other aw controlling substances. We can conclude that *Campylobacter jejuni* is – as it is described in literature – very sensitive to lower water activity values in a very rapid way. The redox-potential measurement method proved to be a rapid, reliable and suitable tool for the evaluation of cell survival experiments.

DETECTION OF HIV CORECEPTOR GENE POLYMORPHISM IN 18TH CENTURY MUMMIES

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The chemokin receptor CCR5 represents the major coreceptor for macrophage tropic HIV. Carriers of the mutated CCR5 (delta32 deletion) gene have a partial protection against HIV infection. Data indicate that this deletion is a relatively recent event in the history of the European population. The aim of this study was the detection and analysis of CCR5 HIV coreceptor gene mutations in the DNA of 256 naturally mummified human bodies from 1731-1838 recovered in the town of Vác as part of a complex microbiological exploration.
One of the biggest technical problems we faced was the recovery of good quality DNA. DNA from archaic biological samples go through substantial oxidative and hydrolytic changes resulting in fragmented and degraded DNA. Therefore, we expected short, maximum 110-150 bp long DNAs. We applied more than 20 different methods for DNA isolation from soft tissues. The silica based,

guanidinium thiocyanate treatment method completed with PTB (N-phenacyl-thiazolium bromide) proved useful. Isolated DNA was analyzed with PCR using specific primer pairs containing the CCR5 deletion fragment designed for the short DNA molecule. The PCR amplified 118/96 bp (*wt* and/or deleted) long specific fragments. With this technique, heterozygous mutations in the CCR5 HIV chemokin coreceptor gene were found in the Vác mummies. The oldest HIV coreceptor gene mutation in Hungary was detected in the case of T.W. from 1766.
The serial analysis of the mummies is in progress.

AFFINITY PURIFICATION OF HOX HYDROGENASE IN THE PURPLE SULPHUR PHOTOSYNTHETIC BACTERIUM *THIOCAPSA ROSEOPERSICINA*

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Hydrogenases are able to catalyze the reversible oxidation of molecular hydrogen and play role in microbial energy metabolism. Most of these enzymes are found in Archaea and Bacteria, but a few are present in Eukarya as well. Nowadays, biohydrogen produced with these enzymes is essential from energetic and environmental points of view. The aim of our study was to purify and characterize a lately discovered Hox hydrogenase. The phototrophic purple sulphur bacterium *Thiocapsa roseopersicina* harbours four different hydrogenases (HynSL, HupSL, HoxEFUYH, and HupUV). The Hox hydrogenase is soluble and consists of five subunits. *Hox* gene homologues are present in *Ralstonia*, *Rhodococcus* and cyanobacteria. Hox is a bidirectional enzyme, but its real function has not been discovered yet.
The purification of Hox hydrogenase in *Thiocapsa roseopersicina* and in other organisms has not succeeded yet with the use of traditional chromatography. Nowadays, affinity chromatography is the state of art method because it is rapid and easy. Accordingly, FLAG and StrepII tags were joined to the C-terminal of the HoxY subunit by gene modification. This subunit with HoxH are responsible for the hydrogen uptake activity. These subunits were connected the strongest. Because of the success of tagging, the hydrogenase could be purified with affinity chromatography in large amount and the examination of the pure enzyme will be possible in the near future. The fact that we know the exact structure and stability condition of Hox allows us to unravel its real cellular functions.

EFFECT OF HORSERADISH EXTRACT ON INDICATOR MICROBES

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The alcoholic extract of horseradish is rich in allyl-isothyocianate (AITC) and allyl-thyocianate (ATC). These compounds may be effective for the preservation of pickled vegetables or fresh salads. Nowadays, there is a new trend: natural plant extracts are preferred to synthetic preserving compounds (like the salts of benzoic and sorbic acid used generally in foods). Unfortunately, the latter conserving agents can cause allergic problems to several people. For this reason, it is very important that a natural plant extract, the alcoholic extract of horseradish (*Armoratia lapathifolia*) may provide an opportunity for food preservation in the future. The aim of our work was i. to produce alcoholic extracts from different sorts of horseradish; ii. to measure the quantity of AITC by GC-MS method; iii. to obtain the effective concentration of the horseradish extract for the inhibition of

Enterobacter cloacae and *Escherichia coli* at different pH-values and temperatures. The growth of the indicator microbes was followed by measuring the absorbance at 610 nm in order to get growth curves. The combination effects of temperatures (37°C, 15°C), pH-values (6, 5, 4) and different concentrations of alcoholic horseradish extract (1, 2, 4, 6%v/v) were studied for 2 weeks.
Enterobacter cloacae was inhibited by 6% of alcoholic horseradish extract at 37°C, in the case of pH 6 and pH 5. In case of *Escherichia coli* total inhibition by 6% alcoholic horseradish extract was exerted only at pH 5. *Enterobacter cloacae* and *Escherichia coli* were inhibited by 4% alcoholic horseradish extract at 15°C, at pH 6 and also at pH 5. The effect of pH 4 was the most pronounced, both at 15°C and 37°C. *Escherichia coli* was unable to multiply under these conditions and *Enterobacter cloacae* was totally inhibited in the presence of 1% alcoholic horseradish extract.

GENOME AND PHYLOGENY OF GOOSE ADENOVIRUS ISOLATED IN HUNGARY

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Adenoviruses isolated from birds are classified into three genera in the family *Adenoviridae*. Complete genome sequences have been determined for fowl adenovirus (FAdV) 1 and 9, belonging to the genus *Aviadenovirus*; egg drop syndrome virus (genus *Atadenovirus*), and turkey hemorrhagic enteritis virus (genus *Siadenovirus*). Regarding aviadenoviruses, phylogenetic relations had been determined from partial hexon gene sequences, and the analysis showed that adenovirus isolates from domestic goose and Muscovy duck represent an independent lineage within the aviadenovirus clade. Our aim was to determine the complete DNA sequence of a GAdV in order to examine its genome organization and exact phylogenetic position.
A Hungarian GAdV isolate, designated P29, was propagated on Muscovy duck embryo fibroblast cells. The DNA was extracted and sheared ultrasonically. The fragments were separated by agarose gel electrophoresis and the population corresponding to 600–1,500 bp was extracted from the gel. The fragments were cloned into plasmids and 750 clones were sequenced. The sequences were processed by the Gap4 program of the Staden package. Inner gaps were filled by PCR. The genome ends have not yet been sequenced. Coding regions were identified using BLAST. Multiple alignments of amino acid sequences were made by MultAlin and analyzed by distance matrix analysis (Phylip program package). Phylogenetic trees were visualized by TreeView.
Up to now, about 37,500 bp, with an average G+C content of 44.7%, was assembled into a continuous sequence. This corresponds to approximately 85% of the size of previously sequenced aviadenovirus genomes. Analysis of the sequence showed that the 16 conserved genes, which comprise the central genome region in every adenovirus studied to date, are also present in GAdV. Moreover, a homologue of the aviadenovirus U exon was also identified. Similarly to FAdV-9 and contrary to FAdV-1, GAdV has one fiber gene only. A number of specific genes, characteristic for the aviadenovirus genus, have been identified, while others do not seem to be present. This fact suggests that GAdV might be more ancient than the known FAdVs. Distance matrix analysis seemed to confirm this hypothesis by placing GAdV on an aviadenovirus branch together with Muscovy duck AdV but distinct from FAdVs or falcon AdV.
Members of the family *Adenoviridae* are thought to have co-evolved with their hosts. The genetic content and phylogenetic position of GAdV fit in well with this view. GAdV is different not only from FAdVs but also from the recently described falcon adenovirus. The three host species belong to three different orders of birds, with the latest taxonomy proposing that *Anseriformes* (where geese and ducks belong) and *Galliformes* have arisen from the ancient taxonomic group *Galloanseri*.

Apparently, adenoviruses of these birds are also monophyletic, having diverged from a common ancestor perhaps at the time of separation of their respective hosts.
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PHYLOGENETIC POSITION OF THE FISSION YEAST *SCHIZOSACCHAROMYCES JAPONICUS* ACCORDING TO THE RECENTLY ISOLATED ACTIN GENE

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There are many dimorphic pathogenic fungi whose pathogenicity can be associated with morphological transitions. *Schizosaccharomyces japonicus* has proved to be an attractive model for studying dimorphism because its dimorphic cycle is similar to that of the pathogenic species, but it is not pathogenic. *Sz. japonicus* is closely related to *Sz. pombe*, a frequently used model organism for cell biological research. Therefore, we wanted to know more about *Sz. japonicus* and its taxonomical and phylogenetic position. In this study, we present further molecular biological data that confirm that the two fission yeasts are closely related.
Since actin is a conserved protein in all living organisms, the nucleotide/amino acid sequences of the actin genes and proteins can be used to get information about an organism's taxonomical and phylogenetic position. We designed degenerate primers (using the sequences of the *act1* gene of *Sz. pombe* and the *ACT1* gene of *Saccharomyces cerevisiae*) and used these primers for PCR amplification of the *Sz. japonicus* counterpart of the *act1* gene. We got a 716 bp long nucleotide sequence, which was a part of the actin gene of *Sz. japonicus*. We used this sequence for phylogenetic analysis and found that the two fission yeasts were closely related. Because the genome sequencing project of *Sz. japonicus* started in the spring of 2006, data are already available at the http://www.ncbi.nlm.nih.gov/blast/mmtrace.shtml website. Therefore, we used this database to find the missing part of the *act1* sequence and probably we have the complete nucleotide and amino acid sequence of the *Sz. japonicus* actin gene/protein.

OPTIMISATION OF BIOMASS PRODUCTION OF STARCH UTILIZING YEAST SPECIES

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The aim of this work was to optimise fermentation media for biomass production of different starch utilizing yeast species. Bio wastes containing starch are produced in enormously large quantities in the food industry, especially during the processing of potato and maize. Processing of these wastes has two important aspects: the high starch content of the waste threatens the environment, and it provides a cheap, yet unexploited fermentation medium. It is expected that such waste/process water does not contain compounds that are inhibitory for yeast growth. Conventional optimisation methods for culture media are laborious and time consuming and are not suitable for detecting the true

optimum due to the interactions of factors. The response surface method (RSM) enables us to examine two factors simultaneously and determine the optimal combination of media constituents.
The central composite design (CCD) was applied for the optimisation and the full second-order polynomial model (response function) was used to fit to the dependent variables of the culture media. The optimisation was carried out in several steps. The combination of two C-sources (saccharose and starch) and two N-sources (yeast extract and ammonium-sulphate) were examined. Strains of two starch-utilising yeast species: *Saccharomycopsis fibuligera* CCY 42-3-1and *Schwanniomyces occidentalis* Y 758 were involved in the experiments. We found that the combinations of the C- and N-sources, shown in the table below, provided the highest biomass yield.

Species	Variables		C/N	Variables		C/N
	C-1	**N-1**		**C-1**	**N-2**	
Schw. occidentalis	2.0	0.7	2.9	2.7	1.2	2.3
Sacch. fibuligera	3.0	0.36	8.6	3.0	0.3	10.0

Species	Variables		C/N	Variables		C/N
	C-2	**N-1**		**C-2**	**N-2**	
Schw. occidentalis	2.3	0.7	3.3	2.2	0.4	6.3
Sacch. fibuligera	2.5	0.8	3.1	1.4	0.4	3.5

C-1: saccharose (%); **C-2**: starch (%); **N-1**: yeast extract (%); **N-2**: $(NH_4)_2SO_4$ (%)

Cultivation of the two strains in starch-yeast extract medium resulted in very similar growth curves. Comparing the biomass production on this medium with that on glucose-yeast extract medium, we found that the biomass yield was slightly lower on starch than on glucose.
Acknowledgement: The research was carried out in the frame of the EU FP6 NMP3-CT-2003 project.

RESISTANCE OF POTATO TO *ERWINIA CAROTOVORA* SSP. *ATROSEPTICA* TESTED BY INOCULATION OF DETACHED LEAVES AND STEMS OF POTTED PLANTS

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Different *Erwinia* species, *E carotovora* ssp. *atroseptica* (van Hall) Dye- (Eca*), E. carotovora* ssp. *carotovora* (Jones) Dye, and *E. chrysanthemi* (Burkh. et al.) - are the causing agents of two of the most important bacterial potato diseases: blackleg on stem and soft rot on tubers. The disease 'blackleg' in general is caused by Eca. Under wet conditions, blackleg symptoms progress from the rotting seed and range from small, black, water-soaked lesions restricted to the stem base to extensive rotting of the whole stem. Under dry conditions, infected stems are stunted and wilt with chlorotic leaves. Resistance to blackleg used to be evaluated under greenhouse conditions using inoculated potted plants or detached leaves. In our experiments, we used both methods for testing resistance of Hungarian potato varieties to Eca.
Seven varieties of the Potato Research Centre (Hópehely, Kánkán, Vénusz Gold, Rioja, White Lady, Lorett and Góliát) were involved in the tests. Leaves of six to nine-week-old plants grown in greenhouse were collected from the middle part of the plants. The bacterial strain "Eca Bn3 Pécs" was used in the stem and leaf tests. Bacteria were grown on peptone-glucose broth at 25 °C. We checked the density of the bacterial suspension using a McFarland densitometer. Detached leaves with petioles were dipped into the inoculum and incubated. We investigated ten leaves per variety.

Three leaves per variety kept with water served as controls. Assessment of the rotted area was done visually using a 1-5 scale. In the other method, nine potted plants at 2-3 cm above the soil level were infected with the bacterial suspension with the help of a hypodermic syringe. Two plants per variety kept with peptone-glucose broth serving as controls. For evaluation we used the same method as before. Based on the data, a sensitivity order was created for the examined varieties. Kánkán, Rioja and White Lady showed the highest resistance in the stem infection test, while varieties Lorett, Hópehely, and Vénusz Gold were the most sensitive. Góliát showed an average resistance. In the detached leaf test varieties White Lady and Góliát were the most resistant. Lorett and Rioja were resistant to Eca. Kánkán and Vénusz Gold showed an average resistance. According to our experiments, the detached leaf method is fast and easy, but considering that stem and tuber soft rot are seed- borne diseases, the stem screening method provides more reliable results.

MOLECULAR EXAMINATION OF ENTEROVIRUSES UNTYPABLE USING CLASSICAL SEROLOGY

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The genus *Enterovirus* (family Picornaviridae) is comprised of more than 80 serotypes, most of which are known human pathogens. The majority of enterovirus infections are asymptomatic or cause only mild illnesses, such as non-specific febrile illness or mild upper respiratory symptoms. Enteroviruses, however, can also cause a wide variety of other clinical illnesses including haemorrhagic cojuctivitis, aseptic meningitis, undifferentiated rash, acute flaccid paralysis, myocarditis and neonatal sepsis-like disease. The neutralisation test, the previous gold standard of enterovirus typing, was found to be generaly reliable, but it was labor-intensive and time-consuming and sometimes might fail to identify an isolate, because of aggregation of virus particles, antigenic drift, recombination within the capsid region or due to the presence of multiple viruses in specimens to be tested.

In 2004 we detetected several enteroviruses from newborn babies and young children from different regions of Hungary by snPCR. After molecular analysis we isolated the viruses on RD (human rhabdomyosarcoma) cell line, but the virus type could not be identified by mixed commercial typing horse sera. We have recently sequenced the gene of the major capsomer (VP1) of untypable enteroviruses. Gene amplification was performed by consensus degenerate hybrid oligonucleotide primers (CODEHOP) which amplified an about 400 nucleotide long fragment.

The results revealed that enterovirus 71 (EV 71) had been isolated from 6 patients of 1 to 6 years of age. The younger ones suffered from encephalitis, acute flaccid paralysis (AFP) and meningitis. Other 3 children had mild symptoms. One patient suffering from meningitis had simultaneously an acute tick borne encephalitis infection detected by serology. Two patients had only mucosal symptoms (herpangina, aphtae in the buccal mucosa). The viruses isolated from the stool samples of two children were shown to be coxsackie A16.

Both EV 71 and Coxsackie A16 belong to the same enterovirus species (called actually cluster B). It is not surprising that EV 71 which did not cause epidemics since 1978, has reappeared in young children. In contrast to the epidemic encephilitis followed by frequent lethal outcome in 1978, only sporadic mild meningoencephalitis occurred in 2004. Immunization with live poliovirus vaccine was already stopped in Hugary from May 2006. On the basis of our preveious findings this might result in the increased circulation of non-polio enteroviruses.

INDUCTION OF EXTRACELLULAR ß-GALACTOSIDASE (BGA1) FORMATION BY D-GALACTOSE IN THE FUNGUS *TRICHODERMA REESEI* REQUIRES GALACTITOL

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The ability of *Hypocrea jecorina* (*Trichoderma reesei*) to grow on lactose strongly depends on the formation of a single, extracellular GH family 35 β-galactosidase, encoded by the bga1 gene. Previous studies, using batch cultures or transfer of pregrown cells, had shown that this β-galactosidase is induced by lactose and D-galactose, and to a lesser extent by galactitol. Here, we used carbon limited chemostat cultivations to study the induction of β-galactosidase at comparable growth rates. Data show that β-galactosidase induction by lactose, D-galactose and galactitol positively correlates with the dilution (= specific growth) rate, and that galactitol induces the highest activities of β -galactosidase. The induction by D-galactose, but not by galactitol, is impaired in a mutant strain disrupted in aldose reductase xyr1, and which cannot oxidize D-galactose to galactitol. On the other hand, induction by D-galactose proceeds normally in an L-arabinitol dehydrogenase (lad1) delta mutant, which is unable to further catabolize galactitol. These results indicate that galactitol is the actual inducer of β-galactosidase formation on lactose and D-galactose in *H. jecorina*.

ISOLATION OF *ARCOBACTER CRYAEROPHILUS* AND *A. SKIRROWI* FROM HUNGARIAN BROILER SAMPLES

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Surveillance and control of food-borne pathogens is a priority task for food safety. Beside traditional pathogens (*Salmonella*, *Campylobacter*), there are emerging pathogens as well, e.g. *Arcobacter spp.,* which are suspected to be food-borne pathogens associated with chicken meat. *Arcobacter* spp. are related to Campylobacters, but are more stress-tolerant. Three species have been associated with human infections, *A. butzleri*, *A. skirrowi* and *A. cryaerophilus*, thus, they are believed to be potential zoonotic agents, though the spread between animals and humans has not been formally proved. All three species are found predominantly in chicken meat, and high prevalence was reported in chicken meat samples collected at slaughterhouse. Few data are available in the English-language literature on their distribution in chicken-derived field samples, and there are no data at all from Hungary.
The present work investigated a week-old broiler stock. Sixty faecal samples were collected as twelve pools of five droppings according to the sampling protocol for *Salmonella* surveillance. Samples were inoculated onto cefoperazone amphotericin B teicoplanin agar plates and incubated for 48 hours at 20 °C. Colonies suspected to be *Arcobacter* were identified using a multiplex PCR capable of differentiating the above-mentioned three species. This PCR was used for the demonstration of Arcobacters directly from the faecal samples. All samples were tested for the presence of Campylobacters using culturing on the same medium and PCR. Out of the twelve pool samples, eleven yielded Arcobacters on culturing. Ten isolates proved to be *A. cryaerophilus*, one culture proved to be a mixed culture of *A. cryaerophilus* and *A. skirrowi*. PCR detected *A. cryaerophilus* in seven samples, while both *A. cryaerophilus* and *A. skirrowi* was found in four samples. The culture negative sample was also negative by PCR. Interestingly, *A. butzleri* was not encountered. All

samples were *Campylobacter* negative both by culture and by PCR.
To our knowledge, the present work is the first to report successful culturing of Arcobacters from chicken droppings in Hungary. Our results indicate that *Arcobacter* contamination in food may occur, which may have food hygiene and public health implications.
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DEVELOPMENT AND OPTIMIZATION OF A PCR ASSAY SPECIFIC TO *SALMONELLA ENTERICA* SEROVAR INFANTIS

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The gold standard method for the characterization of *Salmonellae* is traditional serotyping. This method is time-consuming and its reliability may be corrupted by various environmental factors, e.g. flagellar antigens may temporarily be lost relatively often. Therefore, a number of recent publications concentrate on the development of PCR assays that may replace traditional serotyping in certain situations. Our aim was to develop a PCR assay that can specifically identify *S. enterica* serovar Infantis differentiating it from other, antigenically related serovars.
We chose the *fliC* and *fljB* genes coding for phase I and II flagellar antigens, respectively, as targets for primer design. We amplified and sequenced the whole flagellar antigen genes of five *S.* Infantis isolated from human and chicken faecal samples. These five strains were genetically distinct as determined by pulsed-field gel electrophoresis and enterobacterial repetitive intergenic consensus sequence-based PCR. The resulting respective *fliC* and *fljB* sequences were aligned together with the only *fliC* and the two *fljB* sequences available in the GenBank. The *fliC* and *fljB* consensus sequences were also compared with corresponding GenBank sequences of other, antigenically related serovars, as well as with highly similar GenBank sequences of other flagellar genes. As the *fliC* gene proved to be conserved among antigenically related serovars, we have chosen the *fljB* gene for further study. Primers were chosen to be unique to *S.* Infantis *in silico*, and three forward and two reverse primers were designed. All compatible primer pairs were tested on a collection of further 19 *S.* Infantis strains from various sources and on 40 other serovariants frequently isolated from human or poultry samples. Antigenically closely related serovars (*S.* Bovismorbificans, *S.* Virchow, *S.* Blockley, etc.) were represented by at least two isolates. Two primer pairs proved to be specific to serovar Infantis, yielding a 727 and a 413 bp PCR product. Both showed a detection limit of 10^5 CFU/ml from pure culture and 10^6 CFU/g faeces from artificially inoculated chicken faeces. Neither of them cross-reacted with other serovariants. In conclusion, both primer pairs proved to be highly specific to *S.* Infantis, being capable of differentiating it from other, antigenically related serovars. As the developed assay targets directly the flagellar gene, it can serve as an identification tool when traditional methods fail due to the temporary loss of flagellar antigens, or it can demonstrate *S.* Infantis from cultures containing more than one serovars.
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A PULSED-FIELD GEL ELECTROPHORESIS PROTOCOL FOR TYPING OF HUNGARIAN *HISTOPHILUS SOMNI* ISOLATES

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Histophilus somni is a significant pathogen of ruminants, causing pneumonia and thromboembolic meningoencephalitis in calves and epididymitis and orchitis in lambs. Despite its importance, to date only a few reports aimed at the investigation of its molecular epidemiology. Application of ribotyping, plasmid analysis and different PCR-based fingerprinting methods has been described. Our aim was to develop a pulsed-field gel electrophoresis protocol for typing of *H. somni*.
Twelve isolates from different sources were used during protocol development and the best protocols were tested on a collection of 61 isolates. The isolates were identified on the basis of culture properties and biochemical activity, and in the case of the twelve isolates used during protocol development, identities were confirmed by rDNA sequence analysis.
To isolate the intact chromosomes, log-phase cells were embedded in low-melting point agarose plugs, and were lysed with sodium-lauryl-sarcosyl and proteinase K treatment. After removal of lysis buffer, plugs containing the isolated chromosomes were digested with the restriction enzymes *Apa*I, *Not*I, *Sma*I and later *Xma*I. Digestion products were separated using a CHEF DRIII apparatus.
*Not*I enzyme did not produce enough digestion products, cutting the chromosome into only two separable fragments. The other three enzymes yielded fragment numbers suitable for strain discrimination, but out of the twelve strains used during protocol development, *Apa*I and *Sma*I digested only five and ten, respectively. Therefore, we have chosen *Sma*I for testing our collection. Chromosomes of 17 of the 61 isolates could not be digested with *Sma*I. We attributed this phenomenon to the methylation of DNA, therefore tried out *Xma*I, which is a methylation insensitive isoschizomer of *Sma*I. This enzyme could digest all seven isolates not digested by *Sma*I. The processing of all 61 isolates with the *Xma*I protocol is currently being performed.
Acknowledgement: This work was supported by an OTKA K62663 grant.

WEST NILE VIRUS CAUSED ENCEPHALITIS IN SHEEP

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In the autumn of 2005, the skull of a four-year old ewe showing nervous symptoms before its death (lagging behind, head hanging, ataxia, teeth grinding) was presented for post mortem examination. The histopathological examination revealed nonsuppurative encephalitis characterized by lymphocytic perivascular inflammatory infiltration in all sections of brain that was accompanied with focal and diffuse gliosis. The bacteriological investigations produced negative results. Rabies and scrapie were ruled out by using the fluorescent antibody test and histopathology, respectively.
Vero and rabbit kidney cells were inoculated with the homogenates of brain tissues and diffuse CPE was observed in both cell cultures after 48-72 hours of incubation. The cell culture supernatant was inoculated into the allantoic cavity of embryonated chicken eggs. The embryos died showing severe oedema and haemorrhages. The isolate did not show haemagglutinating activity. In intracerebrally inoculated suckling mice, encephalitis developed and the mice died.
Viral RNA extract was submitted for RT-PCR assay, the obtained 637 nucleotide long amplification product was sequenced and identified. The isolate belongs to lineage 2 of the West Nile virus and the highest sequence identity was found with a WNV strain detected in a goshawk died in CNS symptoms in Hungary in 2004.
Increased WNV activity was observed in Hungary lately involving avian and human encephalitic

cases. Although a lineage 1 strain, similar to the one introduced to the USA, was also detected in 2003, the current sheep case was caused by the lineage 2 strain, which was first isolated in 2004 and caused encephalitic cases in raptor birds also in 2005 in the same area. The current observations indicate that a tropical African strain of WNV successfully maintained in the moderate European climate, found vectors for its spread and overwintering, and caused fatal encephalitis in sheep, which is not a typical host species of the virus in Africa.
This newly emerging strain is a potential threat to veterinary and human health care.

DESCRIPTION OF NOVEL BACTERIAL STRAINS ISOLATED FROM DIFFERENT INVERTEBRATES

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Animals may have several microbial partners, in many cases some of them can be described as novel taxa. During our studies, bacterial strains were isolated from different animals: *Wohlfahrtia magnifica* (Diptera: Sarcophagidae), *Daphnia cucullata* (Crustacea: Cladocera), *Spirostreptida* sp. (Myriapoda: Diplopoda). During the examination of the taxonomic position of the isolated bacteria based on the sequences of 16S rRNA gene, it became obvious that strains S5 and E43, originating from *W. magnifica*, strains D296 and D28, isolated from *D. cucullata* and strains d9 and d10, originating from *Spirostreptida* sp. represented new lineages of descent. Similarity values to their closest authentic relatives are as follows: S5 and E43 showed 93% similarity to *Schineria larvae,* D296 and D28 showed 95% and 98% similarity to *Microbacterium maritypicum* and the sequences of the strains d10, d9 corresponded to *Arthrobacter pascens* in 96,9%. On the basis of these phylogenetic results, further taxonomic analyses were made. All strains were characterized on the basis of their morphological (Gram staining, motility, electron microscopy) properties, physiological and biochemical characters (determination of optimum growth conditions, utilization of 95 different carbon sources as sole source of carbon with Biolog and API systems, urease activity, nitrate reduction etc.). Chemotaxonomical markers were also determined (description of the fatty acids and polar lipid pattern of the cytoplasm membrane, isoprenoid quinone analysis, determination of cell wall constituents). In addition, molecular examinations (riboprinting, full 16S rRNA gene sequence analysis) were done and G+C values were also determined. The results of investigations confirmed that these strains comprised novel species and can be described as novel taxa.

INVESTIGATION OF STAPHYLOCOCCAL BETA-LACTAMASES BY PROTEOMIC METHODS

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Staphylococcus aureus is one of the most widespread pathogenic bacteria and, because of the frequent occurrence of multiresistant strains, it is among the most dangerous ones causing nosocomial infection. Borderline methicillin resistant *Staphylococcus aureus* (BORSA) strains have MIC values

for methicillin between the values of susceptible and resistant strains; the reason of their resistance is the production of beta-lactamases and methicillinases. Previous investigations revealed that the studied borderline methicillin resistant strains produced „A" type inducible beta-lactamases, which were able to degrade methicillin and of which membrane-bound forms were also present in the cells. Extracellular beta-lactamases of several *Staphylococcus aureus* strains were analyzed by two-dimensional gel electrophoresis. After regenerating the enzymes and applying nitrocephin, a chromogenic beta-lactam substance, beta-lactamases were localized on the 2D gels; and corresponding spots from parallel gels were cut, digested and analyzed by mass spectrometry (MALDI-TOF MS and LC-MS/MS). In all cases, the identified extracellular enzyme was BlaZ. We have experimented with different techniques in order to solubilize the membrane-bound enzymes. Both electroelution and cup-loading were efficient to increase the number of protein spots on the gels, but because of the high loss-rate of proteins during regeneration, we were only able to exclude spots that could not be beta-lactamases.

STUDIES ON BACTERIAL POLYPHOSPHATE ACCUMULATION AND PHOSPHATASE ACTIVITY IN THREE HUNGARIAN SHALLOW LAKES

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Diverse species of bacteria are able to store relatively large amounts of polyphosphate in metachromatic granules. Studies indicate that bacterial polyphosphate accumulation, and polyphosphate kinases, have significant importance in water quality, as phosphorus is the major limiting factor of primary production in freshwater ecosystems. The other significant component of the phosphate- and energy-turnover is the phosphatases, which hydrolyse organic phosphorus compounds to liberate inorganic phosphate. In this study, we report on bacterial species taking part in phosphate turnover with their phosphatase activity and/or polyphosphate accumulation in three Hungarian shallow lakes (Balaton [B]: Keszthely-basin, eutrophic; Tihany-basin, mesotrophic and two hypertrophic soda lakes in Kiskunság National Park [KNP]). Samples were taken from the upper 3-5 cm horizon of the sediment in May and August, 2005. 216 [B] and 210 [KNP] strains were isolated using six different media. The cultures were subjected to cell morphology, biochemical and physiological investigations. On the basis of 30 phenotypical tests, cluster analysis was carried out and a phenotypical dendrogram was generated. Strains with 85-90% similarity (using Simple Matching coefficient) formed clusters. Representative strains were chosen for ARDRA from each cluster, and strains with a unique genotypical fingerprint were identified with 16S rDNA sequence comparisons. More than 90% of our isolates had polyphosphate reserve (this property was examined by Neisser staining). The activity of the organic phosphorus compound dissolving enzymes was tested by using Na-phenolphthalein-phosphate containing agar. The proportion of phosphatase activity was variable (38-75%) and irrespective of the trophic state of the lakes. Most of the strains showed the highest sequence similarity with members of the genus *Bacillus* (*B. simplex, B. megaterium, B. pumilus*), other strains were members of the genera *Flavobacterium*, *Sporosarcina*, *Ensifer*, *Sphingomonas*, *Arthrobacter*, *Rheinheimera*, *Pseudomonas*, *Pseudoxanthomonas*, *Aeromonas.*

PRODUCTION OF SOLUBLE FACTOR(S) BY HUMAN CYTOMEGALOVIRUS-INFECTED MRC-5 CELLS EARLY OR LATE AFTER INFECTION INDUCES THE MATURATION OF HUMAN DENDRITIC CELLS

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Human cytomegalovirus (HCMV) infects a large majority of the population and establishes a lifelong asymptomatic persistence. Seroepidemiological data show some association between HCMV-infection and atherosclerosis. Dendritic cells (DCs) are professional antigen presenting cells playing a central role in the immune response. DCs are concentrated at the predilection sites of atherosclerosis. Monocyte populations were isolated from peripheral blood mononuclear cells of HCMV-seropositive and -seronegative donors by the adherence method and treated with GM-CSF and IL-4 for DC maturation. These cells were then further cultured in conditioned media obtained from human fibroblast cells (MRC-5) infected for 1 (early) or 7-9 (late) days with a HCMV strain (Oslo). This strain was passed only 10 times in MRC-5 cells. The HCMV strain failed to replicate and express immediate early antigens in immature DCs. However, both the early and the late HCMV conditioning medium induced an equally increased ratio of DCs expressing maturation markers (CD40, CD83, CD86 and HLA-DR) on the surface of the cells. HCMV conditioning medium, ultracentrifuged to remove virus particles, exhibited a similarly enhanced expression of DC maturation markers. However, functional maturation of DCs, e.g. antigen processing and presentation, was observed only by treatment of DCs with the late HCMV conditioning medium, as measured by increased incorporation of BrdU in the autologous, CD4+ and CD8+ lymphocytes of CMV-seropositive donors, and by increased IFN-g production of these cells. The early HCMC conditioning medium was also able to induce the functional maturation of DCs, as demonstrated by supplementing this medium with a *Chlamydia pneumoniae* antigen. These results suggest that DCs take up, process and present soluble CMV-antigens produced by neighbouring cells, thus contribute to the local inflammatory process at the sites of the developing atherosclerotic plaques.

COMPARISON OF THE BACTERIAL COMMUNITY OF A MESOPHILIC AND A THERMOPHILIC SLUDGE DIGESTER WITH DNA FINGERPRINTING METHODS

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Nowadays, due to the continuously increasing quantity of organic waste and the rapid decrease of fossil energy sources, the use of new, cost-effective and environmentally sound solutions are required to provide our energy requirements. Anaerobic methanogenesis can be an adequate solution to this problem. It’s well known that under thermophilic conditions biogas production is higher and protein degradation is more effective, but this microbial community is more sensitive to environmental changes than the mesophilic microbial community. Our aim was to compare the microbial communities of a mesophilic (35°C) and a thermophilic (55°C) anaerobic digester.
The sludge samples were taken from the experimental meso- and thermophilic reactors of a volume of 3 m^3, of the South-Pest Wastewater Treatment Plant. Because the plating of anaerobic microorganisms is complicated and time consuming, DNA based methods were applied to explore community structures. Following the isolation of genomic DNA, 16S rDNA fragments were amplified using Archaea and Bacteria specific primers. A clone library was created from these 500-

600 nucleotide long PCR products and the clones were digested with two restriction endonucleases. To gain information about the relative abundance of the clones, a fast and cost-effective method called Terminal Restriction Fragment Length Polymorphism (T-RFLP) was applied and clones assigned to the main TRFs were subjected to sequence analysis and identified with the help of an online database. In the mesophilic reactor, the archaeal microbiota is dominated by *Methanosaeta concilii* and by *Methanoculleus bourgensis* while *Methanosarcina* sp. proved to be marginal. In contrast to the mesophilic reactor, the termophilic reactor was clearly dominated by the *Methanosarcina* sp. Concerning the domain Bacteria, a more diverse community was observed. According to our results, most of the TRFs in both reactors belonged to uncultured clones that were isolated from anaerobic digesters and only two clones were found in both consortia. The facts that the Archaea community in the mesophilic reactor can utilize a wider range of substrates and that the Shannon diversity index calculated from the relative ratios of sequenced clones is approximately two times higher in the case of the mesophilic Bacteria community, suggest that the microbiota of the thermophilic reactor is probably more sensitive to environmental changes.
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CHARACTERISATION OF PRESUMED HYBRIDS OF SACCHAROMYCES SENSU STRICTO ISOLATED FROM THE HUNGARIAN BOTRITIZED WINE, TOKAJI ASZÚ

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The aim of our work was the isolation, identification and molecular characterisation of *Saccharomyces sensu stricto* strains participating in the fermentation of the Hungarian botrytized wine, Tokaji Aszú. For the identification of the isolated strains, we amplified the NS1-ITS4 fragment of the rDNA cluster and studied by RFLP analysis (ARDRA). In the case of seven strains, we observed a mixed ARDRA pattern, typical to *S. cerevisiae* and *S bayanus*. We supposed these strains being hybrids and therefore we performed further physiological and molecular characterizations. These seven presumed hybrid strains can be cultivated at a wider temperature range than the type strains; they grow well at the minimum temperature typical to *S. bayanus* var. *uvarum* (9 °C) and at the same time they grow at the maximum temperature typical to *Saccharomyces cerevisiae* (37 C). The hybrid strains - similarly as *S cerevisiae* – are not able to ferment melibiose, while melibiose fermentation is characteristic to *S. bayanus* var. *uvarum*. The sporulation ability of these strains was good, but the frequency of regular tetrads was very low. The viability of the isolated ascospores was, however, very poor.

The molecular characterization and identification focused on the rRNA gene cluster. Several sequences were amplified with PCR with the application of general primer pairs (NL1-NL4, ITS1-ITS2 and NTS2-ETS) and the amplicons obtained were subjected to RFLP analysis. Digestion of the D1-D2 domain (NL1-NL4 sequence) of the hybrids by *HaeIII* yielded a unique RFLP pattern that was the mixture of *S. cerevisiae* and *S. bayanus* type strains, similar to the case of NS1-ITS4 sequences. The RFLP patterns of the ITS1 region obtained by *Hae*III were identical with that of the *S. bayanus* type strains, while heteroduplex analysis of this sequence indicated homology with the *S. cerevisiae* type strain. The NTS2-ETS fragment digested with *Alu*I yielded the same patterns as in the case of *S. cerevisiae* type strain. If the NTS2-ETS fragments were cut by *Rsa*I, a unique RFLP pattern, different from that of the type strains was observed. These preliminary results suggest that certain yeast strains participating in the fermentation of Tokaji Aszú wine are real hybrids of *S cerevisiae* and *S. bayanus* var. *uvarum*, but further confirmation is necessary.
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IDENTIFICATION OF FUNGI WITH REAL-TIME PCR

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The number of invasive fungal infections has been increasing in immunocompromised patients in the last years. The early detection and the rapid and precise identification of fungi are essential for effective treatment and management strategies. Conventional diagnosis of fungal infections is sometimes slow and variable. For this purpose, a real-time quantitative PCR Light-Cycler assay for the most frequent clinically important fungi have started to be developed based on the study of Min-Chih Hsu et al. [1]. Six fungal species were examined: *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, *Candida guillermondii*. Template DNAs of different species were amplified and detected with real time PCR by employing SYBR Green fluorescent dye. The target sequences for species-level detection were located between the 18S and 28S rDNA. The six species could be discriminated with species specific primers and confirmed by melting curve analyses. So far, our examinations show good agreement of identification by real-time PCR method and biochemical identification (100% of 33 strains). For quantification, 10-fold serial dilutions of *Candida albicans* have been made (10^8 – 100 CFU/ml) and the extracted DNAs were amplified. The limit of the detection with this real-time PCR method was 2 CFU/ml. Therefore our assay allows rapid and sensitive detection of the most common clinically important fungi.

1. Min-Chih Hsu et al.: J Med Microbiol **52**, 1071-1076 (2003).

TAXONOMY OF CYANOBACTERIA AND ALGAE: CHALLENGES OF THE PHOTOTROPHS

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Purple and green sulphur bacteria and purple and green non-sulphur bacteria will not be mentioned in this presentation, as only oxygen producing phototrophic microbes are considered as algae by algologists, because the simplest way to define algae is the following: algae are a diverse group of single- (pro- and eukaryotic) or multicellular plants which produce oxygen through photosynthesis and do not have real roots, leaves or tissues. Algae, as a non-phylogenetic taxonomical group, are in many respects the most diverse group of organisms among plants. Algae of the simplest structure are closely related to bacteria, while the most complicated species, the Charophyceae species strongly resemble sea grass. Depending on the taxonomical approach, algae are grouped into 9-10 divisions, several dozens of classes and tens of thousands of species, most of which live in marine, brackish water or freshwater environments, although terrestrial species are also important.

Algal taxonomy, going back to Linné, changed very little from the middle of the 19th to the second half of the 20th century. Plant taxonomies up to the end of the 1980's faithfully displayed the morphology-based taxonomical approach. New results from microbiological, biochemical, molecular biological and electron microscopical research have lead to a real breakthrough and principally changed the former taxonomies. Based on these new results, from the 1990's on, the former, light microscopy-based systematics of divisions, classes and orders has been replaced by the following features: pigment content, storage material, thylakoid type, cell wall, eye spot, flagella structure, and, in phylogeny, DNA based results. However, this does not imply that today's systematics, or description of new species, is based on clear taxonomical principles.

There are hardly any algal groups where the traditional, morphology-based systematics (which often leads to the creation or regrouping of newer and newer taxa), especially at the species level, or the

culture-based microbiological approach (which often leads to the merging of species) are not at least partly maintained. In the case of cyanobacteria, biochemical and molecular biological features identified on the basis of cultures are considered decisive by microbiologists when distinguishing taxa. Algologists, investigating samples from nature, using morphological variety as criteria in identification, consider the separation of many more taxa justified.

In the case of diatoms, electron microscopical research revealed minute micro-morphological structures of the silica wall, which are indiscernible by light microscopy. This led to the description of hundreds of new taxa, especially at the species and genus levels, often based on the features of a handful of silica frustules without considering any other features of the living organism. There is no consensus yet as to what level of variability, or which morphological features can be considered as a species defining value. Naturally, the principal problem of algal taxonomy is often the lack of a clear species concept, as sexual reproduction is unknown or non-existent in the case of a high number of species, referring here not only to the cyanobacteria.

Challenges of phototrophic organisms are of a complex nature. First of all, it has to be stressed that their oxygen production is of primary importance for life on Earth. However, water pollution of the last decades, the process known as eutrophication, has often caused explosion-like multiplication of algae, causing severe problems not only by the sudden advance of potentially toxic species. The challenge for taxonomists is – while exploring structure and function - to create a „reasonable", natural systematics that also helps to get more acquainted with the role algae play in nutrient cycles.

PREVALENCE OF *CAMPYLOBACTER* CONTAMINATION IN BEEF AND PORK SAMPLES AND HASTELET

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Campylobacteriosis, proved to be an emerging disease in Hungary, as it is worldwide. The National Centre of Communicable Diseases (NCCD) registered 6597 cases in 2005, the highest number since 2001. We found sporadic and familiar cases being the most common. Our previous studies targeted the *Campylobacter* prevalence in poultry, thus the present work aimed the examination of retail meat and hastelet, 43 swab samples were of cattle and 57 of swine origin (n = 100) from nine retail shops. Sawbs were transported in peptone water. Parallel swabs were taken of 21 half pork and 13 beef quarter (34 samples) and transported in Charcoal transport medium.

In the laboratory swabs in charcoal transport medium were incubated directly, whereas with swabs in peptone water Bolton enrichment media (with hemin and supplement; Merck) were inoculated. Cultures were incubated in microerophilic athmosphere at 42°C, for 48 hours. Then cultures were plated on Cefaperazon Charcoal Dezoxycholate Agar and incubated as before. Isolated strains were identified phenotypically, using API Campy (BioMerieux). Out of 57 pork samples 17 proved *Campylobacter* positive, in case of beef 21 were positive (of 43 samples). Charcoal transport cultures were positive in 11 cases of 21 pork samples, and 11 of 13 beef samples. *C.jejuni* was found in 9 samples, *C. coli* in 26, *C. lari* UPTC in one case. Both *C. jejuni* and *C. coli* were found in a liver of swine. Beef was absolutely negative in three retail shops of the nine sampled, and pork was negative in one of them. *Campylobacter* contamination was present in 29,8% of pork and 41,8% of cattle samples. Charcoal transport medium proved available to detect *Campylobacter* infection. This noticeable level of contamination of pork and beef with *Campylobacter*, requires fostering the processing hygiene from farm-to-table, according to HACCP, to prevent campylobacteriosis.

RESULTS OF EU FOOD MONITORING ACTIONS PERFORMED BY NPHMS REGIONAL LABORATORIES, HUNGARY

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Actions of the EU Food Monitoring are aimed at the examination of microbiological safety, in salads and products of pasteurized milk. A total of 1213 kinds of cheese made from pasteurized milk, were examined by standard methods (Methods: *Salmonella* [MSZ EN ISO 6579:2002], *Staphylococcus aureus* [MSZ EN ISO 6888-1: 1999], *Escherichia coli* [ISO DIS 7251: 2001], *Listeria monocytogenes* [MSZ EN ISO 11290-1: 1996/Amd.1: 2004 (E) – ALOA]).
Salmonella were not present in any of the samples, as detected in 25 grams. The CFU limits for *S. aureus* were set as $<10^2$/g (satisfactory); 10^2 - $<10^3$/g (acceptable) and 10^3/g (unsatisfactory). Three retail samples proved acceptable and 2 of them were unsatisfactory. The CFU limits for *E. coli* were set as $<10^2$/g (satisfactory); 10^2 - $<10^3$/g (acceptable) and 10^3/g (unsatisfactory). Five retail samples proved acceptable and 10 of them were unsatisfactory. *L. monocytogenes* was present in five retail cheese samples, detected in 25 g; three ripened soft cheeses contained a CFU exceeding 10^2/g.
The presence/absence of *L. monocytogenes* was investigated in 659 salad samples; 551 of these (83.6%) were negative. *L. monocytogenes* was present in 108 samples (16.4%), nine samples contained a CFU exceeding 10^2/g, 5 samples $>10^3$/g, 4 samples were contaminated with $>10^4$/g *L. monocytogenes*. The monitoring, according to EU directions, extended the control of *L. monocytogenes*. It focused attention on the need of examinations of ready to eat food, like salads. Dairy products have been controlled for years, the examination of salads led to new experiences. The samples, contaminated with *L. monocytogenes,* contained components such as mayonnaise, meat, mushroom and spices.

FREQUENCY OF GENES RESPONSIBLE FOR CELL CYTOTOXICITY IN METHICILLIN-RESISTANT AND METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS* STRAINS

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Panton-Valentine leukocidin does not contribute to the intracellular survival of the examined *S. aureus* strains, but the thermostable endonuclease and the haemolysins (the alpha-, delta- and gamma-haemolysins) participate in it. The low frequency of the beta-haemolysin gene in MRSA strains can explain the high organ persistence experienced in our former study, while the absence of this haemolysin can result in weak or no cytolytic effect. In vitro this can explain the delayed or incomplete development of the haemolytic zone around the MRSA colonies. The Panton-Valentine leukocidin gene (*lukS-PV* - *lukF-PV*) was found only in 4.3% of the MSSA strains. Genes of the alpha- and delta- haemolysins (*hla*, *hld*) were carried by both groups in 100%. The *hlb* gene coding

the beta-haemolysin was found in 33% of MRSA strains and 52% of MSSA strains. The gamma-haemolysin gene (*hlg*) was carried in high percentages, 88% of MRSA strains and 68% of MSSA strains. The haemolysin gamma variant gene (*hlg-v*) was found in 100% of MRSA strains and in 84% of MSSA strains. The thermostable endonuclease gene (*nucA*) was also carried by those strains in which the 23S rDNA was not present. The isolation and identification of the strains were done by classical microbiological methods. Strains that were phenotypically proved to be *S. aureus* were confirmed genetically by the detection of the 23S rDNA. We also determined the presence of the thermostable endonuclease gene (*nucA*). The strains were classified according to the presence of the gene responsible for methicillin resistance (*mecA*) into methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains. We examined 110 MRSA and 94 MSSA strains. The genes responsible for the cellular cytotoxicity were detected by simplex or multiplex PCR. The aim of this study was to detect the presence of genes responsible for the cytotoxic effect of *Staphylococcus aureus* strains isolated from invasive infections using polymerase chain reaction (PCR).
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ANALYSIS OF THE GENETIC VARIABILITY OF CLINICAL *CANDIDA PARAPSILOSIS* ISOLATES IN HUNGARY

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Candida parapsilosis is the second most common yeast species isolated from blood stream infections in several surveys. This species has emerged as an important nosocomial pathogen with clinical manifestations including fungemia, endocarditis, endophthalmitis, septic arthritis, and peritonitis, usually occurring in association with invasive procedures or prosthetic devices. This species is more frequent in bloodstream infections of neonates, in transplant recipients, and in patients who received parenteral nutrition or previous antifungal therapy. It had been frequently associated with catheter-associated candidemia and intravenous hyperalimentation.
Previous studies clarified that *C. parapsilosis* isolates can be divided into three groups, which could be distinguished on the basis of several criteria including randomly amplified polymorphic DNA (RAPD) analysis, isoenzyme electrophoresis, sequences of the internal transcribed spacer (ITS) region of the rRNA gene cluster, hybridization to a fingerprinting probe, DNA relatedness, morphotyping, electrophoretic karyotypes, single nucleotide polymorphisms, mitochondrial DNA sequence differences and biofilm producing abilities. Recently, Tavanti et al. (2005) recognized *C. parapsilosis* groups II and III as separate species, *C. orthopsilosis* and *C. metapsilosis*, respectively, based on multilocus sequence typing studies. The two latter species can be recovered relatively rarely from clinical samples. Besides, *C. parapsilosis* group IV has also been found recently among Brazilian clinical Candida isolates by Iida et al. (2005). In this study, we examined the occurrence of *C. parapsilosis* isolates among Candida isolates collected in Hungarian hospitals, and examined the genetic variability of these isolates using sequence analysis of the ITS region, and RAPD technique.
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IDENTIFICATION AND GENETIC VARIABILITY OF *ASPERGILLUS* STRAINS ISOLATED FROM PATIENTS WITH KERATOMYCOSIS IN SOUTH-INDIA

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Keratitis is one of the leading causes of blindness in India. The problem is prevalent in South-India, which comprises largely of agrarian population. *Aspergillus* species are widespread saprophytic fungi that can cause important diseases in immunocompromised humans. They have long been regarded as important pathogens in eye infections, especially keratitis. *Aspergillus* strains are among the most common organisms causing fungal keratitis in the case of rural agricultural workers. Combating fungal keratitis is of special importance as India harbours the largest agrarian population at risk of developing blindness due to fungal corneal infections. Diagnosis of keratomycosis is one of the most difficult problems encountered by ophthalmologists because of its tendency to mimic other forms of infectious 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. The aim of this work was to identify *Aspergillus* strains isolated from Indian patients with corneal ulcers by sequence analysis of the ITS region and to study their genetic diversity.
Morphological examinations, including studies on culture characteristics and microscopy, have been performed for a total number of 25 *Aspergillus* strains isolated from keratomycosis patients in the Aravind Eye Hospital, Tamilnadu, India between September 2005 and March 2006. Most of the isolates proved to belong to the species *A. flavus*, however, other species of the genus including *A. terreus* and *A. fumigatus* were also represented in the sample, although at much lower frequencies. The initial, morphology-based identification of the isolates was confirmed by sequence analysis of the internal transcribed spacer (ITS) region performed by nucleotide-nucleotide BLAST analysis. The genetic diversity of the isolates was examined based on sequence data and ITS-RFLP patterns; the resulting dendrograms and phylogenetic trees are presented. Our data provide significant contribution to the solution of the fungal keratitis problem by revealing information on the current incidence of *Aspergillus* species in corneal ulcers in South-India.
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FATE OF *AZOSPIRILLUM* INOCULUM SPECIES INFLUENCED BY THE ANTAGONISTIC PROPERTIES OF SOME ALTERNATIVE CARRIERS

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The fate of *Azospirillum brasilense* and *Azospirillum lipoferum* strains selected as potential inoculums on maize (*Zea mays* L) was studied *in vitro*. There were two alternative carriers used, such as composted communal sewage sludge (CS) and an anaerobically digested waste (DW) that is humus manure from the wine-preparation process. Colony-forming units (CFU/ml) of the two *Azospirillum* species were estimated in a four-week-incubation study on selective plates with antibiotic markers both in the sterilised and non-sterile initial substrates according to Angerer et al. [1] and Okon et al. [2]. *Actinomycetes* and micromycetes were isolated from both carriers. Antagonism towards the *Azospirillum* bacteria was investigated by an agar-disc method. Means were calculated and significant differences will be shown in the study.
There was a 20 % initial increase in the *Azospirillum* cell-number in case of each substrate used,

independently of the sterilisation process. This finally enhanced cell-number was maintained for two or four weeks in non-sterile or sterilised environments, respectively. Among non-sterilised conditions, the carriers had a substantial influence on the fate (the estimated colony forming units) of the bacteria. The grape-based humus manure in this respect with the anaerobically digesting process could provide better conditions for the survival of both *Azospirillum* species. This fact was supported by antagonistic studies, which showed that less actinomycete (Streptomycetes spp.) isolates were present against the N2-fixing bacteria in the DW manure. Although two different *Azospirillum* species were used, there were individual differences found mainly in their fate. Among the non-sterile conditions, however, the strain of *A. brasilense* showed a higher compatibility with the DW manure. Results showed that the use of alternative carriers of communal or agricultural origin, when considering the antagonistic properties, can be used as potential organics and carriers of the beneficial N_2-fixers.

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ADVANCED APPLICATIONS IN APPLIED MICROBIOLOGY

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Current methods for bacterial and fungal identification in microbiology laboratories are based on the phenotype of the microorganisms and include the analysis of microbial growth on selective media, selective staining, morphology and the analysis of metabolic behaviour. These procedures have the drawbacks that they cannot be easily applied to organisms that cannot be grown in culture, to slow growing organisms and organisms with biochemical characteristics that do not fit into known genera. The combined availability of PCR/DNA sequencing methods with sequence data from a large number of organisms facilitates the application of these technologies for microbial identification.

The MicroSeq® system is the only commercially available, complete system containing reagents, instruments, software and databases for both bacterial and fungal identification based on rRNA gene sequencing. The 16S rRNA gene, which is highly conserved within a species, can serve as the “gold standard” for identification of bacteria at the species level. Due to the 100% accuracy and reproducibility of the MicroSeq® system, thousands of microbial species, including bacteria, yeasts, moulds and fungi can be easily identified from the bacterial and fungal libraries of MicroSeq® (incl. entries for over 1700 and 1000 species, respectively).

Another method, quantitative real-time PCR technology, offers the direct detection of a range of food-borne and environmental pathogens. Taqman® Microbial detection kits for *Campylobacter jejuni, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus* and *Pseudomonas aeruginosa,* in combination with the PrepMan® Ultra Sample Preparation Reagent and the Applied Biosystems 7300 and 7500 Real-Time PCR Systems with the RapidFinderTM software offer fully-integrated and validated single-vendor solutions with superior performance, reliability and ease-of-use. The overview and advantages of these systems with some recent developments from the BioSecurity area will be shown in our presentation.

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IMPORTANCE OF HUMAN NOROVIRUSES IN HOSPITALIZATIONS – EPIDEMIOLOGICAL AND CLINICAL CHARACTERIZATION OF NOROVIRUS INFECTIONS AMONG HOSPITALIZED CHILDREN IN HUNGARY

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Human noroviruses (HuNoV) are one of the four important agents of childhood or adult viral gastroenteritis. Based on the molecular characterization of the virus, four different genera (Vesivirus, Lagovirus, Norovirus and Sapovirus) of *Caliciviridae* family were recognized. Norovirus and Sapovirus are associated with human gastrointestinal infections. The aim of the study was to investigate the epidemiology and clinical characteristics of human noroviruses detected in children hospitalized with gastroenteritis in Hungary.

A total of 449 stool samples were collected from children hospitalized for gastroenteritis at the "Kerpel-Frónius Ödön" Children's Hospital (Pécs, Hungary) between May 2003 and May 2005. The viral RNA was extracted by TRIzol® methods and tested for norovirus using reverse transcriptase-polymerase chain reaction (RT-PCR). Based on the RT-PCR results, 29 (19 single and 10 co-infections) out of the 449 samples were positive. The average age of the norovirus infected children was 43 months (range: 5-217 months). 18 children were male, while 11 were female. Evidence of winter seasonality (15/29, $p<0.001$) was detected. Most common clinical symptoms were diarrhoea, vomiting and fever. In general, single norovirus infections were less severe than cases where multiple enteric pathogens were identified. The most important difference was seen in the number and duration of vomiting. This symptom was more characteristic among patients with multiple infections. We concluded that norovirus infections cause relatively mild enteric infections, however, co-infections with other pathogens may increase the severity of the disease.

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MICROBIAL CONTAMINATION OF SPICE PAPRIKA WITH SPECIAL REFERENCE TO THE MOULD CONTAMINATION

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Spice paprika is a typical Hungarian product and its powder is widely used in Hungarian cuisine. Last year's scandals (including mycotoxin contamination) drew attention to the microbial safety of the product. Foreign products are often used to improve the colour of Hungarian red spice paprika powders. The microbiological investigations involve the whole chain of production from the field to the final product. Here, the results of identification of mould contamination are presented.

Thirty paprika powders of different origin (28 samples from Hungary, the paprika was grown at different places in 2004-2005; 1 sample from South Africa, 1 sample from Brazil) were investigated for their microbiological quality (mesophilic aerobic total count, mould and yeast, *Listeria* sp.). Water activity of the powders was also measured. To study the overall mould contamination, the ergosterol content of the samples was also measured with HPLC.

The mesophilic aerobic total counts of the samples were 10^3-10^7 CFU/g, the fungal count also varied greatly (<10-10^5 CFU/g), yeast counts were low (<10-10^2 CFU/g). The samples could not be grouped by microbial status. Paprika powder from Brazil had higher mould contamination and higher water

activity than the Hungarian samples. Water activity values of the paprika powder samples were low enough to inhibit mould growth (aw 0.284 – 0.561). Ergosterol contents were 4.2-38.5 mg/g, the highest ergosterol content (75.5 mg/g) was measured in the sample from Brazil, indicating an originally high fungal contamination. There was no correlation between mould counts and ergosterol content, indicating that the overall contamination (including field and storage contamination and fungal growth) of the samples were different.
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INSUFFICIENT NUMBER OF CLASSICAL FEATURES – BUT ARE THERE ENOUGH NUCLEOTIDES? TAXONOMY AND SYSTEMATICS IN MYCOLOGY.

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Fungi represent a major group of living organisms. Up to now, almost 80 000 fungal species have been described, which number is perhaps strongly underestimated. One of the main reasons of this underestimation is the high number of possible cryptic species within the morphologicaly homogenous groups where no differences with classical characters can be detected.
Previously the fungi were grouped among the plants. This is the reason why no independent code of the fungal nomenclature exists. The Kingdom Fungi is monophyletic and well-defined, however, several groups belonging to other eukaryotic kingdoms are handled also as fungi and studied by mycologists. So even the interpretation of the name “fungi” leads to systematical problem caused by the inconsistency of common denominations and scientific terms. Similar situation can be found in the case of some fungal groups, like the smut fungi or yeasts, which are no monophyletic groups at all. This presentation addresses some problems and examples of the taxonomy and systematics of “true fungi”.
Three main approaches could be distinguished within the theory and practice of the identification of the fungal species and taxa: the morphological, the biological, and the phylogenetic species concept and recognition. In several cases, the different approaches resulted in discrepancy while other examples showed concordances between the results of the molecular and classical methods. A quite frequent problem was using characters, which were unsuitable for identifying monophyletic groups for taxon delimitation. Several morphological characters used previously in taxonomy and systematics turned out to be phylogenetically non-informative, however others (like subcellular characters, ultrastructural features) might bear important phylogenetic information.
There is no doubt that the advance of DNA based molecular methods and the computer technique revolutionized the mycological taxonomy and systematics. Theoretically any heritable differences between two natural taxa, could be detected with this method. Nevertheless, the approach still has numerous theoretical and practical problems. The pioneer works of the subject focused on the nuclear ribosomal RNA gene complex and the great majority of the molecular studies have been still based on the analyses of different segments of this region. Although the number of studies using different loci (e.g. protein coding genes) or more than one locus, has increased, the analyses of the concordance of multiple gene genealogies are still infrequent. The studied markers not necessarily give enough information, the resolution of the phylogenetic trees might be poor, and branches, particularly the basal, “old” braches, could have low statistical support. The main international phylogenetic projects (AFTOL, Deep Hypha) try to subject these problems.
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IMAIA, A NEW GENUS TO ACCOMMODATE THE HYPOGEOUS *TERFEZIA GIGANTEA* (ASCOMYCOTA)

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The hypogeous ascomycete *Terfezia gigantea,* formerly assigned to the Terfeziaceae in the Pezizales, was described by S. Imai in 1933 from a specimen collected near Sapporo, Hokkaido, Japan. Since then, the fungus has been collected in additional regions of Japan as well as in the Appalachian Mountains in the eastern part of North America. Its habitat of mesic deciduous forests plus its peridial and spore morphology differ strikingly from other *Terfezia* species*,* which occur in xeric to desert habitats of the Mediterranean rim and Western Asia. Our preliminary analysis of the ITS sequence of two American specimens of *T. gigantea* showed that it could not belong to *Terfezia.* Accordingly, we conducted a comprehensive molecular study based on North American and Japanese herbarium materials. The SSU, ITS and partial LSU regions of the nrRNA gene were amplified and sequenced. A species-specific primer pair targeting the ITS region was designed to avoid amplification of fungi potentially contaminating the herbarium specimens. The sequences were used for phylogenetic analyses with GenBank sequences of species from representative families of the Pezizales. The morphological characteristics were studied by light microscopy (peridial structure, asci, spores) and by TEM (mature spores).
Altogether 20 herbarium specimens were studied – 13 were collected in Japan and 7 in North America. The ITS sequences of four North American specimens were identical, and only one base differed in the ITS-2 region of the two Japanese samples. Altering differences of the gene regions between the North American and Japanese specimens' result from their different evolutionary rate. The differences were 0,23% on the SSU, 4,11% on the ITS-1, 1,94% on the 5.8S gene, 6,25% on the ITS-2 and 0,78% on the LSU. No morphological differences could be found between the Asian and American specimens, all of which showed the general characteristics of the species. The species unambiguously separates from the genus *Terfezia* in the phylogenetic analysis. *T. gigantea* clustered with *Leucangium carthusianum* and grouped into the family Morchellaceae. This clear generic separation supports the description of a new genus, named *Imaia* after the author of the species. This new genus is a new example of Eastern Asian and Eastern North American disjunct distributions.
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RIBOSOMAL DNA RFLP ANALYSIS AND HETERODUPLEX MOBILITY ASSAY FOR THE DISCRIMINATION OF FLOATING FILM-FORMING *SACCHAROMYCES CEREVISIAE* STRAINS

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Development of floating yeast film during aging of the botrytized wine, Tokaji Szamorodni, plays an important role in the typical aroma production. This film is similar to the "flor" that is formed during the aging of sherry wines. Our aim was to find a correlation between the two yeast films by the application of molecular techniques.

Fernández-Espinar et al. [1] found that the RFLP analysis of the 5.8S-ITS rDNA region allows the differentiation of "flor" yeasts. Flor-forming yeast strains exhibited different restriction patterns from the non-film-forming *S. cerevisiae* strains due to the presence of a 24 bp deletion located in the ITS1 region. Heteroduplex mobility assay is a simple and rapid tool for the detection of small differences between DNA sequences. Therefore, we supposed that it would also be suitable for the detection of sequence divergence in the rDNA of different yeast strains.
We performed RFLP analysis using the ITS1-ITS4 primer pair followed by restriction enzyme digestion (*Hae*III, *ScrF*I and *Cfo*I) of 26 film-forming, 2 planktonic wine yeast strains and 2 laboratory *S. cerevisiae* strains. According to the RFLP analysis, the 26 film-forming strains grouped into 4 different clusters, which clearly separated from the laboratory and one of the planktonic wine yeast strains. The three flor ("sherry") yeast strains belonged to 3 different clusters, which indicated the rDNA molecular polymorphism of this type of strains. Only one of the flor strains showed the restriction patterns published earlier [1]. The 23 film-forming strains isolated in the Tokaj region belonged to two highly similar clusters.
Heteroduplex mobility assay has a size limit of up to 800 bp, therefore we amplified a shorter region of the 5.8S-ITS region than before. Using the ITS1-ITS2 primer pair, we expected an approximately 440 bp long PCR product, which contained the suspected deletion. First, we tested the self-renaturation of the PCR products. Twenty-two of the 26 film-forming strains resulted in heteroduplex formation in this case, presumably because most of them contained two types of rDNA sequences. Heteroduplex formation between the remaining 4 film-forming strains indicated that one of them contained a heterologous rDNA sequence, while the other 3 strains were probably homologous for this sequence. Our results indicate that the floating biofilm forming capability of certain wine yeast strains is not limited to the sherry flor yeasts, but development of a yeast film during aging may occur in other types of wine, too.

1. Fernández-Espinar, M.T. et al.: *Antonie van Leeuwenhoek* **78**: 87-97 (2000).

GENETIC ANALYSIS OF A NON-CYTOPATHIC BVDV STRAIN ISOLATED IN HUNGARY

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Since the late 1950's, the control of the problems represented by the bovine viral diarrhoea (BVD) infections in dairy farms of Hungary caused increasing difficulties. In 2001, following the serological survey of the stock, an eradication campaign was launched in Tass, which was based on screening of the newborn calves by PLA tests and on the continuous removal of the persistently infected animals that play the most important role in the endemic infection as virus reservoirs. During the program lasting for more than one and half years, several BVDV strains were isolated from the stock. One of these, designated as Tass584, was genotyped and analysed during the investigations described in this work in 2005. Certain genomic regions of the Tass584 strain were amplified following reverse transcription and polymerase chain reaction (RT-PCR), the PCR products were sequenced and aligned to sequences deposited in the international GenBank. The sequence analysis has proved that the Tass 584 strain belonged to the 1f subtype of the Vilcek genetic system.
Our results indicate that the Tass584 strain is the non cytopathogenic biotype of the H3142 cytopathogenic strain, and the two biotypes were isolated independently. The analysis also revealed that a 42 nucleotide–long-insertion (the marker of cytopathogenic ability), which is present in the H3142 cytopathogenic strain, is missing from the NS2-3 region of the Tass584 strain. Further

comparative studies and epizootiological investigations are necessary to support this presumption.

EMERGENCE OF THE FILAMENTOUS FUNGAL OPPORTUNIST *TRICHODERMA LONGIBRACHIATUM* IN HUNGARY

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Several data were published in the last decade about the clinical importance of the filamentous fungal genus *Trichoderma*, indicating that *Trichoderma* species - especially *T. longibrachiatum* - may occur as potential opportunistic pathogens, mainly in immunocompromised patients. In this study, we present the first data about the occurrence of *T. longibrachiatum* as a fungal opportunist in Hungary.
A *Trichoderma* strain was isolated from the blood culture of a 3-year old child with acute lymphoblastic leukaemia. As the patient received previous nystatin treatment, the isolate was considered a culture contaminant. The C-reactive protein (CRP) and procalcitonin (PCT) values of the patient did not suggest a fungal infection, thus no systemic antifungal treatment was administered.
Another *Trichoderma* strain was isolated in the same paediatrics department from the stool of a 15-year-old child with non-Hodgkin lymphoma. CRP- and PCT-levels of the patient were high and the stool was mucous, suggesting the possibility of a fungal infection. The patient was on Diflucan for 3 days before the stool sample was taken; this was changed to intravenous Mycosyst after 2 days, then to intravenous Fungisone after an additional 2 days, when the culture results became available.
A further *Trichoderma* strain was isolated from the sinus lavage sample of a rhinosinusitis patient. The isolate may be responsible for the symptoms of the patient, as the involvement of *Trichoderma* in fungal rhinosinusitis of immunocompetent hosts is known from the literature. Molecular identification of the 3 clinical isolates, performed by sequence analysis of the internal transcribed spacer region and the 4th and 5th intron of the translation elongation factor 1a gene, revealed that all of them belong to the species *T. longibrachiatum*. The sequence data were used to construct phylogenetic trees in order to determine the discrete taxonomic positions of the isolates. Culture characteristics and antifungal susceptibilities were also examined. Based on the three isolations of the fungal opportunist *T. longibrachiatum* in a single clinical microbiology laboratory within a year, the lack of previous reports about its emergence may not be due to the lack of occurrence, but rather to the lack of recognition. It can be suspected that *Trichoderma* is frequently considered a culture contaminant, because its potential role as an opportunistic pathogen is not yet widely recognized. Thus, the frequency of the occurrence of *T. longibrachiatum* in clinical samples may be much higher than can be estimated based on literature, where only the fatal and very serious cases are reported.
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BIODIVERSITY OF *TRICHODERMA* STRAINS OCCURRING IN CLINICAL SPECIMENS

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Trichoderma spp. are known as cosmopolitan soil inhabiting filamentous fungi. Certain members of the genus are emerging as causative agents of opportunistic infections in humans. Here, we present different phenetic and phylogenetic approaches applied for the taxonomic characterization of clinical *Trichoderma* isolates.

Twelve clinical *Trichoderma* isolates were involved in the experiments. Molecular phylogenetic analysis was performed for the sequences of the internal transcribed spacer 1 and 2 (ITS1 and 2) regions of the rDNA cluster and for the 4th large intron of the gene encoding the translation elongation factor 1-alpha (*tef1*). RFLP patterns of mtDNA were generated by *Bsu*RI and *Hin*6I. Phenotype profiles were examined by isoenzyme analysis of 7 enzyme systems with cellulose-acetate electrophoresis (CAE) and by carbon source utilization arrays performed on BIOLOG FF microplates. Based on morphological characters, the 12 clinical *Trichoderma* isolates were originally identified as members of 3 species from section *Longibrachiatum*: *T. longibrachiatum* (5), *T. pseudokoningii* (3), *T. citrinoviride* (1); and 2 species from section *Trichoderma*: *T. viride* (2) and *T. koningii* (1). However, the ITS barcode identification by *TrichO*KEY 1.0 (www.isth.info) revealed that all of them belonged to the duplet of species *T. longibrachiatum*/*Hypocrea orientalis*. Phylogenetic analysis of *tef1* sequences showed that 11 strains belonged to the clade of *T. longibrachiatum*, while one was attributed to *H. orientalis.* The examination of further, non-clinical isolates indicated that the *tef1* marker clearly separated these two species. Restriction fragment length polymorphism of the mitochondrial DNA revealed 7 and 10 different patterns with *Bsu*RI and *Hin*6I, respectively, resulting in 4 groups on the dendrogram, while cellulose-acetate electrophoresis separated the strains into 4 distinct electrophoretic types. BIOLOG Phenotype Microarrays were performed for all clinical and a series of non-clinical isolates from several closely related species. Comparisons were made at 9 time points and at 3 temperatures, however, physiological shifts specific for clinical isolates could not be detected.

Our results support that fungal opportunists belonging to the genus *Trichoderma* are restricted almost exclusively to section *Longibrachiatum*. Beside sequence analysis, the methods of CAE, mtDNA RFLP and BIOLOG Phenotype Microarrays also proved appropriate for studying the biodiversity of *Trichoderma* strains occurring in clinical specimens.

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GREEN MOULD DISEASE OF OYSTER MUSHROOM IN HUNGARY AND TRANSYLVANIA

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Green mould disease caused by *Trichoderma aggressivum* has been extensively reported for *Agaricus*

bisporus. However, in recent years the oyster mushroom (*Pleurotus ostreatus*) was also increasingly affected by green mould. In the last two years, *Trichoderma* outbreaks have been reported to cause serious losses in the commercial production of *P. ostreatus* in Korea, Italy and Hungary.
A series of *Trichoderma* strains have been isolated from *Pleurotus* substrate samples derived from a Hungarian substrate producing and *Pleurotus* growing company, as well as from *Pleurotus* farms in Transylvania that are importing substrate bags from Hungary. The fungal isolates were identified to be co-specific with a yet undescribed phylogenetic species, *Trichoderma* sp. DAOM 175924. Internal transcribed spacer sequences 1 and 2 of the strains belonging to this species proved to be highly similar or identical with those of *Trichoderma* pathogens of *P. ostreatus* in Korea, indicating that the green mould disease of *P. ostreatus* in Hungary and Transylvania is due to the same *Trichoderma* species. The isolates of *T.* sp. DAOM 175924 can be divided into two types on the basis of an A/C transversion at position 447 in ITS2. Interestingly, all the representatives of this species that were isolated from the winter wheat rhizosphere of South Hungarian agricultural fields contain A at this position, while the isolates deriving from Hungarian and Transylvanian *Pleurotus* substrate samples belong almost exclusively to the other, "C" type. Although the isolates of *T.* sp. DAOM 175924 are found world-wide in soil or on decaying wood in the USA, Canada, New Zealand, Europe, Iran and China, the results of the present study cannot rule out that the *Trichoderma* strains causing green mould in Transylvania have been introduced with the substrate bags imported from Hungary.
A large-scale sampling program has been initiated in order to identify the possible sources of green mould infection. The closest environment of a Hungarian substrate producing and *Pleurotus* growing company has been examined. This included sampling of the wheat straw deriving from different locations, the soil within the area of the company, the substrate bags inoculated with different strains of *P. ostreatus* as well as the flow-down water, which is the by-product of the prefermentation phase of wheat straw. The most frequently occurring species in the flow-down water samples were *T. harzianum* and *T. atroviride.* Further progress of the project is presented and discussed.
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MODIFICATION AND REVERSAL OF RESISTANCE WITH SELECTED NON-ANTIBIOTICS AND THEIR STEREO-ISOMERIC ANALOGUES: AN OPTION FOR TREATMENT OF SEVERE RESISTANT AND INTRACELLULAR INFECTIONS IN THE FUTURE?

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Overcoming the growing resistance of pathogenic microorganisms to many drugs requires a critical examination of new treatment possibilities. Traditional combination therapy with classical antibiotics/chemotherapeutics might be failing in treating severe resistant and intracellular infections. Combinatory use of antibiotics and various neuroleptic compounds and their stereoisomeric analogues appears promising as a novel method to counter microbial resistance.

PLANT CELL WALL DEGRADING ENZYMES IN THE ENZYME SYSTEM OF *THERMOBIFIDA FUSCA* – ENZYMES FOR INDUSTRIAL APPLICATIONS

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Thermobifida fusca is a compost inhabiting actinomycete. On the grounds of the results of the team of D. B. Wilson at Cornell University, *T. fusca* became the model organism of the thermophilic aerobic cellulose decomposers. In addition to the taxonomic investigations within the *Thermobifida* genus, I have been focusing on the *T. fusca* strain TM51 cellulose-hemicellulose degrading proteins for the past six years. During this period, we have purified and characterized new hydrolases used by *T. fusca* for cellulose-hemicellulose degradation: one extracellular endoglucanase (Cel5B) belonging to the Glycoside Hydrolase (GH) family 5, two endoxylanases (XylA, XylB) and an endomannanase (ManA) from GH family 10, an acetyl xylan esterase (AxeA), an intracellular GH2 beta-mannosidase (ManB) and a GH42 beta-xylosidase.
We cloned and sequenced the structural genes encoding these enzymes. His-fusion protein technique was used for mass enzyme production needed for biochemical characterization. We also studied the mechanisms that regulate *T. fusca* cellulases and showed that cellobiose and, more effectively, lactose were the inducers and that cellulase synthesis was repressed by any easily consumable carbon source.
On the basis of our results, we conclude that the cellulase system of *T. fusca* consists of seven extracellular cellulases and an intracellular beta-glucosidase, whereas the hemicelullase system of this model organism contains four endoxlanases, a beta-xylosidase, an acetyl xylan esterase, an endomannanase and a beta-mannosidase.
To facilitate further industrial use of these thermostable and robust enzymes, we solved the problem of overproduction of beta-xylosidase and endoglucanase Cel5B in Streptomyces host using the twin-arginine translocation mechanism. This type of enzyme production would be a prerequisite of the industrial application of these proteins e.g. in the paper industry (biobleaching) or animal feed production (feed additive enzymes).

CLONING, RECOMBINANT EXPRESSION AND PARTIAL CHARACTERIZATION OF A THERMOSTABLE FE-SUPEROXIDE DISMUTASE (SOD) FROM THE EURYARCHAEON *THERMOPLASMA ACIDOPHILUM*

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Metabolic biochemical reactions constantly generate in vivo free radicals that can cause damage to almost all biomolecules. The superoxide anion has been shown to be the most readily generated and common intermediate of oxidative stress processes in cells. Superoxide dismutases (SODs) catalyse the conversion of superoxide radicals to molecular oxygen and thus play a major role in reducing cumulative oxidative damage in different cell compartments both in aerobic and anaerobic cells.
Thermoplasma acidophilum is a facultative anaerobic, thermophilic and acidophilic euryarchaeon growing optimally at pH 1.5 to 2 and at 55 to 59°C. In order to adapt to the oxygen-containing environment, this organism possesses an oxygen inducible superoxide dismutase system as we demonstrated by 2D-induction experiments. We purified and partially characterized an iron-containing superoxide dismutase (SOD; EC 1.15.1.1) of this archaeon. The gene encoding this enzyme has been cloned and sequenced. The SOD from *T. acidophilum* could be expressed in *E. coli* RIPL competent host. Both the native and the heterologously overproduced protein have remarkable thermal stability: after boiling for 10 minutes, we measured no loss of enzymatic activity. Based on this character, we could perform SOD activity staining on samples separated on PAGE-gels using a modified photochemical method. We concluded from the zymogram analysis that SOD of *T. acidophilum* may form not only the already known homotetramers but higher macromolecular complexes as well. Inhibition experiments verified the Fe-subtype of the *T. acidophilum*-SOD based on its H_2O_2 sensitivity and CN-stability. By the use of the highly standardized Fluka SOD-kit - based on the xanthin-oxidase inhibition measurement- we measured a 0.07 Unit/microgramm activity. The

enzyme subunit is composed of 205 amino acid residues accounting for a relative molecular mass of 23.799. The calculated isoelectric point of the enzyme is 6.3, which is almost identical to the measured value obtained by Free Flow experiment.
By PFAM structure analysis we could identify the SOD-specific loop connecting the alpha N-terminal domain and an alpha/beta C-terminal domain. The comparison of Ta-SOD and other known protein sequences from this group of enzymes interestingly revealed a higher homology (55%) to the SOD of *Aquifex pyrophilus,* a eubacterial species, than to a related enzyme (32%) of the taxonomically closer crenarchaeon *Sulfolobus solfatarius*.

OPTIMIZATION OF FERMENTATIVE *ACTINOBACILLUS PLEUROPNEUMONIAE* ANTIGEN PRODUCTION

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Actinobacillus pleuropneumoniae is the causative bacterial pathogen of chronic porcine pleuropneumoniae and is the source of severe economic losses worldwide. The effective protection against this Gram negative, aerobic bacterium is vaccination. The major protective factors can be divided into 2 groups: the somatic (outer membrane proteins, lipopolysaccharides, capsule etc.) and the extracellular antigens. While the somatic antigens mostly induce serotype specific immune response, the application of the extracellular Apx toxins provides serotype independent protection. However, the current anti *A. pleuropneumoniae* vaccine of Ceva-Phylaxia Veterinary Biologicals Co. contains both somatic and toxoid antigens, the renewal of the product is in progress as a result of the application of newly developed specific analytical tools (ELISA and cytotoxicity measurements). As a first step, the optimization of the upstream process was performed by maximizing the cell mass and Apx production and minimizing the unwanted endotoxin content. Due to technological development, long debated issues were clarified, such as whether the Ca^{2+} concentration mainly influenced the production rate or the cytotoxic activity of the toxins or whether the dissolved oxygen level affected the production rate of Apx toxins and lipopolysaccharides. Also, sensitivity of some Apx toxins for proteolytic degradation were detected. By the application of the recently gained information, the increase of the production level of some Apx toxins became possible, while lipopolysaccharide concentration was significantly decreased in the fermentation broth.

GENETIC IDENTIFICATION OF NEWCASTLE DISEASE VIRUSES ISOLATED IN HUNGARY

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Since September 2005, over fifty Newcastle disease virus (NDV) strains have been isolated from various domestic and wild birds in the Central Veterinary Institute. More than half of the viruses were obtained from carcasses that were sent to the laboratory during influenza virus surveillance. In order to evaluate their epidemiological significance, phylogenetic analysis based on partial nucleotide sequence of the fusion protein gene was performed.
The vast majority of strains belonged to the group of pigeon-type (genotype VIb) NDV and mainly derived from wild and feral pigeons, but surprisingly, some of them came from other species including water birds (such as swans and wild ducks). A fraction of the viruses were isolated from

chickens associated with outbreaks in the spring. All these were placed in genotype VII, whose progenitors turned up in epizootics in the 1980s and are prevalent in the Far East.

CLONING AND MOLECULAR ANALYSIS OF THE CATALASE 1 GENE FROM THE OPPORTUNISTIC PATHOGEN *RHIZOPUS ORYZAE*

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The aim of our study was the identification and molecular analysis of genetic elements in connection with the virulence of opportunistic pathogen zygomycetous fungi. Thermophilic members of the genus *Rhizopus*, especially *R. oryzae,* are considered as the main causative agent of zygomycosis. The risk factors of this type of mycosis can be immunosuppression, diabetic ketoacidosis, deferoxamine treatment to manage an iron or aluminium overload, leukaemia, and burn injuries. High-risk population has been growing continuously in recent years, which drew attention to these mycoses. Moreover, these fungi have an intrinsic resistance against the recently available antifungal agents, therefore the improvement of new treatments and preventive strategies are highly desirable. Catalases provide protection from reactive oxygen species produced by neutrofilic granulocytes of the human immune system. Neutropenia is a considerable risk factor in zygomycoses, however, genetic feature of these enzymes have been scarcely studied until now.
3 catalase homologous sequences were found in the *R. oryzae* genome database (*Rhizopus oryzae* Sequencing Project, Broad Institute of Harvard and MIT 2004, http://www.broad.mit.edu). The presumed catalase 1 was chosen on the bases of comparisons with known fungal sequences and specific primer pairs designed for PCR. In order to investigate the effect of the gene inactivation on fungal pathogenicity, a vector suitable for site directed mutagenesis was constructed. The vector carries the *R. oryzae pyr4* gene encoding OMP decarboxylase, which provides the selection of the catalase deficient transformants. This construction was transformed with PEG mediated method into a uracil auxotrophic strain of *R. oryzae*. Gene disruption was proved by PCR technique. The catalase activity of selected mutants was tested and a significant decrease could be detected. Further gene expression studies with the wild type and mutant strains are in progress.
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EMERGENCE OF EPIZOOTIC STRAINS OF NEWCASTLE DISEASE VIRUS

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Present relationships of Newcastle disease virus (NDV) and its hosts are used to infer events in the past. In addition to reservoirs that are taken as evolutionary units of virus–host associations, common ancestors are also used as tools in the reconstructions of history.
One type of relationship encompasses wild water-bird species and biologically harmless (in veterinary terms, avirulent) viruses that constitute the primordial reservoir existing today. The genesis of these viruses is supposed to have taken place here, therefore their relationship reflects an evolutionarily primitive state and an indefinably long period of time. The other type of association is represented by NDV in poultry whose history can not go back earlier than the time of the domestication of chicken (5000 years ago). It is hypothesized that the chicken reservoir was first seeded from the primary reservoir with primitive NDV strains, then pathogenic strains (evolutionarily derived state) emerged

in the secondary host. Both colonisation and transition to virulent form could have taken place several times in the period prior to vaccination. Analysis of the genetic composition of genotype I and II viruses that were prevalent in the early epizootic period (that is, prior to immunization) provided evidence for the occurrence of at least two independent colonisations and avirulent → virulent transitions. Introduction of vaccination, however, was tantamount to a change in host (from naïve to immune chicken), which resulted in the collapse of previous strategies and old virulent NDV have gradually become extinct. Apparently, a novel strategy of generation and survival of virulent variants emerged, which entailed recovery after a bottle-neck event and rapid diversification of virulent virus (virulent → virulent). From the evolutionary point of view, it is noteworthy that tertiary reservoirs were only established by seeding novel hosts (cormorants, pigeons and geese) with viruses of recent genotypes (V, VI and VII, respectively).

COMPARISON OF *BIPOLARIS* ISOLATES USING MOLECULAR AND BIOCHEMICAL MARKERS

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Members of the genus *Bipolaris* (Ascomycota, Euascomycetes, Pleosporales, Pleosporaceae) are dematiaceous, filamentous fungi cosmopolitan in nature associated primarily with grasses, but they can also be isolated from plant debris and soil. The plant pathogenic species cause diseases in a wide range of gramineaceous hosts. A few species are also significant as human and animal pathogenic fungi: *Bipolaris* is one of the causative agents of phaeohyphomycosis.
Traditionally, examination of the conidial morphology (including colour, shape, size, numbers and distribution of septa) has been used to describe the species. However, species identification within the genus is relatively difficult due to the possible inconsistencies within isolates, variable cultural conditions, or loss of ability to produce conidia. In the present study, several isolates of *B. maydis*, *B. zeycola*, *B. oryzae*, *B. sorokiniana*, *B. sacchari* and *B. nodulosa* from different origins were compared using various methods, such as analysis of rDNA sequences, secondary metabolite production and carbon source assimilation.
Phylogenetic trees inferred from the nucleotide sequences of the complete ITS (internal transcribed spacer) region suits the results of an earlier analysis based on *Brn1*, a melanin synthesis gene [1]. Species in these trees formed exclusive clusters clearly separated from one another. *Bipolaris* isolates were also assayed for their ability to utilize 70 different substrates as a single carbon source. Significant variability was revealed both on inter- and intraspecific level by this method. Secondary metabolite production was examined by thin layer chromatography; unique patterns could be detected for each species.
All three systems proved to be useful for the determination of markers of distinctive value at a species level. Methods for rapid species identification were established.

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CLASSICAL AND MOLECULAR METHODS FOR LABORATORY DIAGNOSIS OF DANGEROUS PATHOGENS

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Tularaemia, brucellosis and anthrax are the most important zoonotic diseases widely distributed in both humans and animals throughout the world. In the National Reference Laboratory for Dangerous Pathogens, we have introduced rapid and reliable methods to immediately detect and identify rare pathogenic agents such as *Bacillus anthracis, Francisella tularensis* and *Brucella* species. Rapid identification would also be a requirement in the case of a potential bioterrorism attack.
Although the conventional culture and staining techniques are currently the gold standards for the isolation, detection and identification of these agents, these lengthy and complicated assays can provide only preliminary results. The complete laboratory diagnosis takes 72 hours to 2 weeks and requires BSL2 and BSL3 work practices. The novel, rapid detection methods replace the selective and differential culturing steps with nucleic acid amplification techniques. Several sample preparation methods are currently under investigation and there are many commercially available kits for the purification of nucleic acids. In the lecture, we will focus on rapid, automatic and manual sample preparation methods.
F. tularensis is one of the most infectious pathogenic bacteria. There are two subspecies: type A and type B. The routine diagnosis of human tularaemia is usually based on the culture or a positive agglutination test (titre >1:80) that develops in the second week after infection, or on the onset of typical clinical signs (such as in the case of brucellosis). We have developed a polymerase chain reaction (PCR) – based assay for the fopA gene (outer membrane protein) to detect both type A and type B *F. tularensis*. Brucellosis is one of the most serious livestock diseases. The culture requires specialized BSL2/3 laboratory circumstances, but we are able to detect the *Brucella* DNA by a normal PCR targeting a genus specific, 31 kDa cell surface protein gene. Anthrax is also a zoonotic disease caused by *B. anthracis*. Routine culture and biochemical tests are useful for the identification of the *Bacillus*, but definitive identification may take 24-48 hours.
We had the possibility, to make high-level characterization of 8 unknown isolates (soil, blood, tissue, white-powder, biothreat agent etc.) in the U.K., at an Army Medical Research Institute under tightly controlled conditions. During the detection assays, we could compare commercial culture and classical techniques with real-time PCR assays. The virulent *B. anthracis* strain contains 2 large plasmids coding specific non-toxic proteins. The pXO1 carries the protective antigen (pag), and lethal oedema factor (lef); the pXO2 carries the capsule genes (capA, capB, capC). Highly virulent strains can be characterized by carrying both of these plasmids. Considering the limitations of current diagnostic procedures, PCR may become a useful tool for early diagnosis. We found that the complete microbiological result needs classical and molecular methods to be performed in parallel.

ORGANIC AND INORGANIC AMENDED SANDY SOILS STUDIED BY CULTURE DEPENDENT AND INDEPENDENT ENZYMATIC METHODS

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Low-fertility sandy soils generally require organic or inorganic additives to improve the growth and development of agricultural crops. The application of bentonite and fermented liquid manure, etc. may enhance soil fertility through the activation of the soil-biological processes. Our aim was to find the most appropriate parameters suitable for the indication of soil fertility. 0, 5, 10 15 and 20 t/ha bentonite was added to the upper 20 cm layer of soil in a plot experiment, seeded by vetch (*Vicia villosa* L.) and rye *(Secale cereale* L.) co-cultures. In 2005, treatment of 20 t/ha bentonite was combined with fermented liquid manure and sweet corn (*Zea mays* L). Abundance (CFU/g soil) of culturable microbial groups (fast-growing r strategist heterotrophs, slow-growing k strategist oligotrophs and spore-forming l strategist Bacillus) was determined by the method of Angerer et al.

[1]. Microbes isolated from plates were identified with API. Soil enzymatic activities were also measured (invertase, catalase or dehydrogenase were assessed three-times during the vegetation period) (dehydrogenase activity was calculated according to Hungarian Standard MSZ-08-1721/3-86; invertase activity was estimated according to Mikanová et *al*. [2]; Hungarian Standard (MSZ-08-1721/4-86) was used for estimating the catalase activity).

The abundance of some strategic microbial groups correlated positively with the various amendments in the slightly acidic sandy soils. According to the API tests, there were distinct microbial communities recorded in the soils, showing the positive effect of both the organic and inorganic amendments. Microbial total counts, however, are less sensitive in some cases to show the real modification of the microbial parameters in the supplemented soils on a short-term basis. Among the culture-independent techniques, the invertase and catalase enzyme activities proved to be the most applicable parameters. These enzymes were found to be positively correlated with the soil amendments and soil fertility, measured as the dry-matter production of the fodder plant or the yield of the maize. Mikanová et al. (2001) also reported invertase as a good indicator of the soil-biological activity in heavy-metal pollution. Further studies are required to find the functional role of the culturable microbes and specific isolates or the particular microbial communities in those low-fertility sandy soils in the Nyírség region, Hungary.

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EPIDEMIOLOGY AND ANTIFUNGAL SUSCEPTIBILITIES OF *ASPERGILLUS* STRAINS ISOLATED FROM PATIENTS WITH KERATOMYCOSIS IN SOUTH INDIA

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Corneal infections of fungal aetiology are very common and represent 30% to 40% of all cases of culture-positive infectious keratitis in India. The aim of this work was to study the epidemiological features of keratomycosis caused by *Aspergillus* species in South-India and the susceptibilities of the isolates to various antifungal drugs.

Twenty-six *Aspergillus* strains isolated from keratomycosis patients in the Aravind Eye Hospital between August 2005 and February 2006 were involved in this study. 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. The most frequent occupation of male patients was farmer with 53%, while that of female patients was housewives (78%). Corneal trauma has been 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. Regarding other ocular diseases, cataract has been diagnosed in the case of 6 patients (23.1%), while previous ocular surgery was performed in 4 cases (15.4%). Among the further possible predisposing conditions, systemic diseases like diabetes mellitus (26.9%) and hypertension (19.2%) proved to be frequent. The antifungal drugs applied for the therapy of patients included natamycin, itraconazole, ketoconazole, econazole, clotrimazole and amphotericin B. Therapeutic keratoplasty had to be performed in the case of 9 patients (34.6%).

In India, the limited availability of antifungal drugs and the lack of response lead to blindness in a high number of patients. As the knowledge on antifungal susceptibilities is mainly based on the

western literature, a survey of the local susceptibility patterns is vital, therefore the E-test method modified for moulds has been applied for the determination of minimal inhibitory concentration (MIC) values of amphotericin B, fluconazole, itraconazole, ketoconazole and voriconazole towards the *Aspergillus* strains isolated from corneal infections. 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 as follows in μg/ml: 0.064 - 4 for amphotericin B, 0.25 - 1 for ketoconazole, 0.064 - 32 for itraconazole and 0.064 - 1 for voriconazole. The presented data provide ophthalmologists important information about the epidemiological features of *Aspergillus* keratitis in South India, as well as with useful ideas for the apparent initiation of treatment and the selection of the appropriate antifungal agents.

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RECENT DEVELOPMENTS IN BACTERIAL SYSTEMATICS AND TAXONOMY

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The traditional natural or biological species concept does not seem to be applicable to bacteria (prokaryotes), although the exact identification of a strain can often be decisive in questions of life and death. Consensus species definitions helped taxonomists for decades until the early 1970's, when increasing attention was paid to the delimitation of the key taxon (the species) by techniques targeting the blueprint of life, the chromosomal DNA. The 16S rRNA oligonucleotide cataloguing technique, in combination with the DNA-DNA hybridisation and the DNA-rRNA reassociation methodology resulted in the description of the three domain system of life (*Archaea, Bacteria, Eukarya*). An even increasing number of "main phylogenetic lines of descent" has been described. With the adaptation of most different advanced molecular biological techniques, and the application of the methodology of analytical chemistry in the chemotaxonomy of bacteria opened a new era in microbial ecology and applied microbiology. Hundreds of yet uncultured organisms (clones, genospecies) have been unravelled. From 1990 onwards, the results of genome sequencing demonstrated the presence of core genes coding for the cellular design in all organisms, giving the systematists new ideas to attempt a new genetic species concept.

FULL-GENOMIC ANALYSIS OF HUMAN G6P[14] ROTAVIRUS STRAINS FROM A GLOBAL COLLECTION

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Serotype G6P11[14] rotaviruses are rare human pathogenic strains, which were described for the first time in 1988 in Sicily. Subsequent detection of these strains has been reported from Australia (1993, 1996, and 1997), Hungary (1997), Belgium (1997) and the Italian peninsula (2005). The scattered geographical and temporal appearance, the antigenic and genetic relatedness of their outer capsid proteins (VP7 and VP4) to rotavirus strains of ruminant and lapin origin, and the apparent low ability

to spread in the local human population raised conjectures about the origin and epidemiology of G6P[14] rotaviruses. This prompted us to initiate extensive sequencing and phylogenetic analysis on these rare viruses. A total of five strains were selected for the study, including the prototype strain PA169 (Sicily, 1988) and Australian and European strains isolated more recently (MG6, Australia, 1993; Hun5, Hungary, 1997; B10925, Belgium, 1997; and 111/05-27, Italy, 2005). The entire genome sequences (~18.5 Kbp for each strain) were determined using gene specific primers designed for the 5' and 3' ends of each genome segment. Sequence comparison and phylogenetic analysis was performed using cognate genes of human and animal strains. The overall sequence identity of these five G6P[14] strains was 87.9% to 96.2% at the nucleotide level and 95.5% to 98.6% at the amino acid level. However, there were considerable differences when individual genome segments were compared (e.g., 74.7% to 98.6% for NSP1 *vs.* 95.8% to 98.1% for NSP5 at the nucleotide level). This relatively high sequence conservation among geographically and temporally unrelated G6P[14] strains (mainly seen in the genes VP4, VP6, VP7, NSP3, NSP4, and NSP5) suggests that they might have originated from a common, but unknown progenitor. On the other hand, the finding that all five strains shared almost complete sequence identities in a variety of genes (e.g., VP2, VP4, VP6, VP7, NSP1, NSP3, NSP4, and NSP5) to non-G6P[14] strains, including both human and animal (primarily bovine) rotaviruses, implies that their subsequent evolution should have implicated occasional reassortments of cognate genome segments involving locally co-circulating and unrelated rotavirus strains. Interestingly, although the Hungarian strain Hun5 and the Belgian strain B10925 were detected in 1997 alike, no clear molecular epidemiological linkage could be identified between these two strains, as the overall genome sequence identity was only 90.7% at the nucleotide level (range: 76.5% for NSP1 and 96.3% for NSP5). On the other hand, B10925 (1997) and the Italian strain 111/05-27, isolated in 2005, showed an overall sequence identity of 96.2% (range: 90.7% for VP3 and 99.4% for NSP3). This large-scale full-genome analysis study on human G6P[14] rotaviruses provides new insights into the evolution of these strains.
Nonetheless, several questions have remained unanswered, and we believe that a better understanding of the origin and evolution of G6P[14] rotaviruses will be facilitated when more complete genome sequence data are available from both human and animal rotaviruses, such as the G6P[14] and G8P[14] strains recently detected in ruminants.

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VIRAL EXAMINATION OF SEWAGE SAMPLES IN BARANYA COUNTY, HUNGARY

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Sewage plants collect and treat communal and industrial wastewater, so viral content in sewage samples may be used as a good indicator of viruses present in a community. Routine procedures to monitor viruses in water samples have not yet been developed. Enteric viruses can persist under environmental conditions, and they pose a great risk to the public by contaminating natural and drinking water resources. The aim of the study was to detect the four main viruses involved in gastroenteritis of man, such as human astroviruses (HAstVs), adenoviruses (HAdVs), rotaviruses (HRVs) and caliciviruses (HuCVs) from raw and treated wastewater samples, and to qualitatively

determine the treatment efficiency of different sewage treatment techniques.
A total of 36 paired sewage samples – both raw (influent) and treated (effluent) – collected from 4 sewage plants ("A", "B", "C" and "D") in different areas of Baranya County were tested for the major enteric viruses using the polyethylene-glycol method for concentration and the guanidinium thiocyanate-silica procedure for extraction of nucleic acids. Reverse transcription-polymerase chain reaction with virus-specific primer pairs was used for amplification and amplicons were purified and sequenced to test the specificity of the assay.
The most commonly identified viruses were HAdVs (94.7%), followed by HAstVs (55.3%), HRVs 50% and HuCVs 10.5%. On the basis of virus detection rates in the effluent water compared with the influent wastewater, the treatment efficiencies varied from 5% to 57.9%. HuCVs were the most sensitive and HAstVs were the most resistant for any type of sewage treatment procedure. Where corresponding laboratory data were available, no correlation between the presence of viruses and the commonly used indicators of faecal pollution (faecal coliforms) was found.
This study demonstrates for the first time the results of a longitudinal monitoring of enteric viruses in raw and treated sewage samples by using a PCR-based laboratory diagnostic procedure in Hungary. The high detection rate of astroviruses and adenoviruses we encountered in this study contradicts the small number of HAstV and HAdV infections among children admitted to hospitals in Hungary (4% and 10%), suggesting that HAstVs and HAdVs circulate at a higher frequency in the Hungarian population than expected from clinical virological studies. Our results demonstrate that sewage treatment procedures for wastewater have only a limited effect on the viruses we examined, and new techniques of depuration are needed. Our data suggesting that human adenovirus may seem as an indicator of viral pollution is worth further attention.

DETECTION OF GROUP C ROTAVIRUSES BY REAL-TIME PCR IN SEWAGE SAMPLES IN BARANYA COUNTY, HUNGARY

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Group C rotaviruses cause gastroenteritis in all age groups and are responsible for occasional outbreaks in small and large communities. Inapparent infection with group C rotavirus is also common. Although the virus may shed in high quantity into the environment through faeces, there are no published data about the occurrence of group C rotaviruses in wastewater. Detection of these pathogens in environmental specimens may shed light on their epidemiologic features. Viral pathogen detection in water – especially in sewage – is difficult and nowadays PCR is used worldwide to detect these viruses. The aim of the study was to adapt a rapid, reproducible, specific and sensitive fluorogenic RT-PCR technique in order to monitor the activity of group C rotaviruses and determine their quantity released into the environment. We also tried to evaluate the relative efficiency for viral clearance of different treatment procedures used in sewage treatment plants by comparing the viral load in raw and treated sewage specimens.
35 raw and treated sewage sample-pairs collected in 2005 from four sewage plants in different areas of Baranya County were tested using the polyethylene-glycol method for concentration, the guanidinium thiocyanate-silica procedure for extraction of the viral RNA and SYBR Green-based quantitative PCR amplification of the cDNA transcripts of the VP6 gene.
Detection limit of the method was 3 copies of plasmid DNA. When this value was applied to the nucleic acid extracts of sewage samples, the detection limit was found to be 4500 genome copies per 1 L wastewater. Altogether, 54.3% (19 of 35) of raw wastewater samples contained detectable

quantities of viruses, while 22.9% (8 of 35) of treated samples contained at least 4500 copies/l. With a single exception, influent samples always contained a higher viral load ($7.05x10^3$ to $9.44x10^7$ vs. $1.65x10^4$ to $1.36x10^7$ copies/L). The average clearance efficiency of group C rotaviruses varied among different sewage plants (range, ~35% to 93%). Group C rotaviruses were detected only from February to July and in November and December, exhibiting a seasonal pattern of activity in the community. To our knowledge, this is the first report on the detection of group C rotaviruses from wastewater samples. The high prevalence of group C rotaviruses suggests that surface waters contaminated with sewage may be the source of infections with group C rotaviruses. Because of the increasing number of identified water-borne outbreaks in recent years throughout the world and their recognized impact on human health, more efficient sewage treatment procedures are urgently needed.
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ARCHAEA COMMUNITY CHANGES AT TCE BIODEGRADATION IN MICROCOSM EXPERIMENTS

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Halogenated hydrocarbons are unfortunately common groundwater contaminants in Hungary. At present, these types of contaminations are commonly treated with physico-chemical remediation; however, these methods are rather expensive, require serious effort, and take place ex situ. Therefore, it is eminent to develop an effective method of in situ biological remediation.
Since in certain cases methanogens are effective competitors of the dechlorinating communities, they can influence the effectiveness of bioremediation. The analysis of the Archaea community with conventional microbiological techniques is extremely difficult; thus, the diversity of the Archaea community in microcosm experiments was investigated using molecular biological fingerprinting techniques. Contaminated samples derived from different regions of Hungary. Chemical analysis was performed according to standards; the following parameters were examined: pH and concentration of CH_4, TOC, SO_4^{2-}, Cl^- and halogenated hydrocarbons. Genomic nucleic acid was isolated and a section of the 16S rDNA was amplified. The phyletic diversity of the PCR products was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal Restriction Fragment Length Polymorphism (T-RFLP). Moreover, clone libraries were created from microcosm samples to identify species.
Methane production provided indirect evidence for the presence of methanogens in the microcosms. The Archaea community of the samples was dissimilar by DGGE and T-RFLP techniques. In the case of TCE polluted soil samples, the diversity of the community increased with higher pollutant concentration and lower redox values. In active microcosms (TCE degradation), surprisingly simple Archaea community composition was characteristic. The clones were detected as "methanogens with low similarity,, such as: Methanomicrobia, Methanosarcinales and Methanopyri.
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CHARACTERIZATION OF DEHALOGENATING BACTERIAL COMMUNITIES VIA MICROCOSM EXPERIMENTS

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Halogenated organic compounds are produced industrially in large quantities and represent an important class of environmental pollutants. Bacteria evolved several strategies for the enzyme-catalyzed dehalogenation.
In this study, analysis of chlorinated aliphatic compounds (CAHs) and water-chemical parameters was 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. In laboratory microcosm experiments, we used organic industrial by-products as electron donors. Two molecular methods were used to characterize the bacterial community structure of the samples: clone library construction and terminal restriction fragment length polymorphism (T-RFLP) analysis.
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 the 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. *Dehalococcoides* was detected only with specific primers, from groundwater samples J18; J18/2; K7 and a soil sample T29, but we were unable to detect it in groundwater sample T10. In this sample, we were able to detect the presence of *Aneromyxobacter dehalogenans.* Sulphate reducing bacteria were stimulated, FeS production was visible in the microcosms. In the microcosms of samples J18/2, J18 and K7, trichloroethene was almost completely degraded to cDCE and VC, and in microcosm of sample J18/2, ethene was also detected. Our results indicate that the used electron donor is a promising and effective substrate to help remediating a wide range of CAH concentrations at different CAH contaminated sites.
Acknowledgement: This research was supported by the grant GVOP-3.1.2004-05-0407/3.0.

THE ROLE OF SYSTEMATICS AND TAXONOMY IN THE RESEARCH OF APICOMPLEXA AND MYXOZOA PARASITES

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Apicomplexa is a phylum abounding in species that develop both in vertebrates and invertebrates. Some of the species belonging to this phylum cause important diseases in humans and animals. In these days, due to their pathogenic importance, haemosporidians (*Plasmodium* spp.) and cyst-forming coccidians are the most intensively studied parasites; *Eimeria* spp. causing animal coccidioses tend to receive less attention, and relatively little is known about the numerous representatives of gregarinid and haemogregarinid parasites. The taxonomy of apicomplexans, which used to be based exclusively on morphological studies, has become complemented with molecular methods in which primarily the structure of the 18S rDNA sequence serves for the differentiation of species and taxonomic groups. As an example, molecular analysis revealed that cryptosporidia, earlier considered to be closely related to eimeriid coccidians, showed a higher degree of genetic relatedness to gregarines or plasmodia. In Hungary, the use of molecular methods provided new scientific results first of all on *Cryptosporidium* spp., but promising new results were obtained on fish-parasitic coccidia as well. Unpublished data suggest that the DNA sequences of *Goussia* and *Eimeria* spp. infecting fishes are well distinguishable from those obtained from coccidia of warm-blooded animals. Molecular studies on other apicomplexan groups, such as *Plasmodium, Toxoplasma* and *Neospora,* are mostly performed as part of routine diagnostic work in Hungary.

Most myxozoans are fish parasites, and much fewer species have been detected in amphibians, reptiles and invertebrates. New data obtained in recent years prompted general changes in the knowledge of both their systematic position and development. Myxozoans were classified as protistans for many years but molecular studies have proved that they are metazoans. Based on the use of ribosomal DNA (rDNA) sequence analysis, some researchers classified myxozoans as bilateralian nematodes, while others related them with cnidarians, and they stressed their close relation to *Polypodium hydriforme*. It was also demonstrated that the development of myxosporeans was accomplished through the involvement of two alternate hosts. During this life cycle, the myxospores developing in fish infect oligochaetes and, less frequently, polychaetes. Fish became infected through actinospores developing in these worms.
Life cycle studies undoubtedly proved that parasites earlier classified to the phylum Actinosporea correspond to developmental stages of Myxosporea spp. Therefore, the class Actinosporea is synonymous with the class Myxosporea. In the case of some myxosporean species, such as *Myxidium leei,* a direct transmission without alternate hosts is known to exist. In the latter case, vegetative stages of the parasites are transmitted to the new hosts in a way similar to that known for infection with *Toxoplasma gondii*.
The application of molecular methods brought decisive changes in the systematics of Myxozoa and in studying the genetic relatedness of different species. Until now, the 18S rDNA sequences of almost 100 myxosporean species have been identified and deposited in GenBank. Studying the genetic markers of different species enables researchers to replace the long-lasting and complicated experimental infections in the identification of myxospore and actinospore pairs. At present, the phylum Myxozoa comprises of two classes, Myxosporea and Malacosporea. Bryozoans are the alternate hosts in the latter class, which includes *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease of salmonids.

THE ROLE OF INFLAMMATION IN CHRONIC *PSEUDOMONAS AERUGINOSA* LUNG INFECTION IN RELATION TO CYSTIC FIBROSIS: TARGET FOR TREATMENT

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The inherited disease Cystic Fibrosis (CF) is primarily characterized by recurrent lung infections, and the majority of adult CF patients have acquired chronic *Pseudomonas aeruginosa* lung infection, which is the dominating cause of the observed premature death in CF. *P. aeruginosa* reveals a biofilm mode of growth in microcolonies and is thereby protected from the host response and antibiotics. The host response is commonly accepted as domination by numerous polymorphonuclear neutrophils (PMNs) surrounding the microcolonies. However, the massive PMN infiltration gradually degrades the lung tissue instead of eliminating the bacteria. The immunological response is characterized by pronounced antibody response, which results in the formation of immune-complexes and contributes to the destruction of the lung tissue.
Since chronic *P. aeruginosa* lung infection is almost impossible to eliminate, the treatment strategies can be divided into 1) prevention of the chronic lung infection, 2) regular IV courses of antibiotics to decrease the number of bacteria and thereby reduce inflammation, and 3) modulate the inflammatory and immunological response to reduce the loss of lung tissue.
In the presentation, different strategies to manipulate the harmful inflammation and immune response will be presented. The presentation is based on observations in CF patients as well as experimental work of our mouse model of chronic *P. aeruginosa* lung infection.

ABOUT PATHOGENETIC SIGNIFICANCE OF *SALMONELLA INFANTIS* IN BROILERS

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Salmonella reduction of broilers has been initiated in 2001 to be focused first on S. Typhimurium and S. Enteritidis in Hungary, resulting in a decrease of these serovars. However, in 2001 – 2005, an increase in the occurrence of S. Infantis has been observed (with some increase in human cases of *S.* Infantis infection). In order to asses the pathogenetic significance of S. Infantis in broilers, we have tested *in vivo* and *in vitro* pathogenetic characteristics of representative strains of this serovar using a one-day old chick infection model, and Vero-, and chicken embryonic fibroblast culture models for invasiveness and for interleukin stimulation. Furthermore, we used PCR to test representative strains for major virulence genes of Salmonella.
Preliminary results suggested that S. Infantis strains had comparable *in vitro* invasiveness to those of other group C strains, but lower than that of S. Typhimurium and S. Enteritidis. The stimulation capacity of S. Infantis for IL-8 production on these cells was generally of medium-high level. Results on orally infected one-day old chicks indicated more expressed caecal colonization ability and a lower level organ invasiveness of S. Infantis strains than those of the so called invasive serovars. S. Infantis strains lacked *spvC* but all possessed SPAI-1., -2- and -5 -related virulence genes, while the indicator genes of SPI-3., -4 and the TTSS effector (sopE1) were rare or absent. Based on these investigations and on international data, it seems that S. Infantis does have an emerging significance, primarily in broilers, with relatively lower invasiveness and better colonizing capacity, for which the epidemiological and molecular data can be partially explanatory.
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PHYLOGENETIC RELATIONSHIPS AMONG SPECIES OF *COPRINUS*, SECTIONS GLABRI AND AURICOMI BASED ON NRDNA ITS REGIONS

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Critical revision of the taxa *Coprinus*, sections Glabri and Auricomi (Parasola Redhead, Vilgalys & Hopple) have been carried out. Specific limits of the phylogenetic analysis based on ITS regions of the nrDNA repeat were identified and critical revision of herbarium specimens was performed.
Our results of morphological examinations fail to support the traditional species concept and the importance of certain features, such as colour and size of fruiting bodies, and habitat. We found that the most useful morphological characters were spore size and shape. Type studies of almost all taxa have been carried out. These showed that *Coprinus leiocephalus* and *C. galericuliformis* are later synonyms of *Pseudocoprinus lacteus* A.H. Smith; and *Pseudocoprinus besseyi* should be synonymized with *C. auricomus*. Results of ITS sequence analysis correspond well with most morphologically distinct taxa, but the limits between *Pseudocoprinus lacteus* and *C. schroeterii* need the sequence analysis of further specimens. We think that the extreme morphological variability exhibited by *Pseudocoprinus lacteus* may in fact be due to the existence of more species. Furthermore, both molecular and morphological results suggest a close relationship between *Psathyrella conopilus* and *C. auricomus*, both basal to the remainder of the group. This points to the derivation of coprinoid mushrooms from psathyrelloid ancestors and thus contradicts the philosophy of Redhead S. A. et al's taxonomy (Taxon **50**, 203-241, 2001).

INVESTIGATION OF THE DISSEMINATION OF A NDV VACCINE VIRUS WITHIN THE BODY OF SPF CHICKS BY REAL-TIME RT-PCR

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One-day-old and six-week-old SPF chicks were vaccinated intraocularly with one dose of an apathogenic NDV vaccine (Cevac VITAPEST® L), then organ samples were taken postvaccination (at day PV 3, 5 and 9). Strain specific MGB probes were designed for the fusion protein of NDV, and real-time RT-PCR was performed on the collected samples. Relative quantification was done using standard dilutions of the vaccine virus with known infective titre (EID50).
The distribution kinetics was different in one-day-old and six-week-old chicks and higher overall titres were detected in one-day-old chicks. In day-old animals after inoculation the target organs were the trachea and the lung (respiratory system) then the virus propagation moved towards the digestive system (pancreas, caecal tonsil, cloacal swab) and kidney. In six-week-old animals the initial propagation was much weaker in the respiratory system (comparing to the one-day-old chicks) then the virus reached higher titre in the digestive system (caecal tonsil, cloacal swab).

SOME RESULTS OF MEASURING THE UNCERTAINTY OF BIOLOGICAL METHODS IN THE ACCREDITATION PROCESS IN OUR MYCOLOGICAL LABORATORY

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The types of methods we demonstrate are of discrete data type (cf. measured data; e.g. MIC [Minimal Inhibitory Concentration] by E-test, or giving a single name based on special characteristic properties as accuracy of identification methods [cf. observed data determine the result in a complex method] by AUXACOLOR, and measuring error of a procedure like temperature control).
The standard deviation (sd) of MIC values in lognormal distribution was sd = 0.463 (n = 168) measured by an individual person and sd = 0.590 (n = 56) measured among several persons. The F distribution F (55,167) = 1.628* demonstrated different ($P<5\%$) readings of MIC values by persons individually and the readings of MIC values among several persons, however, from a scientific point of view, the values were acceptable as equivalent readings.
It is well known that in the case of biochemical sugar assimilation tests on the basis of the variable tests in AUXACOLOR2, the possible number of code variations should be 2276, however, there are only 316 variations given in the AUXACOLOR2 test specification. The reason for the missing code variations can be explained both by random selections or selections by systematical approaches. Unfortunately, there were species that were completely ignored in the test table according to the code variations. In an identification system, estimation of an error depends on the results of biochemical tests and selected biochemical tests and selected species. On the basis of a binomial probability function, the estimation can be calculated in a special case. Better set of biochemical tests should be proposed for the identification of yeast species. Temperature control is essential at temperature dependent processes like growth of microbiological objects in an incubator. It is also important to estimate not only the actual temperature values in temperature controlled areas, but also the temperature range (min. - max. values) in the sample. A model which is used for the thermometer time constant can be used in “holding temperature between limits in media”.
In an accreditation process of microbiological methods, the validation and the calculation of

uncertainty of the method is required. However, as very simple is to make something acceptable by statistical solutions (distribution types: normal, lognormal, binomial, etc.; estimating error of mathematical functions: adding, subtracting, multiplying, dividing, etc.; determining the deviation for multi processes, etc.) for mathematicians as difficult is to decide the adequate type of method for special cases like biological behaviour. This question is demonstrated on the analysis of carbon source utilisation patterns.

NEW DEVELOPMENTS IN THE FIELD OF GLYCEROL UTILIZATION

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We have been working on new enzymatic processes for glycerol utilization for years. Glycerol is a renewable resource, because it arises during biodiesel production from plant oil. Through the extension of biodiesel production, probably huge amounts of glycerol will be formed, which can not be fully used up by the cosmetic industry, so it is very important to work out alternative processes converting this renewable by-product into valuable articles.
The aim of our work was to produce the three key enzymes of the coupled enzymatic glycerol to 1,3-propanediol and dihydroxyacetone bioconversion by fermentations. 1,3-propanediol is widely used e.g. in inks and medicines to its largest utilization in the polymer industry, and dihydroxyacetone is needed in the cosmetic industry as self tanning agent. 1,3-propanediol is produced industrially by synthetic methods, while dihydroxyacetone production is carried out by microbial fermentations.
We have already presented our first successful bioconversion experiments using coenzyme regeneration as well as a mathematical description that could simulate either the glycerol-1,3-propanediol pathway or the by-product formation via the dihydroxyaceton-acetic acid pathway. This mathematical model shows that the original goal of our work – namely to produce 1,3-propanediol and dihydroxyacetone from one substrate simultaneously (glycerol) with three enzymes and simultaneous coenzyme regeneration in a membrane reactor – can not be reached with the crude enzyme solution of *Enterobacter aerogenes* culture broth. Unfortunately if we can somehow avoid by-product formation, the ATP (re)generating steps will also be eliminated as a consequence, though ATP is needed for the glycerol-dehydratase (E.C. 4.2.1.30) key enzyme reactivation. We found another enzyme source of *Clostridium butyricum VPI 1718,* of which GDHt is not under suicide inactivation with coenzyme B12, but use SAN as coenzyme. We made fermentation runs with this bacterium, and tested its enzymes for glycerol bioconversion. These enzymes were more stable, could be reused, and by-product formation could be eliminated with the addition of an appropriate inhibitor.

THE MICROBIOLOGICAL RELATIONS OF DISINFECTION

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Consumers have novel requirements on foodstuffs due to modern food technologies and new shopping behaviour of consumers. Basic and obligatory requirements of foodstuffs are: safety, long shelf-life, small number of viable bacterial cells and hygienic production. In recent years, the majority (approximately 77%) of *Salmonella* epidemics has been proved to be food-borne. Each year 10-30% of citizens, even in the developed countries, are at risk of food borne diseases.
Foodstuffs are excellent nutrients also for micro-organisms, which is why the use of effective

disinfectants is indispensable during food-production. Unfortunately like all human interventions, the use of antimicrobials has its environmental impact.
Nowadays, resistant human pathogens are also emerging in the food industry. They are resistant to different antimicrobial agents, such as antibiotics and disinfectants, and cross- resistance(s) can emerge. Biofilm formation is an effective self-defence mechanism of micro-organisms against different antimicrobial effects. If the cells are organized into biofilms, they are more resistant than those suspended in planktonic forms. This phenomenon generates serious problems in the food industry, as well as in human medicine. The lecture draws attention to this phenomenon, describes critical points and shows future possibilities.

PHENO- AND GENOTYPIC CHARACTERIZATION OF RECENT *SALMONELLA INFANTIS* ISOLATES FROM HUMAN AND ANIMAL SOURCES

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Salmonella Enteritidis and *Salmonella* Typhimurium are the two most important agents causing salmonellosis worldwide. Mostly due to the national *Salmonella* eradication program, the number of animal infections as well as human diseases caused by these two serotypes significantly decreased in the past few years. In contrast, the number of infections and diseases caused by the serotype S. Infantis, both in the fields of human and animal health (especially in broiler chicks) started to increase in the last years. In order to get information on the genetic diversity in the background of the human and animal diseases caused by S. Infantis, altogether 132 isolates from 2004-2005 [originating from the faeces of broiler chickens (n = 29), chicken meat (n = 31), raw and processed meats of other animals (pork, beef, etc.) (n= 16) and human faeces (n = 56)], were analysed by different pheno- and genotyping methods. All isolates were phage typed and their antibiotic susceptibility was tested by disk diffusion method. Plasmid profiling and macrorestriction analysis by PFGE using Xba1 enzyme have been done on all isolates. The presence of the class 1 integrons, the tetA gene as well as some virulence genes such as spvC, sipA and sopB was also tested with PCR. The examined isolates showed considerably high similarity in respect to their examined traits. 70% of the isolates belonged to the phage type 213 or 217. All animal isolates and all human isolates with a few exceptions were resistant to nalidixic acid. 80% of the isolates belonged to the streptomycin-tetracycline-nalidixic acid resistance type. These isolates uniformly had a 1.0 kb integron, the sequencing of which revealed the presence of the aadA1 gene, which codes for streptomycin-spectinomycin resistance. Most of them also possessed a tetA gene. Although 74% of the isolates had one >170 kb large plasmid, none of them possessed the spvC gene, which codes for Salmonella plasmid virulence in other serotypes. On the other hand, all isolates contained the sipA and sopB genes located on pathogenicity islands of the bacterial chromosome. PFGE profiles of the isolates showed that all of them belonged to one large genetic cluster with an 80-100% similarity. Based on the phenotypic and genotypic characteristics of the above isolates, it is concluded that a S. Infantis clone became widespread in the Hungarian broiler industry and in the food chain, and appeared in the human population during the last few years.
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MYCETOLOGIA CRIMINALIS (FORENSIC MYCOLOGY AND MYCETOLOGIST IN CRIMINOLOGY)

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Since the foundation of Mycenae (murder, escape, divine sign – mushroom = mykes – myketes; conj. = myketo) in history, mycetology was in contact with crime. In the Archaic halucinogenous moulds were used in shamans' ceremony. In the middle ages poisonous Agarics served as "operational" aids (e.g. Borgias). In the New age e.g. arsine produced by *Scopulariopsis brevicaulis* and ethanol served as toxins. The forensic mycologist has a great responsibility, their reports are often decisive: guilty vs. non-guilty! In the first case the Institute of Criminal Technology (ITC) asked us to prove the contact of the suspect's cardigan with the stolen goods (winter salami sausage [WS], already dropped by the thief. With contact sampling specific WS moulds, *Scopulariopsis flava* and *Penicillium camemberti* were isolated from the cardigan, while intact, verticillate, complex conidiophores of *P. camemberti* were detected on it, having been transferred onto it by contact. Thus culprit is guilty, and was condemned. In case II. a controversy erected on the blood alcohol level of a cadaver. At repeated checking more ethanol was detected in a cooled control. With microscopy budding yeast cells and pseudomycelia were observed in the samples. Culture resulted in the isolation of *Rhodotorula mucilaginosa,* and *Candida colliculosa,* anamorph of the *fermenting* wild yeast *Torulaspora delbrueckii* (known from soil and fermenting juices, even in cold circumstances). Total conversion of 0.108 % blood sugar would give 0.055 % ethanol in blood (110 % of the tolerance limit of 0,05 %). Weaker conversion would be compensated by the alarm reactions (adrenalin) elevating starting sugar. In case III. the suspected operator of an illegal "wine" production by fermenting artificial mash (water, sucrose, baker's yeast) supplemented with synthetic aroma could be detected, by identifying the microbes on his jacket, polluted by the mash. In spite of the methodological arsenal used, yeast strains cold not be isolated from the textile samples of the jacket. Fortunately, on Sabouraud glucose agar colonies of Penicillia were grown, that were identical with those of old mash. In old mash yeast cells die and sink, but strict aerobe polluting moulds grow on the surface.

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APPLICABILITY OF THE *AGROBACTERIUM*-MEDIATED TRANSFORMATION IN ZYGOMYCETES

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PEG-mediated transformation, based on the complementation of auxotroph markers, is worked out for some Zygomycetes. The drawback of the system is that a mutant with the required deficiency has to be isolated. Moreover, in these studies, transformants maintained the foreign DNA almost exclusively in autonomously replicating form.

The aim of the present study was to adapt the *Agrobacterium*-mediated transformation (ATMT) method to *Backusella lamprospora*, a Zygomycete, closely related to *Mucor*. This fungus proved to be sensitive to hygromycin B used in the transformation experiments as a selection marker. *Agrobacterium* transfers T-DNA to the host cell, which integrates into the nuclear genome at a random position. This method can integrate heterologous sequences (as the *hph* gene in this study) into the genome, without the need for flanking homologous regions in the transforming vector to direct the homologous recombination.

The transforming vector, pNYI8 contained the hygromycin B phosphotransferase and the green fluorescent protein genes under the control of *Mucor gpd1* regulator sequences. The presence of the *hph* and *gfp* genes in the genome of the transformants was detected by PCR. The *hph* and the *gfp* genes could be amplified directly from the spores of the transformant colonies. The transformation event was also proved by fluorescent microscopy: transformant colonies showed intensive fluorescence. Single-spores of the transformants were repeatedly transferred to selective medium in order to investigate the stability of the introduced gene. A gradual decrease in hygromycin B resistance was observed: growth of the transformants was slower on selective media and the detection of the introduced gene was more difficult. Mitotic instability was observed also in *R. miehei* transformants obtained with ATMT, where bacterial kanamycin resistance gene served as the selection marker [1]. Our results support the possibility of a genome defence mechanism in Zygomycetes, eliminating exogenous DNA as postulated in some of the previous studies. A vector containing the endogenous orotidine-5'-monophosphate decarboxylase (*pyrG*) cassette was also constructed (pNYI9). Experiments are in progress to transform a *Mucor circinelloides pyrG* mutant strain to investigate the applicability of the *Agrobacterium*-mediated transformation in Zygomycetes obtaining stable and integrative transformants.
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PATTERN CHANGES OF BACTERIA STUDIED AT THE NATIONAL INSTITUTE OF DERMATO-VENEREOLOGY, 1993-2004

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Before it was closed in 2005, the Microbiology Laboratory, National Institute of Dermato-Venereology processed 45829 samples from its outpatient venereology clinic and inpatient wards. Pathogenic microbes were detected in 18156 cases. Aerobic cocci (6401) obtained from vaginal and urethral samples were *S. agalactiae*, group D streptococci. Wound samples yielded *S. pyogenes*, group D, α-haemolysing streptococci. *S. aureus* was obtained primarily from urethral or vaginal discharge, ulcus cruris. MRSA occurred infrequently. Gram+, non-spore forming, anaerobic rods were found in wounds, urethral discharge; Gram+, aerobic rods were found in wounds, vaginal and urethral discharge. Gram-, anaerobic rods occurred in wounds. Gram- fermenting rods (3291) originated most frequently from vaginal and urethral discharge, and found frequently in wounds with the dominance of *E. coli* and *Klebsiella* species. *Proteus* and *Serratia* occurred in skin wounds. *Haemophilus* species were detected in urethral, rarely in vaginal discharge. *Acinetobacter* species were detected in wounds, occasionally in the vagina, rarely in the urethra. *Pseudomonas* species were found in wounds, ulcus cruris very frequently. Latent carriage of *C. trachomatis*, *Mycoplasma* and *Ureaplasma* species was detected more and more frequently in the vagina and urethra. From 654 *N. gonorrhoeae* isolates 503 originated from urethral, 150 from vaginal discharge and 1 was pharyngeal. *Candida* species were detected in vaginal (4830) or urethral (210) discharge. Time course alterations in the cultivation are parallel to the changes in sexual behaviour and differences in dermatological disorders. Continuous changes in the normal flora constituents were observed. Profound alterations in the antibiotic sensitivity were detected. Resistance significantly increased in the case of *S. aureus* (ampicillin, gentamycin, trimethoprim, cefixime), *S. pyogenes* and *Klebsiella* species (trimethoprim), group D and α-haemolysing streptococci (gentamycin, trimethoprim), *P. mirabilis* (trimethoprim, nalidic acid, ofloxacin), *Pseudomonas* species (cefuroxime, ceftazidime, nalidic acid), *E. coli*

(trimethoprim, cefuroxime, nalidic acid, ofloxacine), *H. influenzae* (ampicillin, nalidic acid, tetracycline, cefuroxime), *N. gonorrhoeae* (penicillin, tetracycline). Resistance remained infrequent and low in the case of *S. aureus* (tetracycline), *E. coli* (carbenicillin, ampicillin, nalidic acid), *Klebsiella* species (nalidic acid, cefuroxime, ofloxacine, ciprofloxacine), *P. mirabilis* (ampicillin, carbenicillin, cefuroxime, ciprofloxacine), *P. vulgaris* (carbenicillin), *Pseudomonas* species (carbenicillin, ofloxacine), *P. aeruginosa* (ciprofloxacine), *H. influenzae* (carbenicillin, gentamycin, trimethoprim). Fashionable trends in the treatment regimen of this specific cohort of patients might contribute to the selection of resistant bacterial strains. All dermato-venerology specialists must be aware of the altered epidemiology of microbes.

DIFFERENT DIAGNOSTIC METHODS FOR TURKEY RHINOTRACHEITIS VIRUS

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The etiological agent of Turkey Rhinotracheitis (TRT), known as TRT virus (TRTV) or avian metapneumovirus (aMPV) is known almost worldwide to be responsible for respiratory diseases and egg drops in turkeys, chickens and ducks. Together with human metapneumovirus (hMPV), aMPV has been assigned to the genus *Metapneumovirus* (MPV), within the subfamily *Pneumovirinae*, family *Paramyxoviridae*, order *Mononegavirales*. Since the first detection in South Africa in 1978, aMPV-induced infections in turkeys have recurred worldwide and, since 1997, in Hungary as well.
Avian metapneumovirus was divided in four subgroups designated as A, B, C and D. The aMPV strains assigned to subgroups A, B and D were isolated in the mid-80s in Europe in turkeys and/or chickens and subgroup C was first isolated in turkeys in the USA and in ducks in France. In conventional, young turkeys grown under intensive conditions, TRTV causes acute upper respiratory tract infection (both in turkeys and chickens) with a sudden onset and rapid spread through the flocks. The first signs are often mild and transient, but TRT mortality ranges from 3% to 30% depending on the severity of secondary bacterial infections and is highest in six to twelve week old poults.
Due to the sometimes severe economic loss, it is very important to develop reliable diagnostic methods to prove the presence of the virus in diagnostic samples. Macroscopic examination and histopathology usually are not enough to determine a certain diagnosis; these can only be considered additional tools in understanding the pathogenesis of the disease. The techniques applying polymerase chain reaction (PCR) are fast and reliable, and combined with other methods of investigation, such as electron microscopy, are powerful aids in identifying viruses. Two specific primer pairs designed for the N (nucleocapsid) and the G (attachment) protein were used to test the samples and assign the strains into subgroups. After the definite diagnostic use of PCR, the swab samples taken from infected turkeys were examined by electron microscope and pleiomorphic viral particles of about 150 to 300 nm, with a morphology resembling the *Paramyxoviridae* family were observed. The results suggest that RT-PCR using specific primer pairs can be used in rapid detection and preliminary typing of the TRTV field samples. Additionally, further research is required to establish the geographical distribution of TRTV in Hungary and to determine whether other subgroups are present.

PREFERENTIAL LIGATION DURING CLONE LIBRARY CONSTRUCTION – A FACTOR BIASING COMMUNITY STRUCTURE ANALYSIS

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Clone library analysis based on 16S rDNA 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 clone libraries. 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. A widely used, commercially available TA-cloning system was tested. Collection strains of bacteria were selected with differing genomic properties and fully known 16S rDNA sequence. DNA from each strain was extracted and amplified with PCR, quantified and mixed at predefined amplicon copy numbers to serve as 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 heterogeneity of a 16S-23S spacer clone library, preferential ligation of the shorter PCR amplicon was shown. Also, skewed ratios were detected if the inserts of two different strains had the same length and GC content – perhaps due to the unique nucleotide sequence and thus different secondary structure of the 16S rDNA amplicons. These findings indicate that members of a diverse microbial sample may be excluded during clone library construction because of preferential ligation, this way biasing the true community picture.
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TAXONOMY AND BIOSYSTEMATICS: HOW CLOSE/FAR THEY ARE RELATED – SOME PRINCIPLES

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Taxonomy is concerned with the diversity of specimens and populations of organisms. The level of research is not unequivocally idiobiological, however, actual taxonomical work is always carried out on specimens (as for plants and animals: mostly on individuals). Taxonomists aim to collect and evaluate morphological, physiological, phenological, bionomic, etc. data in order to build reference bases for the scientific names of extant or extinct living organisms, thus these names provide a base of a general retrieval system. The legislative aspect of this nomenclature (e. g. the International Code of Zoological Nomenclature and its applications) is not science in itself, rather a useful and unavoidable tool during the collection and retrieval of information. Taxonomy provides information for all other fields of biology, although a causal analysis of this information is limited within this field. Taxonomy has been repeatedly called the foundation stone or the "pioneer" field of biological sciences. This way, our interpretation of taxonomy is close to that of the classical notion, i.e. "the science of species". Practically speaking, a taxonomist is a scientist who publishes descriptions of formerly unknown species and reliable biotical lists based on faithfully identified voucher specimens; a scientist who works in reference collections of living organisms, etc. BioCode, a unified approach for international rules of scientific names of all living organisms, is also shortly discussed.
Systematics, without the specification of subjects, is a field of formal logic, which is concerned with the classification of the body of knowledge. Specifically, biosystematics assembles and processes information from all fields of biology, thus it is the science of biological sciences. Consequently, classifications of living organisms are to be yielded by biosystematical analyses rather than mere taxonomical considerations. The importance of the species concept in biosystematics is paramount. The seemingly close relationship between taxonomy and biosystematics is a consequence of the practical reason that specific names are excellent coding/decoding tools, playing an inherent role in

data retrieval systems in all fields of biology. Our chances to achieve a new synthesis in biosystematics are currently poor because several fields of biology exhibit a rapid development and thus a synthesis is hardly feasible. However, efforts to attain unified theories to explain facts and invariances experienced in seemingly distant fields of biology have an increasing importance today. Unifying concepts such as endosymbiosis, the six kingdoms of life, and the major transitions in evolution, will most probably become major keystones of a future synthesis. In addition to this, surprising recent discoveries modify our view of the living word (Myxozoans are a class of Cnidaria, Microsporideans are microfungi, Archebacteria are not ancient Prokaryotes, etc.).
On the one hand, the necessity of making distinction between taxonomy and biosystematics is apparent, and so is the need for a quicker and more effective information exchange between the two. In our era, mere taxonomical research cannot yield robust classifications, rather an evaluation of diverse information provided by different biological sciences – i.e. a biosystematic analysis – is needed to achieve this goal. The evolutionary concept – with its tremendous heuristic value – is decisively important in all fields of biology, including biosystematics.
The relationship between biosystematics and theoretical biology is not discussed here in detail, although it seems to be in close association as indicated by an overlap of themes and methods. While biosystematics is mainly concerned with the analysis of facts, invariances and concepts based on invariances provided by all the fields of biological science, theoretical biology produces and analyses testable hypotheses on the most general aspects of life, such as its origin and evolution.

IMPROVEMENT OF THE CAROTENE PRODUCTION IN *MUCOR CIRCINELLOIDES*

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Carotenoids are used in the food, pharmaceutical and cosmetic industries and also as feed colour additives (especially to salmon, trout and poultry). Today, most of the carotenoid production is performed by chemical methods, but there is an increasing interest in sources of carotenoids of biological origin. The demand to decrease the amount of chemical additives urges the development and the improvement of the biological production. *Mucor circinelloides* is a beta-carotene-producing fungus that has been frequently used to study the biosynthesis of carotenoids at the molecular level. We intend to use the knowledge acquired in the past years, as well as the genes recently isolated, to develop carotenoid overproducing strains of this fungus. Carotenoids are isoprenoid chemical compounds; their production could be increased by improving the early, non-carotene specific steps of the isoprene biosynthesis. This can be carried out by the over-expression of the genes encoding the rate-limiting enzymes of this pathway. Recently, two structural genes of this biosynthetic route: *isoA*, encoding farnesyl pyrophosphate synthase [1] and *carG*, encoding geranylgeranyl pyrophosphate [2] have been isolated and characterized in *M. circinelloides*.
In the present work, the isopentenyl pyrophosphate isomerase gene (*McIpp1*), also encoding a key-enzyme of the isoprenoid pathway, was cloned and analysed. The gene was isolated by screening a *M. circinelloides* cDNA library. The entire gene with the downstream and upstream flanking regions was determined with the inverse PCR technique. Several expression vectors, containing the *McIpp1*, *isoA* and *carG* genes were constructed and introduced into a double auxotrophic (*leu*-, *ura*-) strain of *M. circinelloides* by PEG-mediated transformations. Co-transformations with the appropriate vector-pairs were also carried out. All studied transformants proved to be autoreplicative: maintenance of the selective conditions was necessary for the transformants to retain the plasmids. Carotene production

of the resulted transformants and the recipient strain was measured by HPLC analysis. Compared to the recipient, transformants harboured extra copies of the gene *McIpp1*, *isoA* or *carG* and produced about 1.5-, 1.4- or 1.7-fold more carotene, respectively. In the co-transformants, carotene production increased about 2 fold.

Applicability of these genes to modify the carotene production of *Mucor* will be discussed.

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CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATES RECOVERED FROM MILK SAMPLES

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Staphylococcus aureus is known worldwide as a frequent cause of mastitis in dairy cattle and also as a principal contaminant of raw milk. Bacterial contamination can originate from multiple sources, such as mastitic cows, dirty udders and poorly cleaned milking equipment. The aim of our research was to examine the genetic diversity and the antimicrobial drug susceptibility of sixty-nine *S. aureus* strains, which derived from bulk tank milk (n = 54) and udder quarter milk (n = 15) of mastitic cows from dairy farms of different size.

The genotypic comparison of sixty-nine *S. aureus* isolates recovered from bulk tank milk from fourteen dairy farms and mastitic cow milk from two farms in Hajdú-Bihar County was carried out. Macrorestriction analysis of *Sma*I-digested chromosomal DNA, using pulsed-field gel electrophoresis (PFGE) was performed for typing and to estimate genetic relationship among the isolates. Antimicrobial drug susceptibility testing was performed on Mueller-Hinton agar by disk diffusion method according to NCCLS guidelines using antibiotic disks.

The sixty-nine strains were classified into eighteen pulsotypes (A-R) and three subtypes (D1, F1, K1). Isolates from bulk milk (n = 54), were divided into sixteen pulsotypes (A-D, F-R) and two subtypes (F1, K1). The mastitis (n = 15) isolates belonged to three different pulsotypes (D, E, F) and one subtype (D1). Only one or two PFGE related patterns were recovered from milk, showing a lack of genetic diversity within each farm. On the contrary, the genetic diversity of the strains was great among the herds. In most instances, we found different strains in each herd. The same types were found in bulk milk and in udder quarter milk samples. This indicates that *S. aureus* from infected udders may contaminate bulk milk. The results of the present investigation showed that pulsotypes D, E, F and subtype D1 seemed to be responsible for most cases of bovine mastitis in two examined farms and partly responsible for contamination of bulk tank milk. All strains were susceptible to methicillin, cefoxitin, lincomycin, tetracycline, erythromycin and sulfamethoxazole/trimethoprim. Forty-four of the sixty-nine isolates (63.8%) were susceptible to all antibiotics tested and twenty-five strains (36.2%) were resistant to penicillin. Resistant strains comprised eleven strains (44.0%) from bulk tank milk and fourteen (56.0%) from mastitic cow milk. Fourteen out of the fifteen isolates (93.3%) derived from udder quarter milk of mastitic cows were penicillin-resistant.

The twenty-five penicillin-resistant isolates belonged to six pulsotypes (D, E, F, J, L and N) and two subtypes (D1, F1), mostly, isolates from the same herd had the same antibiotic resistance profile.

OCCURRENCE OF ENTEROTOXIN-PRODUCING *STAPHYLOCOCCUS AUREUS* ON SEVERAL DAIRY FARMS IN HAJDÚ-BIHAR COUNTY

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Staphylococcus aureus is a very important pathogen of dairy farms and milk processing plants. Subclinical mastitis is often caused by this species, which can contaminate bulk tank milk during the milking of cows suffering from mastitis. Additionally, thermostable enterotoxins produced by some types of this bacterium can cause food poisoning. The aim of our research was to examine the number of *S. aureus* in bulk tank milk in twenty dairy farms of different size and the enterotoxin-producing ability of *S. aureus* strains. We investigated seven large farms (A-G), four medium farms (H-K) and nine small farms (L-T).

We did not find *S. aureus* in the bulk tank milk of six farms (B, G, J, K, S and T). From the bulk tank milk of the other farms, fifty-five *S. aureus* isolates were collected., We investigated the distribution of staphylococcal enterotoxin (SEA, SEB, SEC, SED, SEG, SEH, SEI and SEJ) genes (*sea, seb, sec, sed, see, seg, seh, sei* and *sej*) and the toxic shock syndrome toxin (TSST-1) gene (*tst*) in the *S. aureus* isolates with the multiplex PCR assay. Analysing the connection between farm size and the number of *S. aureus* in bulk tank milk, we found that the number of *S. aureus* was smaller at medium (1.1×10^2 CFU/ml) and large farms (3.3×10^2 CFU/ml), than at small farms (1.2×10^3 CFU/ml). The number of *S. aureus* in bulk tank milk of small farms significantly differed from that of the other farms ($P < 0.05$). The results of the analysis of the quality categories showed that the proportion of the best quality ($<5.0 \times 10^2$) samples was 90.9% in medium, 80.8% in large and only 56.5% in small farms.

Thirteen (24.1%) of the fifty-four isolates carried enterotoxin genes. In three large (A, C, E), one middle (I) and one small farm (N) out of fourteen farms, enterotoxin-producing *S. aureus* strains were found. No strain possessed the *seg, seh, sei, sej* or *tst* genes. The isolates carried just one gene. The *seb* gene was present in five isolates (9.3%) of farm "E" and farm "N", the *sea* gene was detected in three-three isolates (5.6%) of farm "C" and farm "I" and the *sed* gene was present in two isolates (3.7%) of farm "A". The results showed that the number of *S. aureus* was the highest at little farms using tied stall barns and bucket milking installation, and these values often exceeded the limit values. The number of *S. aureus* in bulk tank milk was high in farms where there were problems with the detection and separation of cows suffering from subclinical mastitis. In this way, the milk of mastitic cows could contaminate the bulk tank milk.

During the investigations of the enterotoxin-producing ability of *S. aureus* strains, we found different enterotoxin-producing (SEA, SEB, SEC, SED) *S. aureus* strains in three large farms, one medium and one small farm. The incidence rate of the *seb* gene was the highest. The isolates carried just one gene.

DEGRADATION AND ADSORPTION OF OCHRATOXIN A BY ASTAXANTHIN-PRODUCING YEASTS

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Mycotoxin contamination of agricultural products is a serious health hazard throughout the world. One of the most important mycotoxins is ochratoxin A (OTA), which is produced by several *Aspergillus* and some *Penicillium* species. The occurrence of OTA in several commodities (feeds, foods and beverages) is considered a serious health hazard in view of its nephrotoxic, teratogenic, hepatotoxic and carcinogenic properties.
Several strategies are available for the detoxification of mycotoxins. These can be classified as physical, chemical, physicochemical and (micro)biological approaches. Microbes or their enzymes could be applied for mycotoxin detoxification; such biological approaches are now being widely studied. An adsorption mechanism has also been suggested for OTA removal by lactic acid bacteria, yeasts and conidia of black aspergilli. We examined *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* isolates for their ability to degrade and/or adsorb ochratoxin A in liquid medium. *Phaffia rhodozyma* is a red-pigmented fermentative yeast. Beside producing astaxanthin, *P. rhodozyma* is also able to both detoxify and adsorb OTA at temperatures well above the temperature optimum for growth of *Phaffia* cells. The kinetics of OTA degradation of *P. rhodozyma* CBS 5905 has been examined at two cell concentrations at 20°C in a liquid medium. The *Phaffia* isolates could degrade more than 90% of OTA in about 7 days at 20°C. Previously, an *A. niger* isolate CBS 120.49 was found to be able to degrade more than 90% of OTA after 4 days of incubation. Interestingly, a significant amount of OTA was found to be bound by the cells after two days, indicating that OTA is also adsorbed by the cells. When the effect of temperature was examined, the temperature optimum of this enzyme was found to be above 30°C, which is much higher than the temperature optimum for growth of *P. rhodozyma* cells, which is around 20°C, and the cells are unable to grow at higher temperatures. When the temperature range of the OTA degrading enzyme was further examined, it was found that the enzyme remains active at up to 60°C. Above this temperature, OTA adsorption could take place since OTA turned up in the cell extracts.
We hypothesized that a carboxypeptidase enzyme could be responsible for OTA degradation as observed previously in other fungi. To prove this hypothesis, the effect of various carboxypeptidase inhibitors was tested on OTA degradation activities of *P. rhodozyma* cells. Two of these inhibitors, the chelating agents EDTA and 1, 10-phenanthroline significantly inhibited OTA degrading activities of the *P. rhodozyma* cells, indicating that the enzyme responsible for OTA degradation is a metalloprotease. Further studies are in progress to identify the enzyme responsible for OTA degradation in *P. rhodozyma*.

HUMAN PSITTACOSIS: THE IMPORTANCE OF EARLY LABORATORY DIAGNOSIS

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Psittacosis is caused by an obligate intracellular, Gram-negative bacterium, *Chlamydophila psittaci* (*C. psittaci*; formerly *Chlamydia psittaci*). The main hosts of this bacterium are birds all around the world. Currently seven genotypes of *C. psittaci* are known to infect birds, causing avian chlamydiosis, which is usually systemic and occasionally fatal depending on the species, the health conditions of the birds and the strains of *C. psittaci*. Avian chlamydiosis does not only cause financial losses to the poultry industry, but it is also a potential biological hazard to human health. Human infections usually occur in bird owners, poultry breeders and veterinarians and employees in the poultry industry after handling or processing infected fowl, mainly through inhalation of aerosols from infected bird faeces. The incubation period of psittacosis varies from 1 to 4 weeks and the manifestations of the disease can range from asymptomatic infection to systemic illness and severe pneumonia. The under diagnosed and untreated psittacosis has a case-fatality of 15-20%; therefore, early laboratory testing is crucial to get an exact diagnosis. On the clinical side, in the case of potential psittacosis, it is essential

to reveal the history of bird contact. In the routine laboratory diagnosis of possible psittacosis cases, acute-phase and convalescent-phase (4-6 weeks after onset) paired sera are tested. We summarize our findings with the microimmunofluorescence (MIF) test (Focus Diagnostics) through the data obtained during psittacosis outbreaks in 2005. Applying the MIF test, species-specific antibodies against all of the 3 human pathogen *Chlamydia* species can be detected simultaneously. The limits of serological tests are well known. On the one hand, the amount of specific antibodies in the first serum sample may not reach a detectable level; on the other hand, the high antigenic homology among the Chlamydia species can result in cross-reactions. This is why a second serum sample is essential for the differential diagnosis of *C. psittaci* infection, but it is not always available and thus it may take weeks to confirm the diagnosis. Consequently, direct detection of *C. psittaci* DNA from human respiratory samples could facilitate the correct diagnosis. We describe our PCR methods applied to detect *C. psittaci* in clinical samples in respect of a fatal psittacosis case and also a PCR-RFLP method for genotyping *C. psittaci* strains.
In conclusion, for the correct laboratory diagnosis of human *C. psittaci* infection, serological test results should always be confirmed by molecular methods examining both a first serum and a respiratory sample. For further epidemiological studies and characterization of differences in the pathogenicity of *C. psittaci* strains, genotyping could be a valuable tool.

ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS

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The application of conventional pesticides in plant protection poses a significant risk to the environment and human health. For this reason, the permission of several pesticides has been cancelled. Nowadays, the demand of the use of plant protection products containing natural agents, e.g. plant extracts, is increasing. The possibility of applying essential oils against pests became the focus of interest in the past decade.
In our experiments, 28 essential oils extracted from mediterranean, tropical and continental plant species were tested for their effectiveness against 5 pathogens: *Botrytis cinerea, Fusarium oxysporum* f. sp. *cyclaminis, Monilia fructigena, M. laxa* and *Sclerotinia sclerotiorum.* Conventional fungicides were also involved as controls. The antifungal activity of the oils was compared on the basis of the inhibition of mycelial growth and germination of conidia. The inhibition of mycelial growth was tested by agar diffusion hole test and agar dilution technique using different oil concentrations. The inhibition of the germination of conidia was examined in microtiter plates. Antifungal activity was expressed estimating the EC_{50} and EC_{90} (Effective Concentration) values. The essential oils that gave good efficacy against the pathogens were selected for *in vivo* experiments. The *in vivo* antifungal activity against *Monilia fructigena* and *Monilia laxa* is being tested on mature sour cherry fruits and on strawberry blossoms and on fruits in case of *Botrytis cinerea*. The activity against *Fusarium oxysporum* f. sp. *cyclaminis* and *Sclerotinia sclerotiorum* are evaluated on potted cyclamen plants and lettuce seedlings by soil test.
During the hole-test, almost all oils in a 1% concentration caused total inhibition of mycelial growth. In case of the agar dilution method, great differences could be observed among essential oils in effectiveness at different concentrations. Against *Botrytis cinerea, Fusarium oxysporum* f. sp. *cyclaminis* and *Sclerotinia sclerotiorum,* four oils were effective, resulting in EC_{50} values of less than 0.01% and EC_{90} values between 0.05% and 0.01%. Concerning *Monilia* spp., the EC_{50} value was under 0.01% in ten cases against *M. fructigena* and in fourteen cases against *M. laxa*. These effective oils against *Monilia* species in most cases overlapped with each other. The germination of the conidia

of *Monilia fructigena* and *M. laxa* was completely inhibited by almost all of the 28 essential oils in a 1% concentration. At lower concentrations, great differences could be observed among the oils. The EC_{90} value was under 0.05% in eight cases against *Monilia laxa*, in seven cases against *M. fructigena*, in four cases against *Botrytis cinerea*, and in three cases against *Fusarium oxysporum* f. sp. *cyclaminis*. In case of all 5 tested pathogens, an excellent *in vitro* antifungal activity could be observed with essential oils numbered 16, 21 and 27. Among the essential oils, two (22, 27) proved to be effective *in vivo* against *Monilia fructigena* and *M. laxa* on sour cherry fruits. Disease frequency and incidence was even lower than the control fungicide.
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ISOLATION AND CHARACTERISATION OF ROOT COLONIZING ENDOPHYTIC FUNGI FROM A SEMI-ARID GRASSLAND OF THE GREAT HUNGARIAN PLAIN

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Endophytic fungi colonize the tissues of healthy plants, either inter- or intracellularly, causing no apparent negative effects. Most of the fungal endophytes are ascomycetes. The species belonging to the dark septate endophytic (DSE) fungi colonize the root tissues of the plants and the interaction is mutualistic rather than parasitic. The DSE fungi were named after their melanised inter- and intracellular septate hyphae and microsclerotia produced within the plant cells. DSE fungi have been reported from various ecosystems world-wide.
Majority of the plant species of plant communities of the Great Hungarian Plain studied in previous mycorrhizal status studies were colonized by DSE. The main aim of the work presented here was to isolate and characterise fungi from the roots of plants from a semi-arid grassland.
Root samples of *Stipa borysthenica*, *Festuca vaginata*, *Populus alba* and *Ephedra distachya* were collected from a sandy grassland close to Fülöpháza between October 2004 and November 2005. The melanised fungal strains were isolated on MMN. The ITS region of the nrDNA region was amplified and sequenced for molecular analyses.
Altogether 57 fungal strains were isolated and their characteristics (colony morphology in culture, discolouration of the medium, rate of growth, microscopic features) were determined. The strains clustered into 15 groups based on the analysis of the ITS sequences. *Allium porum* seedlings have been inoculated with representatives of the main clades, to test the microsclerotia forming capability of the strains.
Isolates coming from woody and herbaceous plants always clustered in different clades; no common endophytes of grasses and woody plants were found. The strains belonged to several fungal groups like Helotiales, Pleosporales, Hypocreales and Sordariales. Two of the isolates were *Periconia* species, which is known as a common root colonizing fungus of the tallgrass prairies of North America. The ITS sequences of other strains were almost identical with some sequences of unidentified, uncultured root colonizing fungi from a semi-arid grassland in North America deposited in Genbank. The frequent occurrence of species of Pleosporales might be interesting because of the well-known pathogenicity of the members of this group; however, their common occurrence was also found in other studies of root endophytic fungi. In addition, strains of *Cadophora* sp., considered generally as DSE, were isolated from the roots of *Populus alba*. *In vitro* experiments are planned to study the potential effect of the collected strains on their host plants.
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BACTERIAL DIVERSITY OF DIFFERENT BIOFILM CARRIERS IN AN EXPERIMENTAL NITRIFYING SYSTEM TREATING AMMONIUM-RICH WASTEWATER

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The handling of reject water coming from anaerobic sludge digesters causes a huge problem for wastewater treatment plants because of its high ammonium content. In this study, two experimental nitrifying columns treating digester liquor were examined. The columns were filled with different biofilm carriers: ceramic beads or zeolite, and were loaded with gradually increasing ammonium inflows. The two carriers were compared concerning their nitrifying biofilm community structure.
The diversity of biofilms was analysed by DNA-based methods. For the characterization of the total bacterial community, the 16S rRNA gene was amplified. In the case of ammonia-oxidizing bacteria (AOB), the ammonia monooxygenase (*amoA*) gene was used, and for nitrite-oxidizing bacteria (NOB), the 16S RNA gene was targeted by two group-specific primer pairs. The total bacterial and AOB diversity was examined by Terminal Restriction Fragment Length Polymorphism (T-RFLP). The PCR products obtained by the NOB-specific primers were directly subjected to sequence analysis. At about 500 mg/h NH_4^+-N loading (300 mg/l NH_4^+-N concentration) the total bacterial diversity of the two carriers on the grounds of their TRF pattern differed considerably. On the surface of the ceramic beads, the AOB‘s were represented only by one peak specific to *Nitrosospira spp.*, whereas in the case of zeolite, a TRF corresponding to *Nitrosomonas spp.* also appeared. With the *Nitrobacter*-specific primers, from the surface of both carriers only the family Bradyrhizobiaceae could be identified, which also contains the genus *Nitrobacter*. Using the *Nitrospira*-specific primers, *Nitrospira* species could only be detected from the ceramic bead biofilm. After dismantling the column filled with zeolite - following loads of above 2000 mg/h NH_4^+-N (600 mg/l NH_4^+-N concentration) - five zones from its entire depth were examined. The total bacterial and AOB community structures showed characteristic changes according to the inner ammonium concentration gradient of the experimental column. The *amoA* TRF analysis showed only *Nitrosomonas spp.* specific peaks from the zones with high ammonium content (closer to inflow), while *Nitrosospira spp.* also appeared at lower ammonium concentrations. *Nitrobacter spp.* were detected in all of the zones but *Nitrospira spp.* could not be identified in any of them.
These results demonstrate that various carriers and different ammonium concentration zones within the experimental column both influence the nitrifying bacterial community structures.
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COMPARISON OF BIOAUGMENTATION AND BIOSTIMULATION ON A PARTLY BIOREMEDIATED SOIL

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Diesel oil is a common soil pollutant material. In this study, the effect of nitrogen source (NH_4NO_3) addition and isolated crude oil degrading bacteria was investigated on biodegradation rate in a diesel contaminated soil.

Soil originated from a toxic waste deposit site. The soil was bioremediated, but biodegradation halted before soil TPH reached the values declared in the concerning directive. Diesel degrading strains were isolated from the soil. The possible use of these bacteria in bioaugmentation processes and the effect of addition of nitrogen as “stimulator” was tested in an Oxitop® respirometric measuring equipment and soil lipase activity assay. At the end of the microcosm experiment, selected samples were investigated for their PLFA profile.
Bioaugmentation of the soil with isolated and enriched native bacteria had no positive effect on biodegradation. Biostimulation with NH_4NO_3 had a positive effect at a C:N ratio of 10:1, but not at a C:N ratio of 1:1. PLFA profiles confirmed the data of respirometric measurements. Respirometry and soil lipase activity can both be used for the estimation of biodegradation rate. Strains with high in vitro diesel degrading capacity cannot be dependably used for bioaugmentation of partly bioremediated soils. Stimulation by the alteration of nutrient ratios in the soil can give better results.
Acknowledgement: This research was supported by the grant GVOP-3.1.2004-05-0407/3.0.

HEPATITIS B SEROLOGIC MARKERS IN IMMUNIZED BABIES BORN TO HEPATITIS B SURFACE ANTIGEN POSITIVE MOTHERS

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Infection with hepatitis B virus (HBV) is of global importance because of its potential to cause severe liver disease. It is generally agreed that risk of chronic infection with HBV is inversely related to the age of the child at the onset of infection. The prevalence of HBsAg positive persons among pregnant women is less than 1% in Hungary. In 1995, a passive / active immunoprophylaxis program was introduced for babies of HBsAg positive mothers immediately after birth. Our aim was to investigate the serological status of the immunized children born to HBsAg carrier mothers at 15 month of age to get a deeper understanding of the perinatal prevention of hepatitis B virus infection. Only a small proportion (around 1%) of the children born to HBV carrier mothers became HBsAg carriers, despite the fact that they received immunization. More than 74% of immunised children had >10 mIU/ml anti-HBs titer (protected against HBV infection). Almost 9% of the HBsAg negative children had anti-HBc antibody. According to the literature, anti-HBc detected in babies over 2 years old could be indicator of past infection, so we plan to check the serological status of immunized children at age 2.

DIVERSITY OF REED (*PHRAGMITES AUSTRALIS*) STEM BIOFILM BACTERIAL COMMUNITIES IN TWO HUNGARIAN SODA LAKES - A POLYPHASIC APPROACH

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The efficiency of surface water self purification processes and the biodegradation of organic compounds mainly depend on the composition and activity of biofilms associated with submerged parts of aquatic plants. A better exploration of these complex communities is essential for optimization and improvement of the efficiency of water purification processes. Methods in microbiology are all selective to some extent, which is why detailed investigations on bacterial communities cannot miss the polyphasic approach. The aim of this study was to get insight into the species composition and activity of reed stem biofilms in two Hungarian soda lakes using both traditional cultivation-based and molecular methods. Bacterial isolates were gained after serial

dilutions and plating onto different media. The 164 strains were fairly inactive in the biochemical tests. Sequence analysis of the genotypically and phenotypically identical group-representatives referred to the presence of the genera *Agrobacterium*, *Paracoccus*, *Halomonas*, *Pseudomonas*, *Bacillus*, *Planococcus* and *Nesterenkonia*. The species diversity was also investigated by cloning, whereby the majority of the clones were affiliated with uncultured bacterial clones (sequence similarity between 93 and 99%) originating from diverse environmental samples. DNA sequences of other clones showed the presence of the genera *Flavobacterium*, *Sphingobacterium*, *Pseudomonas*, *Rheinheimera* and *Agrobacterium.* Evaluation of DGGE profiles resulted in unique community fingerprints of the studied samples. Partly due to their metabolic diversity, partly to their plant-growth promoting or their plant pathogenic characteristics, these microbes fulfil diverse roles in the biofilms on the reed stems and can be used for application in different artificial wetland systems.
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ISOLATION AND CHARACTERIZATION OF APATHOGENIC *PSEUDOMONAS* STRAINS ANTAGONISTIC TO *PSEUDOMONAS TOLAASII*

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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. Brown blotch disease caused by *Pseudomonas tolaasii* is well known, but other fluorescent *Pseudomonas* strains, interacting with *P. tolaasii,* can cause light brown coloured symptoms as well. In this study, the *Pseudomonas* strains of an infected *Pleurotus ostreatus* farm in Hungary were investigated. Sixty strains, belonging either to the fluorescent or to the non-fluorescent groups, were isolated on *Pseudomonas* selective S-1 medium, from prefermented oyster substrate and water samples. The molecular characterization of 46 strains was performed by PCR based methods. The PCR reaction with the *P. tolaasii* specific primers, designed from the nucleotide sequence of DNA involved in tolaasin production, revealed that only 2 out of the 46 isolates belonged to *P. tolaasii*, suggesting that other *Pseudomonas* species were present in the samples. The ARDRA and the rpoB-RFLP methods were used for the identification of the strains. After in vitro pathogenicity tests for *Pleurotus ostreatus*, 10 strains were selected, which did not antagonize oyster but were able to antagonize the type strains of *P. tolaasii*. The optimal environmental factors for strong antagonism were determined and competition dynamics between apathogenic *Pseudomonas* strains and *P. tolaasii* strains were revealed by classic and molecular methods.
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THE EFFECT OF "ACENIT A 880 EC" ON THE GROWTH OF SOME SOIL MICROSCOPIC FUNGI AND ON THE SOIL MICROBIOLOGICAL PROCESSES

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We studied the effect of the herbicide "Acenit A 880 EC", used extensively in corn production with acetochlor as active ingredient, on the growth of some microscopic fungi and on the soil microbial processes under *in vitro* conditions. The method of MILLER (1973) was applied, according to which

Acenit was given to peptone-glucose-agar medium in different dosages (1x, 2x, 5x and 10x) and we studied the growth of the microscopic fungi: *Trichoderma* sp., *Fusarium oxysporum* and *Aspergillus niger* on the medium. In addition, we also determined how the bacterium population of calcareous chernozem soil changed under *in vitro* conditions as a result of the herbicide treatment. We studied the effect of different dosages of the herbicide on the nitrate exploration and CO2 formation in calcareous chernozem soil after three and four weeks of incubation. The basic treatments were as follows: control, 1x, 2x, 5x and 10x Acenit dosages. Two series of experiments were set up: a) without glucose, b) treated with glucose.
Our results can be summarized as follows. Even the regular (1x) dosage per hectare greatly inhibited the growth of *Trichoderma* sp. and *Fusarium oxysporum* colonies. The diameter of the colonies was half when applying the tenfold dosage. Acenit did not inhibit the growth of *Aspergillus niger* so tremendously, but significant differences could be observed among the effects of the different dosages. As a result of the "Acenit A 880 EC" treatment, the bacterium population of the calcareous chernozem soil was reduced independently from the increasing concentration of the herbicide to about one third of the control (from 6.97 - 8.25×10^6 CFU/g to 1.90 - 2.77×10^6 CFU/g).
When studying soil respiration, CO_2 concentration was reduced to two-thirds of the former concentration in a week as a result of the herbicide treatment regardless of the dosage. In the second and third weeks, no difference was detected between the control and the treated samples. Although the herbicide treatments did not result in significant differences in the samples treated with glucose, the values increased in the first two weeks compared to the control and decreased in the third week. During incubation time, carbon-dioxide formation increased in both series. In the series treated with glucose, a considerably higher amount of carbon-dioxide was released. In the samples treated with Acenit, nitrate release was higher than in the control for all the four weeks and the inducing effect of the tenfold dosage was always outstanding. After the first week in the glucose-treated samples, the smaller and larger concentrations were of inducing and inhibiting effect, respectively. Nitrate release was considerably smaller in the glucose-treated samples. A partial explanation could be that a considerable amount of the nitrate formed was used by the microbes to decompose the organic material (glucose).

TRADITIONAL AND MOLECULAR CHARACTERISATION OF *PASTEURELLA MULTOCIDA* STRAINS ISOLATED FROM VARIOUS ANIMAL SPECIES IN HUNGARY

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Pasteurella multocida, a facultative pathogen microorganism of mammals, birds and human hosts, causes significant losses for animal husbandry. Chronic carriers with aid of various predisposing factors or introducing the pathogen into naïve herds may cause chronic disease or outbreak of epidemics. It makes the situation more difficult that this bacterium can infect various animal species. *P. multocida* causes fowl cholera in poultry, atrophic rhinitis in swine, haemorrhagic septicaemia in cattle and pasteurellosis resulted inflammation of mucous and serous membranes in several other animal species. Although this pathogen was isolated and identified more than 200 years ago, our knowledge is rather limited about its antigenic structure, infection mechanism and specific immunity generated in various hosts. In order to establish effective preventive measures against *P. multocida,* we need to get more information about its antigens and patho-mechanism.
In our study, 150 isolates from poultry (duck, goose, turkey and chicken), swine and rabbit were examined. Strains were analyzed with traditional fermentation probes, *P. multocida* specific PCR (polymerase chain reaction), capsular multiplex PCR, ERIC-PCR, Heddleston agar gel precipitation

for determining serotypes and so-called biovar test.
Based on capsular specific PCR, 98% of our isolates could be identified in contrast with traditional methods (<50%). Also, traditional fermentation and PCR techniques were compared. The latter proved to be faster. The results of the two methods corresponded with each other. Only half of our isolates could be characterized by the Heddleston serotyping system. Among strains from poultry, serotype 1 was the most frequent finding, while most of the rabbit isolates did not give reaction with any of the 16 serotype specific antisera. The presence of a large number of non-classifiable isolates suggests that new serotypes may occur in Hungary. The same is true for the identification of biovars. Besides Fegan-Blackall biovar 3, in contrast to previous studies, biovar 1 is also dominant. Most *P. multocida* ssp. *septica* were identified as biovars 6 and 7. Some of the strains cannot be grouped into any biovars, which demands the creation of new categories. E.g. indole negative *P. multocida* isolates were described by several authors, and there is yet no adequate biovar group for them.

CELL SEPARATION GENES IN SCHIZOSACCHAROMYCES POMBE

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Cell division is the final stage of the cell cycle, during which the cell is irreversibly split into two daughters. In cell-walled organisms, cell division consists of two phases: cross-wall formation (septation) and cell separation. The genetically tractable yeast *S. pombe* is an excellent organism for studying cell division. During the septation of a *S. pombe* cell, a primary septum is laid down in a centripetal manner, which is then flanked on each side by secondary septa. The concomitant cell separation requires the degradation of the primary septum and the edging region of the mother cell wall. To investigate the genetic regulation of cell separation and the molecular processes involved, we isolated mutants defective in the process.
The genetic analysis of the mutants identified 16 *sep* genes (*sep* for *sep*aration). The gene *sep1+* codes for a fork-head-type transcription factor, which is a key regulator of a large group of M-phase genes, including *ace2+*. The Ace2 protein is a transcription factor that controls the activity of numerous genes coding for proteins and enzymes (e.g. the Eng1 endo-1,3-b-glucanase) directly involved in cell separation. *Sep1+* also interacts with *cdc2+* and *wee1+* to coordinate the initiation of cell separation with mitosis. *Sep9+* encodes a subunit of the chromatin remodelling complex SAGA that loosens the nucleosomal structures to make the DNA available for transcription. The products of *sep10+*, *sep11+* and *sep15+* are subunits of the Mediator complex. The Mediator is composed of a large number of proteins and mediates regulatory information from the gene-specific activators (e.g. transcription factors) to the polymerase II complex that transcribes the genes. Genome-wide transcription profiling of sep- mutants revealed that the Sep10 and Sep15 subunits participate in the control of numerous Sep1-Ace2-dependent cell separation genes and thus contribute to the implementation of the cell separation programme of the cell.

TIME-KILL STUDIES SHOW THAT POSACONAZOLE IS FUNGICIDAL AGAINST SOME *CANDIDA* SPECIES

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Azoles are commonly regarded as fungistatic agents against *Candida* species. However, preliminary

in vitro studies in our laboratory suggested that posaconazole was fungicidal against some *Candida* species. We performed time-kill studies for the following clinical isolates: three each of *C. albicans*, *C. kefyr*, *C. guilliermondii* and *C. lusitaniae*, two *C. tropicalis* and *C. glabrata*, four *C. krusei* and *C. inconspicua*, and ten *C. parapsilosis*. We followed the published time-kill methodology [1]. Drug concentrations ranged from 0.5x to 16x MIC with sampling at 0, 6, 18, 24, 30 and 48 hours. Posaconazole was fungicidal against *C. krusei*, *C. lusitaniae*, *C. inconspicua* and *C. kefyr*. In the case of *C. inconspicua* and *C. kefyr,* there were no viable cells after 30 hours even at such a low concentration as 0.5 x MIC. Similarly, at concentrations of >2 x MIC posaconazole was fungicidal at 48 hours against *C. krusei* and *C. lusitaniae*. The drug was fungistatic against all *C. albicans*, *C. tropicalis*, *C. guilliermondii* and *C. parapsilosis* isolates; for *C. albicans* and *C. tropicalis,* drug concentrations of >4 x MIC were required for optimal growth inhibition. *C. glabrata* isolates were weakly inhibited in the first 24 hours and some regrowth (~1 log increase) was evident after 48 hours. In summary, posaconazole exhibited fungicidal activity against several clinically relevant *Candida* species, including the innately fluconazole-resistant species, *C. krusei*, as well as *C. lusitaniae*, which frequently exhibits reduced susceptibility to amphotericin B.

1. Klepser et al.: Antimicrob Agents Chemother **42**, 1207-1212 (1998).

BIOLOGICAL ACTIVITY OF TOXINS PRODUCED BY ENVIRONMENTAL, INDOOR AND CLINICAL *TRICHODERMA LONGIBRACHIATUM* ISOLATES

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The toxicity of *Trichoderma* strains isolated from different environmental, indoor and clinical sources has been compared. Molecular identification of the 3 environmental (CECT 2412, CECT 2937, CECT 20105), 5 indoor (Tha, Thb, Thc, Thd, Thg) and 3 clinical (CNM-CM-1798, CNM-CM-2171, CNM-CM-2277) isolates performed by sequence analysis of the internal transcribed spacer (ITS1 - 5.8S rDNS - ITS2) region and the translation elongation factor 1a (tef1) gene revealed that all of the examined strains belong to *T. longibrachiatum*.

For toxicity assays, ethanol extracts were prepared from the isolates grown on MEA medium at room temperature. As target cells, boar spermatozoa, feline foetus lung cells (FFL) and murine neuroblastoma cells (MNA) were used. The biological effects of extracts prepared from the isolates could be divided into two categories according to the biological target:

1) Toxins damaging the integrity of the plasma membrane. The definition of toxic effects on spermatozoa exposed to these extracts for 1 and 3 days includes motility inhibition, damage of plasma membrane integrity, depletion of intracellular esterases and mitochondrial depolarization occurring in the same concentrations. Plasma membrane damage is indicated when the cell membrane becomes permeable to propidium iodide. Extracts damaging the plasma membrane were divided into fast-acting extracts and slow-acting extracts.

Fast action was indicated when EC50 on day 1 of the exposure was only twice as high as that on day 3 (e.g. strains CNM-1798, CNM-2277, CECT 20105, Tha, Thb and the reference substance alamethicin). Slow action is indicated when EC50 on day 1 of the exposure is four times higher than that on day 3 (e.g. strains CNM-CM-2171, CECT 2412, Thc and Thg).

2) Toxins depolarizing mitochondria. The definition of these extracts is motility inhibition and mitochondrial depolarization occurring at the same concentrations, whereas plasma membrane damage occurs at 2 to 4 times higher concentrations (e.g. CECT 2937). Selected isolates representing the above categories were chosen for toxicity testing against a continuous (FFL) and a malign (MNA)

cell line. The membrane damaging toxins including alamethicin were equally toxic to FFL and MNA cells. The mitochondrial toxin(s) of CECT 2937 were more toxic to MNA cells than to FFL cells. These results confirmed the observation that the toxicity in the extract of CECT 2937 differed from that recorded for the membrane toxic extracts of CNM-CM 2171 and indicated that toxicity of Thd was more related to the fast-acting and slow-acting membrane toxic substances than to the mitochondrial toxin of CNM-CM 2171.
A further interesting finding of this study is that clinical *T. longibrachiatum* isolates grown on TSA and BHI medium seem to be more toxic than the environmental and indoor isolates when measured with the fast sperm assay including exposure times less than 30 min.
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FISSION YEAST AS AN ANCIENT MODEL ORGANISM IN CELL CYCLE RESEARCH

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The most fundamental requirement for life is cell reproduction. Cell cycle is the sequence of events by which a growing cell duplicates all its components and partitions them more or less evenly between two daughter cells. The events of the cell cycle of most organisms are ordered into dependent pathways in which the initiation of late events is dependent on the completion of early events. For example, chromosome segregation in eukaryotes is dependent on the completion of DNA synthesis. These processes are controlled by a considerably complicated regulatory molecular network called the cell cycle machinery. 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 because of its favourable physiological features, for example, its symmetrical division enables good synchronisation techniques, which are necessary for cell cycle studies. During the last 40 years, *S. pombe* has become an attractive model organism in all chapters of cell cycle research, as well as in other physiological, genetic and biochemical studies. The full genome of *S. pombe* has been recently sequenced, indicating the importance of this species. At the Budapest University of Technology and Economics, we study the fission yeast cell cycle with two different methods. One of them is mathematical modelling; cell cycle progression is regulated by a network of interacting proteins, and these biochemical reactions can be formulated into ordinary differential equations. Afterwards, a computer simulates the cycles of fission yeast cells (for wild type and different cell-cycle mutants as well), and the computational results are compared to the known experimental behaviour of the different strains. The other one is time-lapse microphotography: cells are grown on the surface of an agar pad in a thermostated photomicroscope, and we can later study the growth pattern of length and diameter of individual cells during their cycles, simply by a projector. The presented talk selects some important results of our lab on fission yeast cell cycle during the last decade.

PHENOTYPIC AND GENOTYPIC CHARACTERISATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM A TEACHING HOSPITAL OF DEBRECEN

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The rate of methicillin resistant *Staphylococcus aureus* (MRSA) strains of the University of Debrecen varied between 2.3-3.2 % until 2003, increased to nearly 4 % in 2004, and, despite the well-functioning surveillance system, exceeded 7 % in 2005. The 339 strains isolated from 102 patients in 2005 originated from 18 different clinics. When preparing the statistics, we have taken only the first isolate from each patient into account. The rate of MRSA strains was very high at certain clinics, such as Surgery, Pulmonology and Pediatrics. The distribution of specimens in order of frequency was as follows: bronchial exudate 23. 5 %, wound exudate 17.6 %, nasal exudate 13.7 %, canule 9.8 %, pharyngeal exudate 9.8 %, and the number of haemoculture and abscess specimens was also relatively high (both 7.8 %). The laboratory has possessed phage typing results since 2002. The number of non-typable strains decreased over the years, while the percentage of mixed strains increased, and phage type V was gradually overtaken by type III. In 2005, more than half of the isolates (62.8 %) was mixed, a quarter of them was of type III, and 11.8 % was non-typable. No vancomycin or teicoplanin resistant strains were found. The clindamycin and erythromycin resistance was 99 %, respectively. The ciprofloxacin resistance was also very high, 97 %. Tetracycline sensitivity was 51 %. The trimethoprim/sulphamethoxazole showed better efficacy, sensitivity was 69 %. The sensitivity of amikacin was 61 %. Based on the pulsed-field gel electrophoresis (PFGE) patterns, we could demonstrate 3 types. Type A was the most frequent, followed by types B and C.

INFLUENCE OF Cd AND Pb ON THE ELECTRON TRANSPORT SYSTEM (ETS) DURING EMBRYONIC DEVELOPMENT OF COMMON WATER FROG (*RANA ESCULENTA* L.)

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Presence of living organisms (like fish and amphibians) is particularly important in water life. In aquatic ecosystems, vital functions of these organisms generate irregular pathways in matter circulation, which presumably determine or influence the characteristics of habitats.
Investigations involved the ETS- (Electron Transport System) activity of the samples, which indicated the maximal intensity of respiratory metabolism, because the heavy metal contamination of the experienced critical scale inhibited cellular respiration, while on the other hand the examination the chronic effects of heavy metals deposited in the embryonic development of common water frog (*Rana esculenta* L.) can be regarded as a corner-stone of assessing the ecological–conservational disaster and carrying out biomonitoring activities in the future.
In the course of evolutionary processes, living organisms have become able to give physiological responses to the changes of the natural environment, on both individual and community levels. These abilities provide the stability of ecological systems. However, the perpetuance of stable ecosystems is not ensured under such concentrated impacts as toxic effects of heavy metals, which are results of anthropogenic activities.

CULTURE COLLECTION (HNCMB) OF THE NATIONAL CENTER FOR EPIDEMIOLOGY

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The HNCMB (Hungarian National Collection of Medical Bacteria) was established in 1962. The HNCMB, listed under No. 258 in the World Data Center for Microorganisms, keeps bacteria that are mainly of medical importance. Situated at the National Center for Epidemiology (earlier National Institute of Hygiene) in Budapest, HNCMB has ready access to the wide-ranging expertise of specialists in clinical microbiology. The primarily function of the HNCMB is to supply cultures to public health and hospital laboratories, and to research and educational institutions. At present, the collection holds well over 4000 strains, representing about 80 genera with more than 250 species of bacteria, including 402 strains of *Escherichia coli*, 370 strains of different serovars of *Salmonella* ssp. and 149 strains of *Shigella* spp. The application of cultures includes quality control strains for testing of media, assays of antibiotics, testing of sterilization procedures and commercial diagnostic kits. Within the HNCMB, bacteria are identified using conventional biochemical characters in addition to Biolog MicroPlates and API 32 panels. The strains are stored by freeze-drying or freezing by different methods and temperatures. As of 2006, all new accessions were deposited with the employment of "seed lot system".

MICROBIOLOGICAL QUALITY CONTROL OF FOOD INDUSTRIAL SAMPLES BY REDOX-POTENTIAL MEASUREMENT

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The presentation introduces results of the newly developed redox-potential measuring technique (presented at the 1st Central European Forum for Microbiology, October 26-28, 2005 in Keszthely, Hungary) in the microbiological inspection of some fields of the food industry: i. determination of total count and number of Enterobacteriaceae in raw milk; ii. determination of total count in raw meat; iii. determination of microbiological contamination of surfaces. The rapid microbiological method is based on the linear relationship between the detection time (TTD) and the initial lgN value of the microorganisms tested. These calibration curves proved to be strictly linear in all cases in a very wide range (10^1-10^8 CFU/ml) In the case of raw milk with a typical contamination of 10^4-10^5 CFU/ml, the total count could be determined in 7-8 hours by the new technique, in contrast to classical plate counting, which requires a 3-day incubation. Similarly to raw milk examination, the determination of aerobic total counts prescribed in 2.1 of the European Commission Regulation No 2073/2005 on microbiological criteria for foodstuffs, lasts from 6 to 12 hours depending on animal species and form of meat (carcass, minced etc.). During the hygienic inspection of surfaces, the number of *Salmonella*, *Escherichia coli* and total count were determined with the classical swab-method and using the redox-measurement technique. As a conclusion, we have found that the results obtained by the redox-potential measurement had higher reliability than those of the classical evaluation of swab methods.

EXPRESSION PATTERN OF CD46 ISOFORMS IN OTOSCLEROTIC AND NON-OTOSCLEROTIC STAPES FOOTPLATES

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CD46 is an integral complement regulatory protein ubiquitously expressed on nucleated human cells. It protects host cells against complement-mediated lysis and may have signalling functions that modulate cellular responses. In addition, the CD46 protein can act as a receptor for the measles virus and the host range of this virus may be determined by the specific CD46 receptor on the surface of primate cells. Due to alternative splicing, variable isoforms of CD46 are expressed in a tissue-specific manner. Otosclerosis is a special bone dystrophy of the otic capsule causing conductive and sensoneural hearing loss. Persistent measles virus infection of the temporal bone is supposed to be the main etiological factor in otosclerosis.
Nucleic acid was extracted from stapes footplates of otosclerotic (N = 60) and non-otosclerotic (N = 30) patients. Measles virus RNA was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR). The presence of CD46 isoforms was detected by nested RT-PCR amplification of CD46 RNA. Additionally, the specific transcript variants of CD46 were identified by digestion with restriction endonucleases.
At least five different CD46 isoforms (a, c, d, e and f) could be detected in the studied samples. By our preliminary results, both otosclerotic and non-otosclerotic stapes footplates expressed the same CD46 variants, we only found a difference in the expression level of isoform f, which had a higher expression level in the otosclerotic specimens.
The presence of measles virus derived RNA was shown in all clinically and histologically otosclerotic stapes footplate specimens. The frequent prevalence of certain CD46 variants in the otic capsule might explain the organ specificity of otosclerosis and the localised susceptibility to measles virus.

HUMAN PAPILLOMAVIRUS INFECTION AND MUTATIONS OF THE P53 TUMOUR SUPPRESSOR GENE IN ORAL SQUAMOUS CELL CARCINOMA

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Squamous cell carcinoma is the most common malignant tumour of the oral cavity. Etiological factors include primarily chemical carcinogens (alcohol consumption, smoking), but certain tumour viruses were also proposed to be etiological factors. We examined the presence of HPV DNA and mutations of p53 in primer tumour tissue samples and exfoliated cells from clinically healthy mucosa of 50 patients suffering from oral squamous cell carcinoma. HPV detection and genotyping was performed using the MY/GP consensus PCR and restriction enzyme analysis of the amplicons. Mutations of the p53 gene were studied by means of amplification of exons 5-8 coding for the DNA-binding domain using exon-specific primers, followed by single strand conformation polymorphism (SSCP) analysis of the amplified exons. In case of exon 8, amplimers exhibiting SSCP pattern different from that of the control were sequenced.
HPV-specific sequences were found in 50% (25/50) of tumour samples of patients, high (HPV16, 18, 31, 33) and low (HPV11) risk genotypes were demonstrated in 19 cases and five cases, respectively, in one case we could not determine the HPV genotype using restriction analysis. HPV was present in healthy mucosa in case of four patients; the genotype of the viruses from tumour tissue and the corresponding mucosal cells was the same. SSCP patterns different from those of the control were found in three, twelve and seven cases in exon 5, 6 and 8, respectively, while in exon 7 no differences were found. With the exception of exon 6, these differences were not detected in corresponding healthy mucosal cells. All of the 22 pattern differences were found in p53 of different patients, out of which eleven was HPV positive and eleven HPV negative. Sequence analysis of differently patterned

exon 8 (14452-14588 bp) amplimers revealed two mutations, a guanine insertion at position 14587, and an adenine to guanine replacement causing glutamine to glycine amino acid change at position 14527. Both patients were HPV negative. Sequencing of amplimers with different patterns from the other two exons is currently being performed. Regarding the high (50%) HPV prevalence in tumours, the contrasting low prevalence in healthy mucosa, together with the predominance of high-risk genotypes suggest that HPV plays some role in the development of oral squamous cell carcinoma. Similarly, mutations of the p53 gene may act as predisposing factors. Further investigations are needed to confirm these results and to clear up the connection between HPV carriage and mutations of the p53 gene as well as their impact on survival.
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HUMAN PAPILLOMAVIRUSES IN ORAL LEUKOPLAKIA AND ORAL LICHEN PLANUS

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Human papillomaviruses (HPVs) can infect not only the anogenital region, but mucosal surfaces of the upper airways and the oral cavity as well. Consequently, they may play a role in the development and malignant transformation of praemalignant lesions of the region, e.g. oral leukoplakia (OL) and oral lichen planus (OLP), as well as in the development of head and neck cancers. We studied the HPV prevalence in exfoliated cells collected from lesions and clinically healthy mucosa of 23 OL and 66 OLP patients. Prevalence data were compared with those of a control group of 66 healthy individuals. HPV was detected by the MY/GP consensus nested PCR, and, we performed restriction enzyme analysis of amplimers for genotyping. HPV prevalence between groups was compared using chi-square tests, correlation between clinical manifestation and HPV carriage, age, and gender was analysed by means of logistic regression.
In groups with OL and OLP, we detected HPV significantly more frequently ($p<0.001$) than in the controls (47.8% and 33.3% *versus* 3.0%, respectively). Dominantly high risk genotypes (HPV16, 18, 33, 51) were found in both patient groups, while controls carried the low risk HPV11 and the high risk HPV16. The risk of lesions with higher oncogenic potential (atrophic, erosive forms) was higher in the younger age group (<50 years), in males and in HPV positive patients (OR*age* = 6,25, Or*gender* = 2,40, OR*HPV* = 2,50), but none of these correlations was significant, most probably due to the low number of cases. In case of OLP, none of these parameters increased the risk of development of the more serious clinical forms. In conclusion, beside chemical carcinogens (alcohol consumption, smoking), the role of HPV is also presumable in the aetiology of premalignant lesions of the oral cavity, but this needs to be confirmed by further studies. Further studies with larger patient numbers are also required to assess the utility of HPV carriage as a prognostic indicator.
Acknowledgement: The work was supported by OTKA F046749 and OTKA T046588 grants.

MOLECULAR FINGERPRINTING IN MICROBIAL TAXONOMY

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Human fingerprints have been used for personal identification for more than 100 years. In the second half of the 20th century, due to the development of genetics and molecular biology, the use of

molecular fingerprinting techniques (e.g. repetitive regions of the human genome) have become more and more widespread in criminological identification.
In microbial taxonomy, identification is also one of the main challenges and, similarly to modern criminology, molecular biology methods are widely used nowadays. The first step of a common microbial taxonomy research is generally the screening of huge strain collections. This step is often carried out with the comparison of fingerprints derived from different cell components (e.g. genomic DNA, fatty acids, quinones), then the final identification of the representative strains is done by sequence analysis of the 16S rRNA gene, which is the universal molecular name-tag of microbes.
Modern microbial ecology proved that only the minority of microbial community members could be identified with cultivation-based microbial techniques. Therefore, cultivation-independent methods suitable for fingerprinting of whole communities have emerged and become prevailing in environmental microbial research.
In my presentation, I would like to present the most commonly used chemotaxonomical and nucleic acid-based fingerprinting techniques, such as PLFA analysis, comparison of respiratory quinones, methods amplifying repetitive genomic structures (REP-PCR, BOX-PCR, ERIC), separation processes based on secondary structure (DDGE, TGGE, SSCP) and DNA sequence (e.g. restriction digestion-based methods). Beyond reviewing the different methods, the advantages and limitations of each technique will be demonstrated by the presentation of practical examples.

COMPETITIVE INTERACTION BETWEEN LACTIC ACID BACTERIA AND FOOD-BORNE PATHOGENIC/SPOILAGE BACTERIA ON SURFACES

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Lactobacilli are known for their ability to inhibit growth of spoilage and pathogenic microorganisms by producing antimicrobial compounds and competing for available nutrients or attaching sites. Therefore, lactobacilli could play a role in controlling colonisation of harmful bacteria on surfaces in the food industry and - as probiotics - in the gastrointestinal tract.
The adhesion ability of altogether twelve *Lactobacillus* strains of dairy origin was tested on stainless steel and human intestinal cells in vitro. Two strains with good adhesion capacity were chosen for further experiments: on stainless steel, the impact of *Lactobacillus delbrueckii* ssp. *bulgaricus* was investigated on *Listeria monocytogenes* and *Pseudomonas fluorescens*; on epithelial cells, the interaction between *Lactobacillus casei* ssp. *pseudoplantarum* and *Escherichia coli* was examined. Cell counts of adherent bacteria were determined by plating on selective media and with microscopic enumeration: on stainless steel surface, microbes were stained with fluorescent dyes and Gram-staining was used on epithelial cells.
No evidence was found that *Lb. delbrueckii* ssp. *bulgaricus* hinders the attachment of *Ps. fluorescens* or *L. monocytogenes* to stainless steel, in contrast, we found that the investigated *Lactobacillus* strain significantly ($p<0.05$) aided the attachment of these bacteria. It has been noted that *Lb. delbrueckii* ssp. *bulgaricus* forms chains and often clumps and these aggregates attach to the stainless steel coupon. *P. fluorescens* and *L. monocytogenes* cells stick to or between the lactobacilli cells (coaggregation), and this phenomenon can be accounted for the increased bacterial adhesion in mixed cultures. In case of adhesion to the epithelial cells, the two detection methods gave different results: plate counting did not indicate a significant difference in bacterial attachment in case of single and

mixed cultures. However, the microscopic analysis showed that lactobacilli and *E. coli* mutually aid the adhesion. The difference in the detection methods is probably due to bacterial clump formation. In the background of the mutual increase in bacterial counts in the mixed culture, coaggregation or the alteration of biding sites on the epithelial cell surface could stand.
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PEPTIDE-ANTIBIOTICS PRODUCED BY MEMBERS OF THE GENUS *TRICHODERMA*

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The efficiency of antimicrobial chemotherapy is increasingly challenged by the emergence of pathogenic strains exhibiting high levels of antibiotic resistance. Therefore, it is very important to search for novel compounds produced by living organisms, such as peptide-type antibiotics, which are undergoing intensive investigations. Detailed knowledge of their properties may provide important information about their potential applicability as drugs against pathogens.
Peptaibols and related peptaibiotics (PrPs) are linear, amphipathic polypeptides constituting a family of peptide antibiotics of fungal origin, whose number is constantly growing since the first member, alamethicin, was reported from cultures of *Trichoderma viride* in 1966. A considerable part of these antibiotics was isolated from the soil borne filamentous fungal genus *Trichoderma*, the members of which are well-known as potential biological control agents. The PrPs of *Trichoderma* species are composed of several amino acids (7-20) containing nonproteinogenic amino acid residues as characteristic building blocks in a number of cases. For these peptides, the N-terminal residues are usually acetylated, and an amino alcohol is linked by a peptide bond at the C-terminal end. Large multifunctional enzymes, known as peptide synthetases, assemble these molecules by the multiple carrier thiotemplate mechanism from a remarkable range of precursors that can be N-methylated, acylated or reduced. These types of antibiotics show interesting physico-chemical and biological properties, such as the formation of pores in bilayer lipid membranes as well as antibacterial, antifungal and, occasionally antiviral activities, and may elicit plant resistance. In consequence of the high Aib content, they form helical structures, either a-, 310- or mixed a/310-helices and in the case of (Aib-Pro)n sequences, they show a b-bend ribbon spiral helix-like motif. These helical structures play a role in their widespread biological activity. These naturally occurring, small-sized molecules are potential candidates for computational simulations of ion binding and translocations. PrPs are valuable model systems providing insight into complex processes including the synergistic biocontrol mechanisms of *Trichoderma* strains.
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NEW GENOTYPES OF *TRICHODERMA* DETECTED IN HUNGARIAN SOIL SAMPLES

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The identity and diversity of *Trichoderma* strains isolated from roots of winter wheat grown in agricultural fields were examined based on morphological and molecular characters, and a detailed phylogenetic analysis was performed. Morphological data were collected by measuring the structure and shape of conidiophores, phialides and conidia as well as colony morphology and growth characteristics. In the case of the isoenzyme analysis, 38 electromorphs belonging to 26 electrophoretic types were determined based on the running patterns of five selected enzymes. The ITS 1 - 5.8S rRNA- ITS 2 region was amplified by polymerase chain reaction (PCR) and applied as a marker to identify the strains at the species level with the aid of oligonucleotide barcodes. Data collected during the taxonomical investigations were analysed phylogenetically with the methods of parsimony, distance matrix and Markov chain Monte Carlo in separate analyses of the ITS 1 and 2 sequences and the isoenzyme data. Finally, in order to identify the taxonomic position of all examined isolates, a combined phylogenetic analysis was carried out with the Bayesian method using the Markov chain Monte Carlo algorithm.
In the analysis, five well separated *T. longibrachiatum* strains belonging to the section *Longibrachiatum* were used as the outgroup. Fourty-one strains proved to be *T. harzianum* representing 4 ITS-genotypes that have already been described, and 4 further Hungarian genotypes that were firstly detected during this study. The isolated *T. virens* strains (31) could be classified into 4 genotypes, one of them was identical with the ex-type, while three proved to be novel. The *T. atroviride* isolates (9) formed a twin-branch of the ex-type strain on the phylogenetic trees. Three isolates proved to belong to *T. oblongisporum*, four to *T. brevicompactum*, one to *T. spirale*, while five isolates to two genotypes of *T. rossicum*.
Fourteen strains appeared to be completely separated from the species described previously in the literature. Nine of them proved to be members of the *Rufa* clade, while 5 belonged to the Stromatica clade. Based on our investigations, these isolates probably represent five new species. Furthermore, to our knowledge, the present study is the first to demonstrate the occurrence of *T. virens*, *T. rossicum*, *T. spirale*, *T. brevicompactum* and *T. oblongisporum* in Hungary.
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PEPTIDE ANTIBIOTICS PRODUCED BY HUNGARIAN *TRICHODERMA* ISOLATES

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Species of the imperfect filamentous fungal genus *Trichoderma* with teleomorphs belonging to the Hypocreales order of the Ascomycota kingdom are of great economic importance as sources of enzymes and antibiotics, as plant growth promoters, decomposers of xenobiotics, and as commercial biofungicides. The peptaibols and related peptaibiotics (PrPs) are secondary metabolites constituting a family of fungal peptide antibiotics, whose number is constantly growing since the first was reported from cultures of *Trichoderma viride*. These compounds are linear, amphipathic polypeptides composed of several amino acids. They usually contain several nonproteinogenic amino acid residues,

which are important in forming their characteristic structures.
One hundred and twenty *Trichoderma* strains were isolated from roots of winter wheat grown in agricultural fields of Southern Hungary. The identity of species was examined based on morphological and molecular characters. The presence of PrP-producing strains among the isolated *Trichoderma* strains were obtained with biological tests and the antibiotics were partially purified using a multistep chromatography procedure involving exclusion chromatography, adsorption chromatography and thin-layer chromatography. About 20% of the isolates proved to produce PrPs, which showed high variability among the taxonomical order of the examined strains. The antibacterial activity of the compounds were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus* and *Escherichia coli*, while the antifungal effects were recorded against *Fusarium oxysporum*, *F. culmorum*, *Rhizoctonia solani* and *Pythium debaryanum*.
Acknowledgement: This work was supported by the Hungarian Government with the grant OM-00083/2004. L. K. is a grantee of the Bolyai János Scholarship of the Hungarian Academy of Sciences.

GENETIC CHARACTERISATION OF AVIAN INFLUENZA VIRUSES ISOLATED IN THE 1970S IN HUNGARY

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The emergence of the Asian H5N1 avian influenza (AI) has directed attention to some of the earlier cases, particularly those that were caused by the H5 and H7 subtypes. In order to get some idea on the significance of these outbreaks, a number of early AI-strains (collected by Dr. János Tanyi and maintained in the Veterinary Institute, Debrecen) have been subjected to phylogenetic analysis.
In the 1970s, these viruses caused relatively severe disease in ducklings, turkeys and guinea fowls but occasionally wild ducks were affected, too. Symptoms included general and respiratory signs (with 10-30% mortality) and reduction of egg production (between 10-60%) in layers. The serological examinations placed isolates into subtypes H4, H5, H6, H7 and H10.
Phylogenetic analysis of the nucleotide sequences of the haemagglutinin (HA) gene of these AI-viruses, on the one hand, have confirmed subtype assignment obtained previously by serological methods. On the other hand, present studies classified these historical strains to the Eurasian lineage of the respective subtypes. Amino acid analysis of the proteolytic cleavage site of the HA showed that the early H5 subtype strains belonged to the low pathogenicity category. Studies are in progress to elucidate the epidemiological relationship of the early AI-viruses isolated in Hungary.

THE ROLE OF PHOSPHORYLATION OF THE SEP1 FORK HEAD TRANSCRIPTION FACTOR IN THE REGULATION OF CYTOKINESIS IN FISSION YEAST

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Fork head transcription factors are characterized by a conservative DNA-binding domain, which is a modification of the helix-turn-helix motif. This type of regulator was originally identified and

designated for fruit fly fork head mutant fifteen years ago, but many counterparts have been discovered from many eukaryotes during those fifteen years. These include mammals (human), other multicellular and also unicellular organisms. The information available on their function indicates a pivotal role in the regulation of cell cycle and cell differentiation and also in embryonic development.
Functions of fork head genes have also been analyzed in the budding yeast *Saccharomyces cerevisiae,* a model system which was proved to be applicable to study gene function. Results of these studies substantially contributed to the understanding of the function that fork head genes play in the regulation of cell cycle and differentiation.
Another crucial model organism for the study of gene function is the distantly related fission yeast *Schizosaccharomyces pombe,* which has been successfully employed in cell cycle research for decades. The genome of this yeast contains four fork head type regulators; three of them (*sep1*, *fkh2* and *fhl1*) were identified and characterized to different extent by our laboratory. This presentation is focused on the results obtained from the analysis of the sep1 gene and protein. We showed that mutation and deletion of this gene resulted in the omission of cell separation, which normally occurs by the degradation of the primary septum.
Further whole genome microarray analysis disclosed that *sep1* was a regulator of many genes involved in the control of mitosis and cytokinesis, although the mechanism of regulation is not known. We wanted to elucidate this control mechanism with emphasis on the role of sep1. The sep1 mRNA does not fluctuate during cell cycle, indicating that it is not regulated at the RNA level, which raised the possibility of the control by post-translational modification. Indeed, sep1 protein is subject to phosphorylation as revealed by detecting the protein and subsequent phosphatase assays. Furthermore, the pattern of phosphorylation changes as the cell enters into mitosis, suggesting that sep1 modification by phosphorylation is important to accomplish its function. Synthetic mutant alleles of *sep1* were created by *in vitro* gene manipulation to map regions of sep1 important for phosphorylation. Results of this analysis and a possible role of sep1 phosphorylation in the regulation of cytokinesis will be presented.

REAL-TIME PCR ASSAYS ON CYTOKINE RESPONSE TO *SALMONELLA* IN VITRO AND ITS RELATION TO INVASIVENESS

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Invasion of epithelial cells is essential for pathogenesis by the so called invasive serovars of non-host specific *Salmonella* (S. Enteritidis and S. Typhimurium). These serovars are known to posses and activate a wide array of virulence genes during epithelial cell invasion, several of which are residing on the SPI-1 or are acting as effectors. However, in this respect, much less is known about less invasive serovars (i.e. S. Hadar, S. Infantis), which also seem to be of significance in Hungary, especially in broiler flocks.
The aim of these studies was to investigate the *in vitro* invasive abilities of some representative chicken isolates of *Salmonella* Typhimurium, Enteritidis, Hadar and Infantis and to determine the expression of selected bacterial virulence genes. Furthermore, the cytokine response of Vero cells and chicken fibroblast cells to the above *Salmonella* strains was investigated. In further studies, deletion mutants of *S.* Hadar-18 were produced by the Lambda-Red (Datsenko and Wanner, 2000) and Overlap Extension (Steffan et al., 1989) mutagenesis techniques. The resulted mutants were tested for IL-8 and TNF-α response.
First, the expression variability of different *Salmonella* genes (*sipB*, *spvC*) during the invasion procedure was studied. For the above purpose, a Vero cell system and real-time RT (SybrGreen) PCR system were used. In comparison with the overnight broth added to the cells (pre-infection),

Salmonella gene expression outside (pre-invasion step) and inside the Vero cells (post-invasion step) was analysed. As a result, an accentuated *sipB* activity was found outside the Vero cells, but no or only a very slow response from the invaded bacteria. The expression of *sipB* was not related to the invasive ability of the given *Salmonella* strain. Secondly, real-time RT PCR system was also used to test the IL-8 and TNF-α response of Vero cells to different *Salmonella* serovars (*S.* Typhimurium, *S.* Enteritidis, *S.* Hadar) and some mutants (*S.* Hadar-18). The expression data resulted in a great variety among the different *Salmonella* Hadar-18 mutants in reference to their IL-8 stimulating capacity. Data indicate that invasion, gene expression and host interleukin response are not directly related.

TAXONOMIC INVESTIGATION OF *RHODOCOCCUS* STRAINS ISOLATED FROM OIL CONTAMINATED SITES OF HUNGARY

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In the last decade, almost one hundred bacterial strains were isolated by our group from oil contaminated soils and groundwater in different parts of Hungary. By phenotypical analysis, eight strains from this collection were identified to belong to the genus *Rhodococcus*. All the strains were Gram positive, aerobic, sensitive to lysozyme, producing yellowish, orange or red pigments and having a thermal optimum around 30 °C. They were capable to degrade up to 30% of the diesel oil-crude oil mixture content of the medium in submersed culture after 120 hours of incubation.
According to electron microscopic examination of the uranyl-acetate stained preparations, cells were rod- and branched filament-shaped during the early growth phase (1 day), then fragmented into short rods or 1 µm diameter cocci during prolonged incubation (up to 8 days), thereby completing the growth cycle characteristic to the members of the genus *Rhodococcus*.
Strains could not be identified at the species level with the use of conventional microbiological tests, such as resistance to antibiotics, utilization of different carbon sources, degradative tests, etc. 16S rDNA sequence comparisons were performed for the correct taxonomical positioning of the strains. The partial 16S rDNA sequence similarity of seven strains (AK35, AK36, AK37, AK40, AK41 and AK42) reached 99-100% to the corresponding rDNA sequences of type strains, while strain AK38 shared 99% 16S rDNA similarity to a yet undetermined *Rhodococcus* sp. AN22.
Based on these results, strains AK35, AK36 and AK42 were identified as *R. erythropolis*, strain AK37 as *R. pyridinivorans*, strain AK40 as *R. rhodochrous* and strain AK41 as *R. rubber*, respectively. Strain AK38 seems to be a good candidate for a new taxon as it shares only 94-97% similarity to the valid *Rhodococcus* species. Description of strain AK38 as a new species will be based on a polyphasic approach.
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PREVALENCE AND GENOTYPES OF ANELLOVIRUSES IN HUNGARY

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TT and SEN viruses belong to the *Anellovirus* genus. The aim of this study was to characterize TT

and SEN viruses carried by healthy individuals and patients suffering from hepatitis of unknown origin in Hungary. TTV DNA was detected by seminested PCR with the commonly used N22 primers. Twenty of the 108 sera taken from healthy persons and 115 of the 228 sera of hepatitis patients were found positive. The nucleotide sequences of 26 clones derived from 17 hepatitis patients and 15 clones from nine healthy persons were determined and a phylogenetic tree was constructed. Genotype 2 (group 1) was found to be the most frequent, but other group 1 genotypes and genotype 8 and 17 of group 2 were also detected. Mixed TTV infections were found in eight cases.
Sera of healthy persons were tested for SEN virus DNA by PCR. Fifty of the 100 sera of young males between 18 and 21 years of age and 59 of the 96 health workers of a hospital were found positive. Prevalence was extremely high in the surgery and the maternity ward. Co-infection with different SENV genotypes was quite common.
The sequence changes of SENV were also followed in the serum of a patient over more than eight years. Over the years, the number of variable positions grew to 18 (about 7% of the region tested), some of which resulted in changes of the amino acid sequence, and others were silent.

ISOLATION AND EXPRESSION OF THE *RHIZOMUCOR MIEHEI* β-GLUCOSIDASE GENE

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Rhizomucor miehei is a thermophilic zygomycete frequently used in biotechnological applications as consequence of its effective extracellular enzyme production (e.g. proteases, lipases).β-Glucosidases (β-D-glucoside glucohydrolases) constitute a group of well characterized, biologically important enzymes that catalyze the transfer of glycosyl group between oxygen nucleophiles. Filamentous fungi are considered as good producers of β-glucosidases. Although several fungal glucosidase genes have been isolated and analyzed, Zygomycetes are poorly characterized from this aspect. The aim of this study was to identify and analyze the *R. miehei* β-glucosidase gene (*bgl*).
β-glucosidase activities of several *R. miehei* strains were checked by different methods. Some *R. miehei* strains showed intensive enzyme activity. Degenerated β-glucosidase specific primer pairs were designed to conserved regions of fungal glycoside hydrolase family 3 genes and a 493 bp long fragment has been amplified by PCR from the genomic DNA of the *R. miehei*. The sequence of the amplicon was determined. The analysed fragment showed high homology with the C-terminal domains of β-glucosidases belonging to family 3. Based on this sequence, specific primers were designed for inverse PCR. The original fragment was lengthened in four steps to a 4063 bp long sequence, in which the β-glucosidase gene is 2826 bps encoding a 743 amino acid long protein. This is the first known β-glucosidase gene sequence from a fungus belonging to the class Zygomycetes.
Rhizomucor bgl showed the highest homology with the β-glucosidases of *Phanerochaete chrysosporium*, *Trichoderma reesei* and *Piromyces* sp. strain E2. For gene expression studies, two transformation vectors were constructed: the plasmid pTM1 contains the *bgl* gene under the control of the regulator sequences of the *Mucor circinelloides gpd1* gene, while the plasmid pTM4 harbours the promoter region of the *bgl* gene fused with a green fluorescent protein (GFP) gene. In lack of an efficient transformation system in *R. miehei*, genetic transformations were started in a heterologous system: PEG-mediated protoplast transformations were performed in an uracyl auxotrophic strain of the related *M. circinelloides*.
The resulted transformants maintained the plasmids in autoreplicative way. Induction of the *bgl* promoter by different substrates was studied in the *M. circinelloides* transformants harbouring the pTM4 plasmid. Strong fluorescence was observed only in the transformants growing on cellobiose containing medium. Fluorescence was most intensive in the growing tips of the hyphae and near the cell wall. Analysis of the transformants containing pTM1 is in progress.

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DETECTION OF FUNCTIONAL GENES IN AROMATIC HYDROCARBON DEGRADING BACTERIA

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The monocyclic aromatic hydrocarbons like benzene, toluene, xylene and phenol are toxic pollutants for higher organisms, but these compounds can serve as sole sources of carbon and energy for several microorganisms. Detection of catabolic genes and the knowledge of the degradation potential of a microbial community living in a BTX contaminated environment, are important for the selection of the right bioremediation method. Several microbes possess the genes of aromatic ring cleavage enzymes like catechol dioxygenases, but the diversity of these genes is so high, that it's not easy to detect them by PCR with universal primers. On the other hand, the activity of the mentioned enzymes can be easily detected spectrophotometrically. Enzyme activity measurements and the detection of the aromatic ring cleavage enzyme coding genes give us the chance to investigate the degradative capability of BTX degrading strains and the diversity of an aromatic hydrocarbon degrading bacterial community.

Hydrocarbon degrading bacteria were isolated from gasoline polluted soil samples. Bacteria were isolated from the samples by an enriched medium that contained BTX-compounds. Strains, which were able to use BTX compounds as sole carbon source, were identified by the 16S rDNA sequence comparisons. A *Rhodococcus erythropolis*, *Acinetobacter calcoaceticus, Streptomyces lateritius, Alcaligenes sp.* and a *Rhizobium* strain proved to tolerate high benzene, toluene or xylene concentrations, and grow on these compounds as sole source of carbon. The enzyme activity assays were carried out in the case of *Rhodococcus erythropolis*, *Acinetobacter calcoaceticus* and *Rhizobium* sp. In the case of these three strains, benzene and toluene induced the ortho cleavage pathway of the aromatic ring cleavage degradation and catechol 1,2-dioxigenase activity was detected. For the molecular analysis, the detection of the catechol 1,2-dioxygenase and the catechol 2,3-dioxygenase gene was chosen. To detect these genes in our strains, group specific PCR primers were designed instead of universal primers. The catechol 1,2-dioxygenase gene was detected in *Rhodococcus erythropolis* and in *Acinetobacter calcoaceticus*, and the catechol 2,3-dioxygenase gene was detected in *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Comamonas testosteroni* and in *Delftia acidovorans*. Moreover, with the designed primers, these genes were also detected in DNA samples isolated from a phenol degrading community and from gasoline contaminated soil samples. This result demonstrates the high specificity of our primers, and gives the possibility to monitor an aromatic hydrocarbon degrading community.

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COMPARISON OF PHENOTYPIC PROPERTIES OF *KLEBSIELLA* SPECIES ISOLATED FROM BLOOD AND URINE

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Klebsiella species are important opportunistic pathogens causing infections mainly in immunocompromised patients suffering from underlying diseases. It is estimated that *Klebsiella* spp.

cause 8% of all hospital acquired infections throughout the world. The prevalence of *Klebsiella* isolates is ranked 5th-7th (6-17 %) among pathogens causing urinary tract infections and 3rd-7th (4-15%) among blood stream infections. The aim of this study was to examine and compare the distribution of virulence factors of *Klebsiella* isolates originating from different types of infections. We examined the presence of particular virulence factors such as type 1 and 3 fimbriae, production of two different siderophores, and serum resistance. 44 blood culture isolates from septic patients and 83 urinary isolates from patients with significant bacteriuria (10^5 CFU/ml). Only patients with *Klebsiella* as the single isolate were selected. In both infection types, *Klebsiella pneumoniae* was the dominant species: (88,7%), {39/44} and (81,9%),{68/83}, while *K. oxytoca* was found (11.3 %), {5/44} and (18,1 %), {14/83} in the blood and urine cultures, respectively. Type 1 fimbria (mannose-sensitive haemagglutinin) was expressed by (50,0%), {22/44} of the isolates from blood cultures and (69,9%), {58/83} by the isolates from urine. In case of the blood culture isolates, (47,7%), {21/44} were positive for type 3 fimbria {mannose-resistant and Klebsiella-like haemagglutinin (MR/K)}, while (32,5%), {27/83} were presented with positive phenotype among the urinary strains. The siderophore production pattern showed high similarity in both isolate types. Enterobactin production was found in (88,6%), {39/44} among blood culture isolates and (88,0%), {73/83} among strains isolated from urine. Among blood culture strains, (2,2%), {1/44} showed aerobactin positivity, while (2,4%), {2/83} of the urine isolates produced this siderophore. The percentages of non-producers were (11,3%), {5/44} and (9,6%), {8/83} among the blood culture and urinary isolates, respectively. Serum resistance patterns could be ranked into seven categories on the basis of survivor counts in the three-hour incubation period of the experiment. Among the urinary strains, (91,6%),{39/44} belonged to the four sensitive categories ranging from very sensitive to moderately sensitive. Interestingly, a high proportion of the invasive blood culture isolates (88,6%), {39/44} also belonged to one of the four sensitive categories. Further characterisation by capsular typing and molecular analysis, including the magA status of the isolates, is planned to reveal possible relationships between the virulence phenotypes and the clonal types of Klebsiellae.

A NOVEL TYPE SULPHUR REDUCTASE IN THE HYPERTHERMOPHILIC ARCHAEON *THERMOCOCCUS LITORALIS*

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Thermococcus litoralis is a strict anaerobic, hyperthermophilic archaeon growing optimally at 85°C. It is an obligate heterotrophic microorganism capable of utilizing both peptides and carbohydrates as carbon and energy sources. Elementary sulfur stimulates its growth with a concomitant evolution of H_2S. In Thermococcales species, the hydrogenase and sulfur reductase enzymes have a basic role in the maintenance of redox equilibrium in the cells, and the excess electrons formed during the fermentative metabolism are removed as hydrogen and/or H_2S.

Thermococcales species usually contain two cytoplasmic and one or two membrane bound NiFe hydrogenases. The latter are responsible for the hydrogen evolution, while the soluble NAD(P)-reducing enzymes are involved in the fine-tuning of the redox balance. In many strains, upstream from the soluble hydrogenase II operon, the genes encoding a heterodimeric sulfur reductase (sulphide dehydrogenase) were identified. In *T. litoralis*, upstream from the appropriate hydrogenase operon, a polycistronic region containing four open reading frames was recognized. Their putative gene products were not similar to the dimeric sulphide dehydrogenases, however, three out of the four ORFs resembled various eubacterial oxidoreductases and one of them had archaeal homologues. Comparison of the genomic context of these and the homologous genes revealed this gene arrangement to be unique. A similar locus was found only in the genome of *Thermococcus*

kodakariensis. The protein encoded by the largest *orf* is similar to that rare eubacterial NuoG-type enzyme that contains an integrated glutamate synthase (GltD) domain. For detailed biochemical analysis, this *orf* was overexpressed in *Escherichia coli* and purified by an affinity tag. The recombinant protein had sulfur, polysulfide, 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB), O_2 and benzyl viologen reducing activity but reverse reaction could not take place. The enzyme was able to utilize NADPH specifically as electron donor.
Based on the activity measurements, we propose that this novel type sulfur reductase might participate in the maintenance of the redox balance in the *T. litoralis* cells. This physiological function corresponds to the role of the known sulphide dehydrogenases in other Thermococcales, although the two enzymes are apparently dissimilar. Moreover, the enzyme can also play a role in the protection of the cells against oxidative stress based on its O_2 reducing activity.

COMPARISON OF MICRONAUT-CANDIDA, A NEW YEAST IDENTIFICATION SYSTEM WITH API ID32C

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Identification of *Candida* species is extremely important for the clinical mycologists. The performance of the identification system MICRONAUT-Candida (Merlin Diagnostika GmbH) was compared with API ID32C (BioMerieux). MICRONAUT-Candida is based on testing 21 biochemical reactions. Evaluation is automated, results are available in 24 hours.
128 clinical and reference strains of 18 different yeast species were tested with the MICRONAUT-Candida system, including the most common human pathogenic *Candida* species, i.e. *C. albicans* (19 isolates), *C. tropicalis* (14 isolates), *C. glabrata* (17 isolates), *C. krusei* (23 isolates), *C. parapsilosis* (14 isolates), *C. inconspicua* (12 isolates), as well as less frequently found *Candida* species *C. kefyr* (6 isolates), *C. famata*, *C. lusitaniae*, *C. guilliermondii* (4 isolates each), *C lipolytica* (3 isolates), *C. dubliniensis, C. rugosa* (2 isolates each), *C. norvegensis, C. catenulata* (1 isolate). Single isolates of the medically important yeast species *Cryptococcus neoformans, C. humicolus, Rhodotorula spp*. were also tested. Results of the API ID32C system were regarded as reference. In case of discrepancies between the two methods, both tests were repeated.
MICRONAUT-Candida never yielded unsuccesful identification (results of *Candida spp*. or unidentified yeast). In 92.2% (103/128) of the cases, identification with MICRONAUT-Candida gave the same results as that with API, including all *C. albicans, C. tropicalis, C. glabrata, C. krusei, C. parapsilosis, C. inconspicua, C. kefyr, C. lipolytica* and *C. lusitaniae* isolates and the single *C. catenulata, C. neoformans* isolates. Three of four *C. guilliermondii* isolates were identified with excellent results, while the remaining one (0,7% of all isolates) repeatedly exhibited questionable identification. Out of the consistently identified 103 isolates, 16 (12.5%) required the performance of the additional tests recommended by MICRONAUT-Candida, including all 12 *C. inconspicua* isolates, the *C. neoformans* and one of the *C. rugosa* isolates. Inconsistent results were found at 6.3% (8/128) of the isolates, i.e. the *C. norvegensis* and *C. rugosa* reference strains, one *C. dubliniensis* and the *C. humicolus* isolate as well as all four *C. famata* isolates, which were surprisingly identified as *C. lusitaniae* with 'excellent reliability'. It can be concluded that MICRONAUT-Candida and API ID32C equally identified all common *Candida* species, discrepancies could be observed with some of the rarer yeasts. Consequently, MICRONAUT-Candida can be a faster alternative of the widely used API ID32C identification system.

MOLECULAR COMPARISON OF PERSISTENT *CHLAMYDOPHILA PNEUMONIAE* INFECTIONS IN IN VITRO MODEL SYSTEMS

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Chlamydophila pneumoniae (*C. pneumoniae*) is an obligate intracellular bacterium causing respiratory diseases in humans and may play a role in the development of atherosclerosis and related complications. The persistent infections with *C. pneumoniae* are especially linked to atherosclerosis. There are several ways to induce persistent chlamydial infections *in vitro*, including the withdrawal of amino acids or glucose from the culture medium, or the treatment of the infected cultures with interferon-g or certain antibiotics. The molecular characteristics of persistent chlamydial infections are not well defined. In our study, as *in vitro* models for persistent infections, susceptible HEp-2 cells infected with *C. pneumoniae* and treated with penicillin, and semipermissive human monocyte-derived dendritic cells infected with *C. pneumoniae* were used. Human dendritic cells may function as host cells for *C. pneumoniae* infections. We compared the expression profile of some of the important chlamydial genes at different time points in these persistency models. 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 and ftsW genes (involved in binary fission). We found that the infectious bacterium production and the expression patterns of these genes were different in the *in vitro* persistency models.

COMPARATIVE CHARACTERISATION OF MINISATELLITE SEQUENCES FROM *BOTRYTIS CINEREA* POPULATIONS IN THE EGER AND TOKAJ WINE REGIONS

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Botryotinia fuckeliana (anamorf: *Botrytis cinerea* Pers.) is a haploid, heterothallic, ascomycetous fungus that causes both grey mould and noble rot on grapevine and destroys many other important crops in the temperate zone worldwide. On the one hand, presence of grey mould on the grape berries induces a significant growth spillage and quality decrease, on the other hand, infection can result in a highly prized, sweet, special quality wine called aszú. Information on the populations and knowledge on the size and structure of *B. cinerea* populations are essential for the effective and economic protection against grey mould and for the production of quality grapes and wine.
Based on these considerations, a first-of-its-kind study to characterize *B. cinerea* populations of two Hungarian wine producing districts was undertaken. The isolates of grapevine berry-growing *B. cinerea* were collected from various locations of the Eger and Tokaj wine regions. Individual strains were obtained by single-spore isolation. Characterization of their genotype was done by analyzing MSB1 minisatellite sequences located in the intron of the ATP synthase. Its 37-bp repeat unit is AT-rich and found at only one locus within the genome.
Based on the minisatellite sequences, there are at least three subpopulations in the Eger, and two subpopulations in the Tokaj wine district, both of which are dispersed within the territory. We could

not find any geographical preference for the different genotype groups and *B. cinerea* populations of the Tokaj wine district. Their MSB1 pattern was identical to those isolated from the Eger wine region, but were clearly different from the sequences detected by other researches in France.

COURSE ON ALTERNATIVE ENERGY SOURCES WITH SPECIAL REGARD TO MICROBIOLOGICAL PROCESSES FOR ENGINEER STUDENTS

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The question of energy demand has become an extremely important problem all over the world. It seems that energy demand should be covered in an increasing ratio from renewable sources. The fossil sources – due to their disadvantages – have to be substituted with alternative energy sources. In this work we give an overview on alternative energy sources, compare them from various aspects, and outline a global, comprehensive picture. According to our knowledge, complex course material on all areas of alternative energy sources has not been compiled so far in Hungary, since most of the experts deal with their particular area of interest. We believe that engineers of the next generation should have a correct, complex knowledge on this area. The course givs a comparative survey on all types of alternative energy sources (including solar, wind, water, geothermic, biomass, …etc.), and energy storage systems, drawing special attention to microbiological processes. Having this complex view on alternative energies, ideas on integrated and/or combined processes may be risen providing novel, reliable and economically more attractive solutions for long-term operations.

MONITORING THE CHANGES OF MICROBIOTA DURING OYSTER MUSHROOM (*PLEUROTUS* SP.) SUBSTRATE PRODUCTION

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Oyster mushroom (*Pleurotus* sp.) is the second largest commercially produced mushroom after button mushroom in Hungary, whose production is in the medium range in the European Union. The success of mushroom production is mainly based on the quality of the substrate. Substrate production has three phases. Shredded and moistened straw is composted under aerobic conditions, which serves as the pretreatment of the straw. The second step is the pasteurization in tunnels, which kills pathogens. Finally, during maturation, it is conditioned under thermophilic conditions. Although the cultivation of oyster mushroom has a long tradition, there is insufficient knowledge about the microbiota of the substrate, which is an important parameter of its quality. Our aim was to isolate the dominant microbes from the substrate and characterize the substrate with microbiological fingerprinting methods. Microbes were isolated from the first phase on oatmeal agar and on agar-media prepared with the extract of the substrate. All samples were incubated in parallel at mesophilic and thermophilic conditions. After purification of the isolates, a collection of 113 strains was established. The strains were grouped with BIOLOG-plates for further investigations. For the fingerprinting methods, total DNA were isolated from the straw, the moistening water, the leechate and from seven samples of substrate-production. Then, different regions of the DNA were amplified: the 16S rDNA region with universal eubacterial primers, and the ITS region with fungal primers. The eubacterial 16S rDNA were investigated with terminal restriction fragment length polymorphism (T-RFLP). The

fungal ITS region was investigated with the automatic rRNA intergenic spacer analysis (ARISA). The results were analyzed by principal component analysis (PCA). Our results show that the different straw samples had a similar bacterial but a different fungal community. The moistening water and the leechate had unique microbiota, which differed from all other samples and also from each other. During the substrate production, a clear trend was observed in the changing bacterial community, which was absent from the fungal microbiota.
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EPIBATHE – AN EU FUNDED STUDY ON HEALTH EFFECTS OF BATHING WATERS

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EPIBATHE is an EU funded international project involving a partnership of 6 EU research institutions for the investigation of the health effects of bathing in natural, untreated fresh and marine waters. The project aims to assess whether current EU standards provide suitable protection for bathers and it addresses the need for further epidemiological data as noted in Article 14 of the EU Bathing Water Directive published in 2006. As well as a detailed meta-analysis of existing data, Epibathe will initiate a series of 8 field studies, 4 of which are being conducted in Hungary with 4 identical studies in Spain in the 2006 and 2007 bathing seasons. The protocol employs a randomized controlled trial of the same type, which underpins the risk assessment in the recent WHO Guidelines for Safe Recreational Water Environments. This protocol was previously implemented in the UK and Germany. The current presentation summarizes the experiences of the first 2 studies in Hungary.
The first study was conducted on 16th July, 2006 in Dömsöd, at the Ráckeve Danube branch. The second study was on 13th August, 2006 in Dombori, at the Fadd-Dombori dead channel of the Danube. Both selected beaches were designated bathing sites with several years' history of good water quality. Volunteers were recruited for participation in the study. The target volunteer number in 2006 was 1000 for the two studies, but the number of participants completing the studies exceeded expectations. Volunteers were randomized into bather and non-bather groups. Bathers spent 10-15 minutes in the water on the study day, immersing their heads in the water several times.
Health effects were assessed by four questionnaires (1-3 days before exposure, on the exposure day, then 1 week and 3 weeks after exposure). Simultaneously, water samples were taken every 20 minutes during the bathing period in 6 different sampling locations to characterize microbial water quality. Samples were analyzed for enterococci, *E. coli* and somatic coli phage by standard methods. A unique water quality was thus attributed to each bather, which was measured at the time and location when they bathed. Final conclusions on the correlation of detected water quality and the observed health effects are to be drawn after all 8 studies have been completed and will be available in 2008. Hungarian studies were successful, serious illness caused by bathing was not recorded.

EXOGENOUS INTERFERON-GAMMA RESTRUCTURES THE ANTI-CHLAMYDIAL TRANSCRIPTOME RESPONSE OF MURINE EPITHELIAL CELLS

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Chlamydia trachomatis is an obligate intracellular pathogen that primarily infects columnar epithelial cells of the eye and the oculogenital tract. It is well described that the main cytokine involved in the clearance of chlamydia is the interferon-gamma (IFN-gamma) produced locally by T cells and macrophages, however, a genome-wide detailed screen of the IFN-gamma effect on the chlamydia infected host epithelial cells has not been performed.

We treated BM.12.4 murine oviduct epithelial cells with *Chlamydia trachomatis* serovar L2 alone and in combination with IFN-gamma, and explored the host transcriptome changes using the Affymetrix Mouse Genome 430A 2.0 whole genome array 24 hours post infection. Functional gene classes that were altered after chlamydia infection alone included regulation of transcription, antigen processing and presentation, chemotaxis and signal transduction.

The IFN-gamma and chlamydia infection together had a significantly deeper effect on the host gene expression. The host upregulated genes were related to similar functional classes as with the chlamydia infection alone, however, the extent and number of changes were remarkably higher. IFN-gamma enhanced the expression of key genes that were involved in the anti-chlamydial innate and adaptive immune responses, including chemokines: CXCL1, CXCL2, CXCL5, CCL2, CCL7, CCL20, genes involved in antigen recognition and presentation: TLR2, TLR3, H2-T23, H2-L, PSMB8, PSMB9 and genes involved in the intracellular elimination of different pathogens: IRG-1, TGTP, IIGP, GBP2, MX1.

The exogenous IFN-gamma had a dramatic effect on the host downregulated gene set. The chlamydia infection alone resulted in the downregulation of 27 host genes, while the combined treatment of the host cells with IFN-gamma and chlamydia increased this number to 490. This significant downregulating effect of IFN-gamma was only observed when it was used in combination with chlamydia infection; IFN-gamma treatment alone downregulated only 5 host genes. Interestingly, the simultaneous presence of IFN-gamma and chlamydia resulted in the downregulation of several genes related to the Golgi system and Golgi-ER transport, amino acid and lipid metabolism. These cellular processes are required for efficient chlamydial growth, therefore, shutting these systems down can inhibit the intracellular replication of the pathogen. Our results show that the locally produced IFN-gamma alters multiple, potentially anti-chlamydial metabolic pathways of the chlamydia infected host epithelial cells and has a significant enhancing effect on the epithelium-mediated natural and adaptive local immune responses to chlamydial infection.

EFFECTS OF MEDICINAL PRODUCTS FROM NATURAL SOURCES ON PATHOGENIC FUNGI

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There is a tendency to treat patients by alternative medical treatment in the case of suspected fungal diseases. Real scientific control should be done to evaluate the effects of the used drugs. For this purpose, we have investigated several drugs that were produced from natural sources. The fungicidal effects and the effects of inhibition were examined. The tests were done on human pathogenic yeasts such as *Candida albicans*, *Candida krusei*, *Candida glabrata* etc. Fungicidal effects were tested by the fungal species CFU (Colony Forming Unit) decreasing (in time and concentration) in water solution. The development of the colonies were tested in Sabouraud glucose agar in Petri dishes. In the case of oils (e.g. tea-plant oil, etc.) or volatile oil (e.g. thymol), we first made an alcoholic mixture which was diluted by water or physiological NaCl solution. The end-concentration of the natural compounds were 5000x or 500x. In these experiments, the product that was a composition of natural plant volatile oils used in foraging and veterinary showed a very high fungicidal effect. In further examinations, grapefruit-seed extract, lavender-oil, tea-plant-oil, geranium-oil, rosemary-oil and

orange-peel extract were tested. Three of them, tea-plant oil, geranium-oil and orange-peel extract, showed fungicidal effect; others had no effect on fungi. The fungistatic effects were also tested by the reduced growth of the species in growth media containing acid/alcalic indicators. The effects were visualised by colour change depending on the amounts of acid(s) produced by the yeast species.

THE EFFECTS OF AGROTECHNICAL FACTORS ON SOME PARAMETERS OF CARBON –CYCLE IN MEADOW CHERNOZEM SOIL

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The biological activity of soil was measured on meadow chernozem. Our aim was to obtain reliable data on how the different cultivation methods would influence the microbiological processes in a long-term experiment. Three different measurement methods were applied to approximately determine the biological activity of the soil. These three methods may be added to the biological activity of soils having different agrotechnical processes.
Soil cultivation and utilisation field trials were set up and conducted on meadow chernozem soil (Vertisols) in 1997. In the experimental field, the effects on some parameters of microbiological activity of two types of cultivation methods – *Traditional ploughing* and *Conservation tillage* - were measured in the eighth year of the experiment (in 2005). In the case of conservation tillage, there was no ploughing but direct sowing. The plants were barley and sunflower. During the growing season, soil samples were taken three times per year: in the spring, in the early summer and in the autumn, from a depth of 0-10 cm and 10-20 cm, respectively. In the course of laboratory analyses, the total number of bacteria, the total microscopic fungi, the number of aerobic cellulose decomposing bacteria, as well as the activities of urease, dehyrogenase enzymes and CO2 production were determined and evaluated.
Long-term minimal soil tillage had no negative effect on soil biological activity in the eighth year of the experiment. Shallow loosing and rotation at a depth of 20 cm increased the number of soil microorganisms and the activity of urease and dehyrogenase in the arable layer. It is especially true in the 0-10 cm layer, when compared with conventional ploughing of depth of 22 cm. Only the activity of phosphatase was generally higher in the parcels of conventional ploughing both in the 0-10 and the 10-20 cm layers. Regarding to the aerobic cellulose decomposing bacteria, there was no significant differences between the treatments of the two cultivation methods; higher bacterium number was measured in the soil of sunflower. In case of microscopic fungi, both the total number of fungi and the cultured genera were higher in the parcels of sunflower, especially in the case of conventional tillage.